A Soybean Disease Caused by Neocosmospora vasinfecta

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ABSTRACT

Neocosmospora vasinfecta, isolated from soybean plants with internal stem browning, caused similar symptoms in inoculated soybean plants. It invaded the pith extensively, but did not invade vascular tissues and apparently caused little damage to the host. Internal stem browning was induced in bean and cowpea, but not in plants of 11 other genera.

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Additional key words: Glycine max, Phaseolus vulgaris, Vigna sinensis.

Smith (14) observed fungus hyphae and microconidia in vascular tissues, and macroconidia and perithecia on dead stems of cotton, watermelon, and cowpea affected by a wilt disease. Cultures derived from ascospores did not cause wilt in his experiments, but since these cultures produced perithecia as well as macro- and microconidia, he concluded that all were stages of the fungus causing wilt and named it *Neocosmospora vasinfecta.*

The work of Butler (3), Higgins (6), and Wollenweber (16) demonstrated that *Neocosmospora vasinfecta* E. F. Smith was not the perithecial stage of a *Fusarium* of the section Elegans. Butler (3) and Higgins (6) regarded *N. vasinfecta* as a saprophyte, whereas Wollenweber (16) called it an obligate saprophyte. Since that time, only a few reports have appeared on pathogenic activity of *N. vasinfecta* (9, 10, 12, 13), but it has been found commonly in soil and on roots of plants in certain areas (1, 5, 7, 8).

Since *N. vasinfecta* was the only organism isolated from several soybean (*Glycine max* [L.] Merr.) plants with internal stem discoloration, this study was made to determine the relation of this fungus to internal stem discoloration in soybean.

MATERIALS AND METHODS.—Soybean plants used for inoculation were grown in solution culture in growth chambers as previously described (11), in pots of steam-sterilized soil, or in soil fumigated with methyl bromide in the greenhouse. Field plots were fumigated with methyl bromide (175 g/m^3).

Two *N. vasinfecta* isolates from soybean and five from peanut pods or kernels were used for inoculations. Unless specified otherwise, the cultures used were single ascospore isolates from soybean.

Inoculum preparation and the stem puncture inoculation procedure have been described (11). I inoculated roots of plants growing in soil by cutting them on one side with a knife and pouring inoculum into the hole made in the soil.

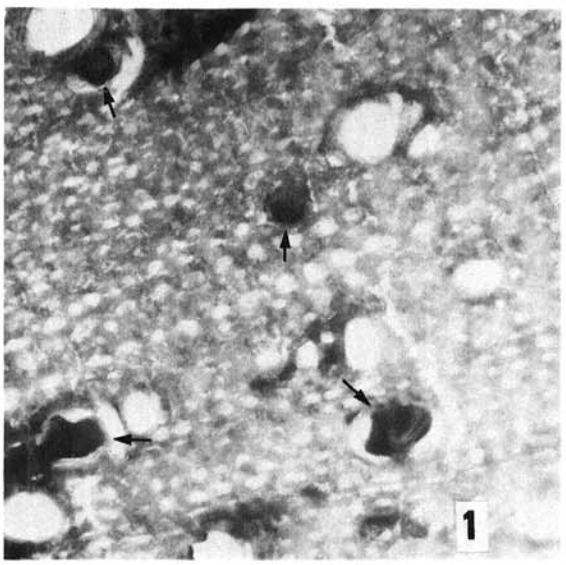
I usually determined disease severity 28-30 days after inoculation by splitting the stem longitudinally and visually checking for discoloration in the pith and xylem. The length of internal discoloration was measured, or the stems were rated on a scale of 0 to 4 (11) (0 = no discoloration, 4 = maximum discoloration).

RESULTS.—Inoculation with *N. vasinfecta* resulted in disease symptoms in 15 cultivars of soybean; Lee, Semmes, Bansei, Hardee, York, Hill, Hood, Jackson, Dare, Pickett, Davis, Bragg, Coker 102, Coker 207, and Coker 318. The primary symptom was a reddish-brown to dark brown discoloration in the pith and xylem of the stem (Fig. 4, 5) with occasional yellowing and loss of lower leaves. In one experiment, external lesions appeared on the stem of some inoculated plants (Fig. 3). Numerous perithecia developed on stem sections from inoculated plants after 6-7 days in a moist chamber, but were not observed on the stems of living plants. The fungus was reisolated from the discolored portion of the stem of inoculated plants but not from apparently healthy tissue above the discolored portion.

All inoculated soybean plants held at 27 or 21 C, and 91% of those at 15 C, developed symptoms, but the internal browning was more extensive at 27 C than at lower temperatures. At 27 C, the mean discoloration rating was 2.5, significantly higher (1% level) than the mean of 1.5 at 21 C or 1.2 at 15 C.

Internal stem discoloration was present in 83% of a total of 538 soybean plants 4 weeks after inoculation. Stem puncture inoculation resulted in a higher percentage of diseased plants than did inoculation of injured roots (Table 1). The length of

Fig. 1-6. **1)** Plugged xylem vessels in the stem of a soybean plant inoculated with *Neocosmospora vasinfecta.* **2)** *N. vasinfecta* hyphae in the pith of an inoculated soybean plant. **3)** External lesions on soybean stems inoculated with *N. vasinfecta.* **4)** Split soybean stems showing internal discoloration caused by *N. vasinfecta* (left) and *Cephalosporium gregatum* (right). Healthy plant in center. **5)** Split soybean stems showing internal discoloration; (left to right) healthy Semmes; inoculated Semmes, Lee, Bragg, York, Hood. **6)** Chlamydospores in an old *N. vasinfecta* culture.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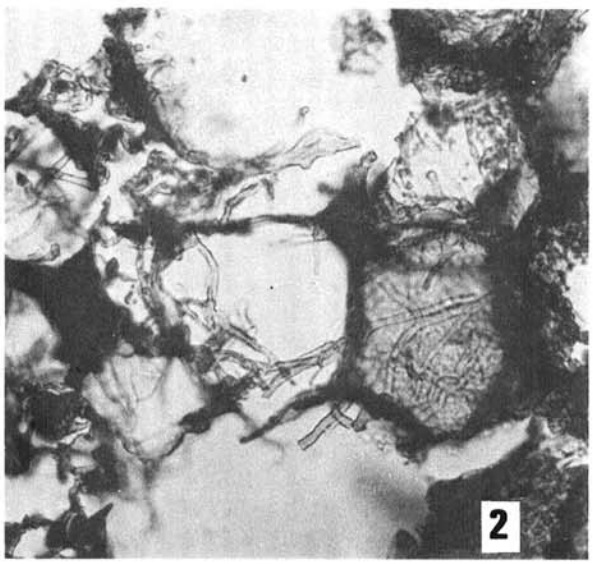
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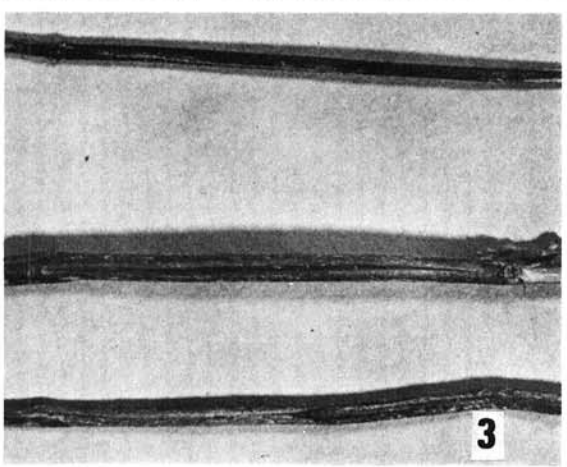
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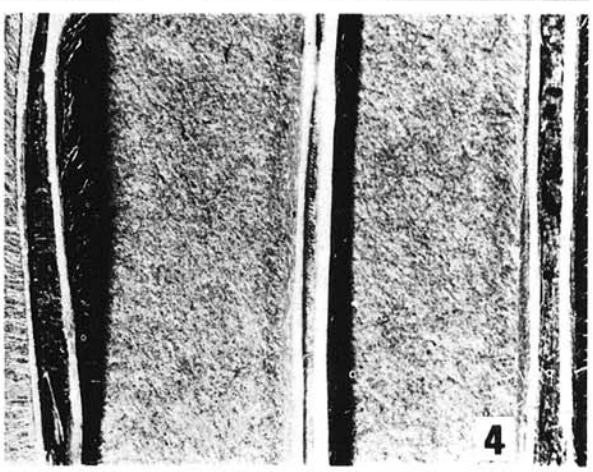
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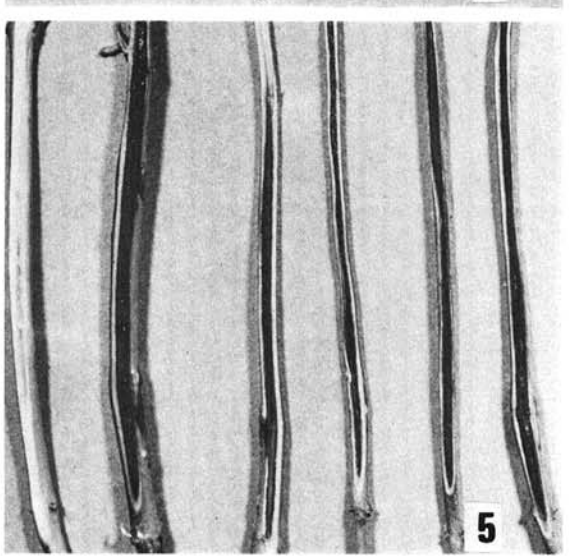
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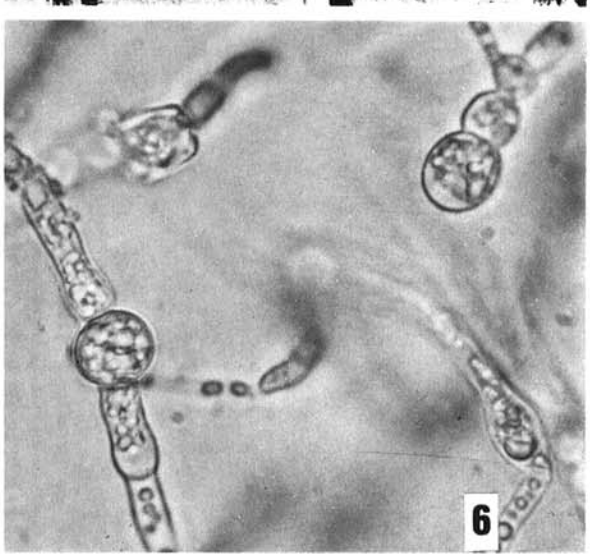
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TABLE 1. Effect of inoculation method on disease development 4 weeks after inoculation with *Neocosmospora vasinfecta*

Host plant	Inoculation method	Plants with symptoms	Internal stem browning[a]	
			Maximum	Mean[b]
		%	*cm*	*cm*
Glycine max	Stem injection	82.6	10	3.4 a
	Injured root	23.0	12	5.3 ab
Vigna sinensis	Stem injection	88.2	11	4.4 ab
	Injured root	28.1	9	5.7 b
Phaseolus vulgaris	Stem injection	100.0	11	6.8 b
	Injured root	31.2	9	6.9 b

[a] Measured from the soil surface.

[b] Mean of those plants with symptoms only; numbers followed by the same letter are not significantly different at the 1% level.

internal stem discoloration in plants 4 weeks after inoculation did not exceed 13 cm, and the mean was 4.5 cm. Soybean plants were examined 4, 5, 6, and 7 weeks after inoculation in one experiment; there was no significant difference in length of internal browning. No inoculated plants died, and their growth was equal to the controls. Noninoculated plants had no symptoms in any of the greenhouse or growth chamber experiments, but internal stem browning was present in 3% of the control plants in field plots. Internal stem discoloration in plants inoculated in June and examined in October (Table 2) was more extensive, and differences among cultivars were greater than in greenhouse experiments. The length of internal stem browning in inoculated plants was equal to or exceeded that in plants inoculated with *Cephalosporium gregatum* Allington & Chamberlain isolate 36, but was less than that in plants inoculated with *C. gregatum* isolate 5 (Table 2).

The internal browning in soybean stems inoculated with *N. vasinfecta* sometimes differed in color or intensity from that caused by *C. gregatum*, but the differences were not consistent enough to distinguish between these two diseases (Fig. 4).

It is possible to distinguish between the diseases in young soybean plants 4 weeks after inoculation by the examination of transverse stem sections microscopically. In several hundred sections, hyphae were not observed in the xylem vessels of plants inoculated with *N. vasinfecta*, but were commonly found in the xylem vessels of soybeans inoculated with *C. gregatum*. In contrast, hyphae are abundant in the pith of plants inoculated with *N. vasinfecta* (Fig. 2), but not in the pith of young soybeans inoculated with *C. gregatum*. *C. gregatum* hyphae, however, have been observed in the pith of plants with brown stem rot (2). Although hyphae of *N. vasinfecta* have not been found in the xylem, a low percentage of vessels are commonly filled with a brown material (Fig. 1) which apparently causes the browning observed in the xylem of split stems.

TABLE 2. Comparison of disease development in field-grown soybeans inoculated with *Neocosmospora vasinfecta* or with *Cephalosporium gregatum*

Soybean cultivar	Inoculated with	Plants with symptoms	Internal stem browning[a]	
			Maximum	Mean[b]
		%	*cm*	*cm*
Lee	Nv[c]	95	17	8.8 a
	Cg 36[d]	100	25	8.9 a
	Cg 5[e]	100	62	43.5 c
Bragg	Nv	95	16	8.6 a
	Cg 36	89	20	9.7 a
	Cg 5	100	88	59.1 e
Hampton	Nv	100	25	12.5 ab
	Cg 36	90	48	8.6 a
	Cg 5	100	70	50.9 d
Coker 102	Nv	100	47	16.0 b
	Cg 36	94	20	10.3 a
	Cg 5	100	103	66.1 f

[a] Measured from soil surface.

[b] Mean of 40 determinations; numbers followed by the same letter are not significantly different at the 1% level.

[c] *Neocosmospora vasinfecta* Isolate No. 6-1 (single ascospore isolate).

[d] *Cephalosporium gregatum* Isolate No. 36 (ATCC 11073).

[e] *Cephalosporium gregatum* Isolate No. 5.

In addition to soybean, *Phaseolus vulgaris* L. 'Top Crop', 'Contender', 'Tender Crop', 'Pinto', 'Kinghorn Wax', and *Vigna sinensis* (Torner) Savi 'Early Ramshorn' were susceptible to *N. vasinfecta* (2 isolates from soybean and 5 from peanut pods or kernels) in greenhouse tests. The primary symptom was internal stem browning similar to that in soybean.

Plants which were inoculated but were not susceptible include: *Arachis hypogaea* L. 'Argentine' and 'Virginia Bunch'; *Gossypium hirsutum* L. 'Coker 413-67' and 'Empire WR-61'; *Nicotiana tabacum* L. 'Havana 425'; *Zea mays* L. 'Seneca Chief'; *Lycopersicon esculentum* Mill. 'Rutgers'; *Cucurbita pepo* L. 'Early Yellow Summer Crookneck' and 'Genuine Mammoth'; *Cucumis melo* L. 'Pride of Wisconsin'; *Citrullus lunatus* [Thunb.] Mansf. 'Charleston Gray'; *Beta vulgaris* L. 'Detroit Dark Red'; *Brassica oleracea* var. *capitata* L. 'Golden Acre'; and *Brassica juncea* (L.) Coss 'Tender Green'.

DISCUSSION.—This disease has been found in soybeans in the field, but random surveys of soybean fields in Georgia indicate that its frequency is low. The low percentage of infected plants resulting from injured root inoculation indicates that *N. vasinfecta* is probably not an aggressive root invader, which may in part explain why this disease is found infrequently in the field.

Nisikado & Yamauti (10) reported that a strain of *N. vasinfecta* causing a seedling wilt of silk tree (*Albizzia julibrissin*) also attacked cotton and watermelon. None of the *N. vasinfecta* isolates used

in this study caused symptoms on cotton or watermelon. Sarojini (12) reported that conidial and chlamydospore isolates of *N. vasinfecta* were more virulent to pigeon pea than the original strain or its ascospore derivatives. Four single ascospore isolates and one single microconidial isolate were as virulent as the parent isolate on Lee soybeans in this study. Chlamydospores were found only in old cultures (Fig. 6).

N. vasinfecta appears to do little damage to soybean plants, as it did not kill or reduce growth of inoculated plants. This lack of apparent damage to the host may be explained by the fact that it was confined to the pith. In contrast, *C. gregatum* invades the vascular system extensively and has been shown to inhibit water movement (4) and reduce yields (15).

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