Epidemiological and Mycofloral Relationships in Cotton Seedling Disease in Mississippi

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Published with the approval of the director, Mississippi Agricultural and Forestry Experiment Station as Journal Series Paper 4745.
This research was supported in part by Environmental Protection Agency (EPA) Contract CR-806277 and Subcontract L800152. The results published in this article are not to be represented as views, findings, or policies of the EPA and mention of trade names or commercial products is not an endorsement or recommendation of them over others that also may be suitable.
Accepted for publication 11 December 1981.

ABSTRACT

Roy, K. W., and Bourland, F. M. 1982. Epidemiological and mycofloral relationships in cotton seedling disease in Mississippi. Phytopathology 72:868-872.

Cotton seedlings and soil adjacent to seedlings were collected from various locations in Mississippi. Seedlings were evaluated for incidence of fungi on root and hypocotyl tissues. Selected seedling, fungal, and soil variables were analyzed for possible correlations. Of more than 50 different fungi isolated from seedlings, *Fusarium oxysporum* was the most frequent, followed by *Rhizoctonia solani, Thielaviopsis basicola, Fusarium equiseti,* and *F. solani.* Most fungi were isolated more frequently from young than old root and hypocotyl tissues. In pathogenicity tests, isolates of *F. oxysporum, R. solani,* and *T. basicola* were pathogenic to seedlings. Incidence of *F. oxysporum* on seedlings was positively correlated with root disease index, hypocotyl disease index, and total soil N/ha and negatively correlated with soil pH. Incidence of *T. basicola* on seedlings was positively correlated with soil pH, root disease index, and hypocotyl disease index and negatively correlated with seedling population. Incidence of *R. solani* on seedlings was positively correlated with hypocotyl disease index. Root disease index was positively correlated with hypocotyl disease index and negatively correlated with seedling population.

Seedling disease, a disease complex of cotton (*Gossypium hirsutum* L.), causes annual economic losses in Mississippi. *Rhizoctonia solani* Kühn, *Thielaviopsis basicola* (Berk. & Br.) Ferr. and species of *Fusarium* and *Pythium* are generally considered major fungal components of the complex, although *Fusarium* is considered by some to be a secondary invader (3,6,26,33). Any one or a combination of these fungi may incite seed rot, root rot, or damping-off, resulting in stand failure (14,16–18,24,25).

Data from previous investigations (13,25) generally characterized the mycoflora of cotton seedlings in Mississippi. However, there is little data on incidence (not only in Mississippi, but elsewhere) of selected fungi, particularly species of *Fusarium*. A 1942 report from Oklahoma by Ray and McLaughlin (26) apparently is the only major study of the incidence of *Fusarium* species on cotton seedlings in the United States. There also is a paucity of data on the ecology and epidemiology of cotton seedling disease fungi.

The major areas of cotton production in Mississippi were surveyed, with the following objectives: to determine the incidence and severity of seedling disease; to identify fungi associated with diseased seedlings, particularly species of *Fusarium;* and to identify ecological and epidemiological relationships among selected seedling, fungal, and soil variables.

MATERIALS AND METHODS

Seven locations in north central Mississippi and 29 in the Yazoo-Mississippi Delta were surveyed for the incidence of cotton seedling disease and seedling mycoflora in May of 1978 and 1979. Each year, 30 seedlings were randomly sampled from a 30-m section of row per location. Seedlings were wrapped in moist paper towels, placed in plastic bags and stored on ice prior to examination at Mississippi State. After storage for less than 48 hr, seedlings were rated for root and hypocotyl symptoms. Seedlings were washed in running tap water for 20 min; from each one, two contiguous 5-mm sections were excised from the root tip, intermediate portion, and root-hypocotyl transition zone of taproots. Sections of tissue were surface-sterilized for 30 sec in 0.5% NaOCl, rinsed in sterile water, and blotted on sterile filter paper. A section from each of the three taproot positions was plated on potato-dextrose agar (PDA) amended with 100 ppm streptomycin B sulfate and 2 ppm aureomycin (PDASA). Three additional contiguous sections were plated on cornmeal agar (CMA) amended with 100 ppm pimaricin, 50 ppm penicillin G, and 50 ppm polymyxin B sulfate (CMAP). During a 10-day incubation period at 22 C, colonies of *R. solani, T. basicola, Fusarium,* and other fungi on PDASA, and *Pythium* on CMAP, were enumerated. *Fusarium* isolates were transferred to a modified Bilay medium (9) and cultured under continuous light at 22 C to facilitate identification. Pythiums were not identified to species in 1978; in 1979, they were cultured on CMA and on grass blades in water (31) for species determination.

In 1979, soil adjacent to roots of randomly selected seedlings was sampled and seedling numbers (stand) was determined from a 30-m section of row at each location. Soil samples were obtained from the upper 10–15 cm of soil with a hand spade and were composited. Composite soil samples from each location were stored in sealed plastic bags at 10 C for a maximum of 4 mo prior to analysis for chemical and physical characteristics by the Soil Testing Department of the Mississippi Cooperative Extension Service. Length of the first true leaf of each seedling collected was measured and used as an estimate of seedling age. Selected combinations of the following data were statistically analyzed for possible correlations: incidence of *Alternaria alternata, Diaporthe* spp., *Fusarium equiseti, F. oxysporum, F. solani, Penicillium* spp., *Phoma* spp., *Pythium* spp., *R. solani, T. basicola,* and total fungi (ie, the group of fungi listed in Table 1) on seedlings; amounts (in kilograms per hectare) of Ca, K, Mg, total nitrogen, P, and S in soil; percent soil organic matter; soil pH; seedling age; root disease index; hypocotyl disease index; seedling numbers (stand); and percent seedlings with abnormal radicles.

The three most frequently isolated fungi were tested for pathogenicity on cultivar Stoneville 213 cotton seedlings. Eight isolates of *F. oxysporum* and 10 isolates each of *R. solani* and *T. basicola* were cultured for 1 wk on modified Bilay medium (9), PDA, and V-8 juice agar, respectively. Inoculum of each isolate was prepared by macerating one culture in 125 ml of water in a Waring Blendor. Each isolate of *R. solani* and *T. basicola* was

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0031-949X/82/07086805/$03.00/0

TABLE 1. Incidence and severity of symptoms on cotton seedlings collected in Mississippi

Percentage plants with root disease index:[a]						Percentage plants with hypocotyl disease index:[b]			Range and mean of disease indices (%) over all locations					
									Root			Hypocotyl		
1	2	3	4	5	6	1	2	3	Low	Mean	High	Low	Mean	High
7.8	37.4	25.8	14.4	9.1	5.6	9.6	65.8	24.6	1.7	3.2	4.6	0.6	2.1	3.8

[a] 1 = No root necrosis (browning) and 2–6 = increasisngly severe necrosis. Figures are percent of total plants sampled.
[b] 1 = No hypocotyl lesions, 2 = nongirdling hypocotyl lesions, and 3 = girdling hypocotyl lesions. Figures are percent of toal plants sampled.

inoculated onto 50 seeds by distributing inoculum uniformly over seeds that were in furrows of sand contained in 20-cm-diameter clay pots. After covering seed with a 2.5-cm layer of sand, treatments were incubated in a growth chamber at 19 C. Sand was kept moist throughout the incubation period. Seeds treated with aqueous suspensions of V-8 juice agar and PDA served as controls for *T. basicola* and *R. solani* tests, respectively. Stand and disease severity were evaluated after 2 wk. Eight 10-day-old seedlings were inoculated with each isolate of *F. oxysporum* by dipping roots momentarily in inoculum. Control seedlings were dipped in an aqueous suspension of the modified Bilay medium (9). Seedlings were transplanted to sand contained in 20-cm-diameter clay pots and incubated in the greenhouse. Symptoms on seedlings were recorded and disease severity evaluated during and at the end of 3 wk.

RESULTS AND DISCUSSION

More than 90% of seedlings sampled had root and hypocotyl symptoms. Most seedlings had moderate root necrosis and nongirdling hypocotyl lesions (Table 1).

The diverse mycoflora associated with diseased cotton seedlings is presented in Table 2. Of the fungi isolated, *Aureobasidium pullulans, Chaetomium trilaterale, Curvularia lunata* var. *aeria, Diaporthe* (? *phaseolorum*), *Fusarium fusaroides, F. graminearum, Helminthosporium sorokinianum, Papulaspora* (? *rubida*), and *Pithomyces chartarum* apparently had not been previously isolated from cotton seedlings. Such fungi were relatively infrequent. The most frequent isolates were species of *Fusarium, R. solani, T. basicola,* and species of *Pythium,* which comprised 42, 11, 10, and 6%, respectively, of the total number of isolates.

Nine species of *Fusarium* were isolated from diseased seedlings (Table 2). On PDASA, *Fusarium* isolates either did not produce the microconidia and/or macroconidia that are required for species determination or such structures were morphologically variable. When cultured on a modified Bilay medium (9) for 5–10 days under continuous light all species produced conidia. The resultant increase in production and decrease in morphological variability of conidia greatly facilitated identification.

Based on our survey and one by Davis (13), which encompassed over 40 locations in Mississippi, *Fusarium* is more frequent on cotton seedlings in this state than previously indicated (25). *Fusarium* spp. were the most frequently isolated fungi from cotton seedlings in surveys conducted in Arkansas (14), Oklahoma (26), and in one survey conducted throughout the Cotton Belt (33). Although studies (26,33) indicated that *F. moniliforme* was the most prevalent *Fusarium* species on cotton seedlings, *F. oxysporum* was the most frequently isolated species in our survey. It was isolated from more than 90% of seedlings at seven locations and from seedlings at 27 of 36 locations. The positive correlations between its incidence on seedlings and the root disease index (0.58) and hypocotyl disease index (0.44), both significant at $P = 0.01$, suggest that it is an active seedling disease pathogen. Others have shown that it can cause seed rot, root rot, and damping-off of cotton (6,14,26,27). Therefore, it appears that *F. oxysporum* may play an important role in the seedling disease complex, as Rosen (27) implied in an earlier report. Further, the distribution of *F. oxysporum* indicates that most cotton soils in Mississippi are infested with this fungus, strains of which cause Fusarium wilt (24). In pathogenicity tests, two isolates of the fungus caused typical wilt symptoms on seedlings leading to their eventual death; four isolates caused wilt symptoms, but were not as aggressive as the latter two; and two isolates were nonpathogenic. This suggests that some of the *F. oxysporum* isolates recovered from the survey were the wilt fungus, *F. oxysporum* f. sp. *vasinfectum*.

TABLE 2. Incidence of fungi on cotton seedlings in Mississippi[a]

Fungus	Frequency of isolation (range and mean, %) over all locations		
	Low	Mean	High
Fusarium oxysporum	0	36.4	100
Rhizoctonia solani	0	17.7	65
Thielaviopsis basicola	0	16.0	100
Fusarium equiseti	0	13.5	60
F. solani	0	13.2	35
Pythium spp.[b]	0	8.8	35
Pencillium spp.	0	8.8	75
Diaporthe spp.[c]	0	8.0	40
Phoma spp.	0	7.5	20
Alternaria alternata	0	6.8	45
Trichoderma viride	0	5.1	20
Macrophomina phaseolina	0	4.6	40
Fusarium moniliforme	0	2.8	15
Auriobasidium pullulans	0	2.4	40
Thielavia basicola	0	2.3	10
Fusarium semitectum	0	1.7	15
Botryodiplodia theobromae	0	1.6	10
Cephalosporiopsis sp.	0	1.6	15
Cladosporium herbarum	0	1.6	15
Curvularia lunta	0	1.5	20
Epicoccum purpurascens	0	1.5	10
Verticillium dahliae	0	1.3	15
Pithomyces chartarum	0	1.1	15

[a] Seven of the locations were sampled in 1978 and 29 were sampled in 1979. For each of the following fungi, mean frequency over all locations was less than 1%: *Aspergillus flavus, A. niger, Chaetomium globosum, C. trilaterale, Colletotrichum capsici, C. gloeosporioides, C. gossypii, Curvularia lunata* var. *aeria, Fusarium acuminatum, F. fusaroides, F. graminearum, F. tricinctum, Gliocladium* sp., *Helminthosporium Sorokinianum, Helminthosporium* spp., *Humicola grisea, Mucor mucedo, Phomopsis* spp., *Papulaspora* (? *rubida*), *Rhizopus nigricans, Sclerotium rolfsii, Verticillium dahliae.*
[b] *Pythium ultimu, P. irregulare,* unidentified *Pythium* isolates *Nigrospora oryzae, Nigrospora* spp., and *P. heterothalicum* comprised 52.9; 35.9, 10.6, and 0.4%, respectively, of total *Pythium* isolates in 1979.
[c] Some isolates tentatively identified as *D. phaseolorum.*

In addition to *F. oxysporum,* eight other *Fusarium* species were recovered from diseased seedlings. There is substantial evidence that *F. moniliforme* is pathogenic to cotton seedlings (6,14,26,33,34). The pathogenicity of *F. solani* was demonstrated (5,26), as was that of *F. equiseti, F. acuminatum,* and *F. semitectum* (5).

Considering their pathogenic potential, the incidence of *Fusarium* species on cotton seedlings, particularly *F. oxysporum,* should be monitored more closely. As an estimate of the potential for Fusarium wilt, monitoring of *F. oxysporum* takes on added significance.

R. solani was isolated from seedlings with a frequency less than one-half that of *F. oxysporum,* occurring on seedlings at 23 of 36 locations, but was not isolated from more than 65% of seedlings at any location (Table 2). In pathogenicity tests, eight isolates of the fungus killed all seedlings prior to emergence; one isolate caused preemergence and postemergence damping-off on 81% of seedlings and one caused preemergence damping-off on 92% of the seedlings. Other workers have shown that *R. solani* is strongly pathogenic (14,16,22). Yet considering the frequency with which it was isolated, it falls far short of accounting for root and hypocotyl symptoms observed during the survey. Incidence of the fungus on seedlings was positively correlated with the hypocotyl disease index (0.52, significant at $P = 0.01$) but was not correlated with the root disease index. Perhaps the relative importance of *R. solani*, at least as a pathogen of emerged cotton seedlings, has been somewhat overestimated, as has been suggested (3,27).

T. basicola, which occurred on seedlings with essentially the same frequency as *R. solani* (Table 2), was isolated from seedlings at 18 of 36 locations. This fungus was difficult to detect on the assay medium (PDASA). It produced numerous chlamydospores and endoconidia on plated tissues, but usually did not advance on the agar surface, apparently due primarily to competition from faster-growing fungi. Consequently, direct observation of the fungus in situ was necessary for its enumeration, suggesting that *T. basicola* may sometimes be overlooked in routine assays for seedling disease fungi. In surveys conducted in Mississippi for the periods 1959–1961 (25) and 1972–1974 (13), the fungus was recovered from an average of less than 7.5 and 1.0% of seedlings, respectively. In our study *T. basicola* was recovered from 16% of diseased seedlings (mean of all locations) and was isolated from 100% of sampled seedlings at four locations.

The frequency with which *T. basicola* occurred on seedlings, its occurrence on 100% of seedlings at some locations, its distribution in the state and its reported pathogenicity (14,22,29) indicate that it is a substantially important seedling disease pathogen in Mississippi. This is supported by its pathogenicity to seedlings (Table 3) and the negative correlation between its incidence on seedlings and seedling stand (0.52, significant at $P = 0.01$). Moreover, positive correlations occurred between incidence of the fungus on seedlings and the root disease index (0.44) and hypocotyl disease index (0.40), both significant at $P = 0.05$.

Pythium spp. were relatively infrequent (Table 2). They occurred on seedlings at 14 of 36 locations, but were not isolated from more than 35% of seedlings at any location. They were not correlated with any of the selected variables. The most frequently isolated species, *P. ultimum*, has often been recovered from diseased cotton seedlings (4,16,17). Whether it is the prevalent species on cotton in Mississippi can only be determined through an examination of a larger collection of *Pythium* isolates, since species of *Pythium* on cotton seedlings in Mississippi have not been reported before.

Several other isolated fungi appear to be noteworthy (Table 2). Some isolates of *Diaporthe* were morphologically similar to the *Diaporthe* species that cause pod and stem blight and stem canker of soybean (*Glycine max* (L.) Merr.). Several isolates of *Diaporthe* from cotton caused stem cankers when toothpick tips infested with mycelium were inoculated into stems of 7-wk-old cultivar Bragg soybean plants in the greenhouse. Since such isolates agreed closely with Wehmeyer's description of *D. phaseolorum* (32) they were tentatively identified as such. Isolates of *Colletotrichum gloeosporioides* (sensu Arx, 7) were identical to isolates from soybean seed found to be pathogenic to soybean seedlings in another investigation (K. W. Roy, *unpublished*).

Seven fungal species, including the two most frequently isolated ones, and total fungi occurred more frequently on young than on old root tissues (Table 4). Whether this indicates competition among fungi or a preference of fungi for tissues of a certain age cannot be explained by the present study. Nevertheless, the location of fungi in root and hypocotyl tissues seems of practical significance in monitoring their incidence on seedlings. Assays of young rather than old root tissue should provide better estimates of

TABLE 3. Pathogenicity of *Thielaviopsis basicola* isolates to Stoneville 213 cotton seed and seedlings[a]

T. basicola isolates	Emergence (% of control)	Root disease index[b]
T101	87.5	2.3
T102	79.2	2.5
T103	95.8	2.4
T104	87.5	2.1
T105	95.8	2.0
T106	93.8	2.0
T107	97.9	2.0
T108	100.0	2.1
T109	95.8	2.0
T110	95.8	2.4

[a] In the greenhouse, mycelial suspensions of each isolate were distributed uniformly over 50 seeds, which were in furrows of sand in clay pots. Seeds were covered and incubated at 19 C for 2 wk, after which emergence and incidence of root symptoms were recorded. None of the emerged seedlings damped off.

[b] 1 = No or slight lesions, 2 = moderate lesions, and 3 = severe lesions (sunken, often extending from soil surface to tip of taproot).

TABLE 4. Relationship between age of cotton root-hypocotyl tissues and the incidence of fungi

Fungus	Incidence (%) of fungi[y] on cotton seedling tissue classed as:		
	Young (%)	Intermediate (%)	Old (%)
Fusarium oxysporum	42.0 a[z]	25.2 b	32.8 ab
Rhizoctonia solani	52.0 a	28.0 b	20.0 b
Thielaviopsis basicola	16.7 c	52.4 a	30.9 b
Diaporthe spp.	90.1 a	0.9 b	0.0 b
Phoma spp.	61.5 a	7.7 c	30.8 b
Alternaria alternata	64.3 a	21.4 b	14.3 b
Trichoderma viride	55.6 a	33.3 b	11.1 c
Macrophomina phaseolina	66.7 a	22.2 b	11.1 b
Total fungi isolated	42.6 a	27.9 b	29.5 b

[y] Relative incidence (percent of total isolates of a fungus) among young (root tip), intermediate, and old (root-hypoctyl transition zone) root-hypocotyl tissue.

[z] In analysis of variance, data from 29 Mississippi Delta locations were used as replications. Numbers within a row followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

TABLE 5. Correlations between selected cotton seedlings, fungus, or soil variables

Independent and dependent variables	Correlation coefficient[a]
Percent seedlings from which *F. oxysporum* was isolated and:	
Soil pH	−0.67**
Kg Mg per hectare of soil	+0.53**
Seedlings (%) from which *F. equiseti* was isolated	+0.44*
Kg total N per hectare of soil	+0.41*
Percent seedlings from which *T. basicola* was isolated and:	
Soil pH	+0.41*
Percent total fungi isolated from seedlings and:	
Root disease index	+0.75**
Hypocotyl disease index	+0.56**
Seedling age	+0.51**
Soil pH	−0.45**
Kg Mg per hectare of	+0.43*
Kg total N per hectare of soil	+0.39 ($P = 0.06$)
Root disease index and:	
Hypocotyl disease index	+0.77**
Seedling age	+0.57**
Seedling numbers (stand)	−0.51 ($P = 0.06$)
Kg total N per hectare soil	+0.41 ($P = 0.06$)
Kg Mg per hectare of soil	+0.40 ($P = 0.06$)
Seedling numbers (stand) and:	
Seedling age	−0.55*
Seedlings (%) with abnormal radicles[b]	−0.53*

[a] Probabilities: *, $P = 0.05$; **, $P = 0.01$; and as noted in parentheses.

[b] Lacking positive geotropism.

the incidence of most seedling disease fungi.

With one exception, the incidence of a given fungus on cotton seedlings was not significantly correlated with the incidence of any other fungus. Incidence of *F. oxysporum* and *F. equiseti* were positively correlated (Table 5). The correlation, though modest, suggests that the two fungi may have some ecological similarities or may interact in some way.

Incidence of fungi on seedlings was correlated with soil pH (Table 5). *F. oxysporum* and total fungi were most frequent on seedlings from acid soils, whereas *T. basicola* was most frequent on seedlings from basic soils. These relationships could have resulted from direct or indirect effects of soil pH. Fungi are generally more abundant in acid soils (30). Bagga (8) reported that the most frequently isolated fungi in Yazoo-Mississippi Delta soils with an average pH between 5 and 6 were species of *Fusarium* (15 unnamed species). In our study, the average pH of the Delta soils sampled was 5.7, a pH that favors growth of *F. oxysporum* in culture (10). The acidity of soil may have indirectly affected the incidence of *F. oxysporum* and total fungi isolated through predisposition of seedlings. For example, it has been suggested that toxic levels of manganese, which may occur in soils of pH 5.2 or less and which are associated with poor stands of cotton seedlings, predispose seedlings to pathogens (11). Eight of the soils we surveyed had a pH of 5.2 or less. It does not appear to be coincidence then, that the incidence of *F. oxysporum* and total fungi on seedlings was negatively correlated with soil pH. In the case of *F. oxysporum,* the negative correlation is consistent with the high incidence of Fusarium wilt of cotton in acid soils (19).

We are not aware of previous correlative field data showing a relationship between the natural incidence of *T. basicola* on cotton seedlings and soil pH. Increasing the pH of soil increased the severity of *T. basicola* root rot of tobacco (2) and poinsettias (20). Anderson et al (2) showed that the optimum pH for growth of the fungus in culture paralleled closely the soil pH at which black root rot of tobacco was most severe. In the present study, it is probable that the growth of *T. basicola* was enhanced in basic soils, reflected by its greater incidence on seedlings from such soils.

Incidence of *F. oxysporum* on seedlings, total fungi isolated from seedlings and root disease index were each positively correlated with total soil nitrogen (Table 5). Other investigators reported that a high level of soil nitrogen was associated with an increased incidence of soilborne diseases of various hosts, including Fusarium wilt of cotton (1,15,17). It was suggested that excess nitrogen fertilization may predispose cotton seedlings to pathogens by injuring roots (11). An increase in the succulence of plant tissue associated with excess soil nitrogen generally has a predisposing effect (15). Incidence of fungi on seedlings may have been affected by nitrogen in a more indirect manner. For example, Sadasivan (28) found that nitrogen amendments to soil increased the severity of Fusarium wilt of cotton and correspondingly decreased populations of bacteria and actinomycetes antagonistic to the wilt fungus. In the present study, similar effects of nitrogen may be responsible in part for the increased incidence of fungi on seedlings.

The amount of soil magnesium was positively correlated with the incidence of *F. oxysporum* on seedlings, total fungi isolated from seedlings, and root disease index (Table 5). This finding is inconsistent with data on other host-parasite combinations. Foliar sprays of magnesium sulfate reduced the total number of rhizosphere fungi (28) and deficiencies of magnesium in the soil increased the susceptibility of soybean roots to *R. solani* (21). However, applications of magnesium sulfate to soil increased the susceptibility of peanut to pod rot by *R. solani* and *Pythium* (12).

Seedling age and hypocotyl disease index were positively correlated and seedling population was negatively correlated with root disease index. Seedling numbers was negatively correlated with seedling age and incidence of seedlings with abnormal radicles (Table 5). These data suggest that the earlier seed were planted the more susceptible they were to infection. The oldest seedlings (presumably from early plantings) were exposed to temperatures that favored infection; ie, cool temperatures (23), and were exposed to fungi for the longest period of time. Infection of roots and hypocotyls and abnormal development of seedling radicles contributed to the reduction in stand.

Seedling disease of cotton is an extremely complex phenomenon. The traditional major fungal pathogens do not account totally for the incidence of disease. Whether fungi participate in the disease complex solely as individuals or in combination with other fungi is still unclear. Further research to determine the pathogenicity of individual fungi and to elucidate interactions among fungi is necessary for understanding the complex. As suggested by the correlations (Table 5), other factors indirectly affect the incidence of seedling disease. An understanding of their effects must be integrated into our knowledge of the seedling disease complex. The correlations suggest that production practices, particularly those affecting soil fertility and pH, may be strategically managed to reduce the incidence and severity of seedling disease.

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