Seedling Diseases Caused in Soybean by Species of *Colletotrichum* and *Glomerella*

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ABSTRACT

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Infestation of soybean seeds with *Colletotrichum dematium* var. *truncata, C. gloeosporioides, C. graminicola,* and *Glomerella cingulata* reduced seedling emergence in the greenhouse and field. *Colletotrichum destructivum* reduced emergence only in the greenhouse, *Glomerella glycines* only under field conditions. *C. dematium* var. *truncata, C. gloeosporioides* and *G. cingulata* were the only species that caused severe hypocotyl and cotyledonary infection and significant stunting of seedlings. As potential pathogens of soybean seedlings in the field, *C. dematium* var. *truncata, C. gloeosporioides,* and *G. cingulata* appear to be more significant than the other species investigated.

Additional key words: alfalfa, apple, bell pepper, cocklebur, corn, purple nutsedge.

Anthracnose of soybean (*Glycine max* (L.) Merr.) is found in all soybean-producing areas of the United States. Historically it was considered a minor disease (3,9) but in recent years has become increasingly important, especially in humid regions of soybean production, causing substantial yield reductions in the South (1). Disease loss estimates, however, usually do not account for preemergence and postemergence killing or blighting of seedlings by anthracnose fungi.

Colletotrichum dematium (Fr.) Grove var. *truncata, C. destructivum* O'Gara (10), *C. gloeosporioides* Penz. (5), *C. graminicola* (Ces.) Wilson (8), *Glomerella glycines* Hori (10), and *G. cingulata* (Stonem.) Spauld. & v. Schr. (6) are the anthracnose fungi reported on soybeans in the United States. However, in the most current and comprehensive treatments of soybean diseases (3,9), only *C. dematium* var. *truncata* and *G. glycines* are referred to as causes of anthracnose, with the former considered the more prevalent of the two species.

The ability of *C. dematium* var. *truncata* to cause seed rot, preemergence and postemergence killing, and blighting of soybean seedlings is well documented (11). However, comparable data are lacking for the other anthracnose fungi associated with soybeans.

This investigation was made to determine the ability of selected *Colletotrichum* and *Glomerella* species to cause preemergence and postemergence infection when inoculated onto soybean seeds.

MATERIALS AND METHODS

Isolation and identification of fungi. The sources of the *Colletotrichum* and *Glomerella* isolates used for inoculation of soybean seeds are presented in Table 1. Fungi were isolated from field collections of soybean, alfalfa, purple nutsedge, cocklebur, and bell pepper. Seeds or sections of tissue from mature stems and from leaf spots of these plants were surface-sterilized with 1% NaOCl, plated on potato-dextrose agar (PDA), and incubated at 22 C. Species of *Colletotrichum* and *Glomerella* growing from plated material were identified or, to facilitate their identification, were cultured on sections of sterilized soybean stems in test tubes. Descriptions of *Colletotrichum* and *Glomerella* species reported by Arx (2) and Tiffany and Gilman (12) were used in species determination.

Increase of inoculum. Both mycelial and spore suspensions were used as inoculum.

To obtain mycelial colonies, fungi were grown in total darkness on PDA (20 ml per petri dish) for 10 days at 22 C. A mycelial suspension of each fungus was obtained by comminuting the entire contents of one petri dish culture in sterile distilled water in a Waring Blendor. The final volume of inoculum per fungus was 125 ml.

To obtain spores for inoculation, fungi were grown in continuous light (1,600 lux) on sterilized soybean stems in test tubes for 10 days at 22 C. *G. glycines* produced ascospores only, while all other species produced conidia. Stem sections were transferred to test tubes containing sterile distilled water and were shaken in the water to dislodge the conidia. Conidial suspensions were filtered twice through two layers of cheesecloth and adjusted to 5×10^5 conidia per milliliter with sterile distilled water containing two drops of Tween-20 in 100 ml. The epidermis of stem sections bearing perithecia of *G. glycines* was peeled off and macerated between two sterile microscope slides. The macerate was washed off the slides and the resultant suspension was filtered through two layers of cheesecloth. The ascospore suspension was adjusted to 10^5 ascospores per milliliter with sterile distilled water containing two drops of Tween-20 per 100 ml.

Pathogenicity tests. Seeds of the soybean cultivar Bragg were used in all pathogenicity tests. They were examined and only those with no visible damage were selected for the tests.

In the first greenhouse experiment, seeds were inoculated by using mycelial suspensions of the different fungi. Thirty seeds in each of three replicates were inoculated with each fungus. The seeds were in shallow furrows in sterile sand contained in 20-cm-diameter clay pots and were inoculated by distributing 40 ml of inoculum uniformly over them. Seeds were covered with a 2-cm layer of sand and watered. The treatments and untreated controls were randomized and maintained on a greenhouse bench. Emerging seedlings were observed periodically for symptoms. Emergence and the incidence of stunted seedlings were recorded after 2 wk, and 10 randomly selected seedlings per replication were uprooted, washed, and evaluated for symptoms. This experiment was conducted twice.

In the subsequent greenhouse experiment, one isolate of each species was selected and spore suspensions of it were used to inoculate the seeds. Ten seeds in each of four replicates were

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TABLE 1. Sources of *Colletotrichum* and *Glomerella* isolates used for inoculation of soybean seeds

Fungus	Isolate number	Host	
		Common name	Scientific name
C. dematium var. *truncata*	T1	Soybean	*Glycine max* (L.) Merr.
C. dematium var. *truncata*	T2	Cocklebur	*Xanthium pennsylvanicum* Wallr.
C. dematium var. *truncata*	T3	Purple nutsedge	*Cyperus rotundus* L.
C. destructivum[a]	D1	Alfalfa	*Medicago sativa* L.
C. destructivum	D2	Alfalfa	*M. sativa*
C. destructivum	D3	Alfalfa	*M. sativa*
C. gloeosporioides	GL1	Soybean	*G. max*
C. gloeosporioides[b]	GL2	Apple	*Malus sylvestris* Mill.
C. gloeosporioides	GL3	Bell pepper	*Capsicum frutescens* L.
C. graminicola	GR1	Alfalfa	*M. sativa*
C. graminicola[c]	GR2	Corn	*Zea mays* L.
C. graminicola[c]	GR3	Corn	*Z. mays*
G. cingulata	C1	Soybean	*G. max*
G. cingulata	C2	Soybean	*G. max*
G. cingulata	C3	Soybean	*G. max*
G. glycines	G1	Soybean	*G. max*
G. glycines	G2	Soybean	*G. max*
G. glycines	G3	Soybean	*G. max*

[a] ATCC 12088.
[b] Culture received from L. E. Trevathan, Department of Plant Pathology and Weed Science, Mississippi State University, Mississippi State 39762.
[c] Cultures received from R. L. Nicholson, Department of Botany and Plant Pathology, Purdue University, W. Lafayette, IN 47907.

TABLE 2. Effects of *Colletotrichum* and *Glomerella* species on emergence of seed-inoculated Bragg soybeans in the greenhouse and field

Fungus	Isolate number	Emergence (% of control)[a]		
		Greenhouse[b]	Greenhouse[c]	Field[d]
C. dematium var *truncata*	T1	8.6*[e]	0.0*	32.2*
C. dematium var. *truncata*	T2	61.0*		
C. dematium var. *truncata*	T3	45.8*		
C. destructivum	D1	79.6*	76.0*	93.1
C. destructivum	D2	89.6*		
C. destructivum	D3	85.1*		
C. gloeosporioides	GL1	84.9*	75.6*	87.3*
C. gloeosporioides	GL2	87.5*		
C. gloeosporioides	GL3	93.1*		
C. graminicola	GR1	70.3*	98.5	86.2*
C. graminicola	GR2	100.0		
C. graminicola	GR3	100.0		
G. cingulata	C1	88.9*	85.7*	81.2*
G. cingulata	C2	93.8*		
G. cingulata	C3	90.7*		
G. glycines	G1	98.1	100.0	80.5*
G. glycines	G2	99.4		
G. glycines	G3	100.0		

[a] Emergence (stand) determined 2 wk after inoculation and planting of seeds.
[b] Greenhouse experiments in which mycelial suspensions were used as inoculum. Data are averages of two separate experiments.
[c] Greenhouse experiments in which spore suspensions were used as inoculum. Data are averages of one experiment.
[d] Experiments in the field in which mycelial suspensions were used as inoculum. Data are averages of one experiment.
[e] An asterisk denotes a significant difference from control ($P = 0.05$). Data were analyzed by analysis of variance and means separated by Duncan's new multiple range test.

inoculated with each isolate. Seeds were submerged for 3 min in spore suspensions, immediately placed on sterile sand contained in 10-cm-diameter clay pots, covered with a 2.5-cm layer of sand, and watered. Treatments and untreated controls were randomized and maintained on a greenhouse bench. Seedlings were observed periodically for symptoms and emergence was determined after 2 wk.

Mycelial suspensions of each of the isolates of *Colletotrichum* and *Glomerella* tested in the preceding experiment were inoculated onto seeds in the field. One-row plots each 0.6 m long were established in a randomized complete block design. Twenty seeds in each of five replicates were inoculated with each isolate by distributing 25 ml of inoculum suspension uniformly over seeds in the furrow. Due to dry field conditions, the plots were irrigated with an overhead sprinkler after planting. Seedlings were observed periodically for symptoms and emergence was determined after 2 wk.

RESULTS AND DISCUSSION

C. dematium var. *truncata* was isolated from leaf spots of 35 and 15% of the cocklebur and purple nutsedge plants sampled, respectively.

The occurrence of *C. dematium* var. *truncata* on çocklebur and purple nutsedge and the high frequency with which the two weed species occur in soybean fields (7) suggest possible epidemiological roles for cocklebur and nutsedge. They could, for example, serve as sources of inoculum and as overwintering hosts for *C. dematium* var. *truncata.* Weeds have been implicated as sources of fungal inoculum for infection of soybean (4,5), although the occurrence of *C. dematium* var. *truncata* on these weed species has not been previously reported.

Isolates of *C. gloeosporioides* from soybean and bell pepper were morphologically indistinguishable from the bitter-rot fungus isolated from apple. Apparently, this form of *C. gloeosporioides* has not been previously described on soybean. It could be referred to as *G. cingulata* (2), but will be referred to herein as *C. gloeosporioides* to distinguish it from the *G. cingulata* isolates from soybean (Table 1). When cultured on PDA, *C. gloeosporioides* could be readily distinguished from *G. cingulata; C. gloeosporioides* produced pink to red colonies, elliptical conidia measuring 13.3 × 3.6 μm, and did not produce perithecia; *G. cingulata* produced olive-green colonies, oblong to cylindrical conidia measuring 12.9 × 3.8 μm, and often produced perithecia.

The effects of *Colletotrichum* and *Glomerella* on emergence of soybeans in the greenhouse and field are presented in Table 2. When mycelial suspensions of the fungi were inoculated onto seeds in the greenhouse, emergence was significantly reduced by all isolates of *C. dematium* var. *truncata, C. destructivum, C. gloeosporioides,* and *G. cingulata,* and by one isolate of *C. graminicola. G. glycines* did not reduce emergence. With the exception of *C. graminicola* and *G. glycines,* mycelial and spore suspensions of the fungi induced similar symptoms under greenhouse conditions. Of the fungi tested, only *C. destructivum* failed to significantly reduce emergence of soybeans in the field.

In greenhouse tests, *C. dematium* var. *truncata, C. gloeosporioides,* and *G. cingulata* were the only species that

consistently caused high levels of infection on hypocotyls and cotyledons and significantly retarded the growth of seedlings (Table 3). All isolates of *C. destructivum* and one isolate of *C. graminicola* caused lesions on hypocotyls, and one isolate of *C. destructivum* caused lesions on cotyledons, but the lesions were neither as frequent nor as severe as those caused by the former three species. *G. glycines* did not cause lesions on hypocotyls and cotyledons. Comparable results were obtained when selected isolates of the different species were inoculated under field conditions, although stunting of seedlings by *C. dematium* var. *truncata, C. gloeosporioides,* and *G. cingulata,* and disease severity in general, was not as great as that observed in the greenhouse. This was probably due in part to dry field conditions following the inoculation.

Lesions incited on emerged soybean seedlings by the fungi tested were similar in some respects; ie, they were usually sunken and were initially reddish brown to light brown and became dark brown to black with age. However, there were differences among the species in the types and severity of symptoms they incited. *C. dematium* var. *truncata* caused more severe symptoms than did the other species. Cotyledons were often stunted and, because seed coats remained attached to them, they often failed to unfold. Discrete

TABLE 3. Effect of inoculating Bragg soybean seeds with mycelial suspensions of *Colletotrichum* and *Glomerella* species on incidence of hypocotyl and cotyledonary infection and stunted seedlings[a]

Fungus	Isolate number	Seedlings with hypocotyl infection (%)	Seedlings with cotyledonary infection (%)	Stunting index[b]
C. dematium var. *truncata*	T1	100.0[c]	100.0[c]	3.8[c]
C. dematium var. *truncata*	T2	97.4*[d]	97.2*	3.8*
C. dematium var. *truncata*	T3	95.3*	92.9*	3.4*
C. destructivum	D1	26.3*	15.4*	1.8
C. destructivum	D2	18.7*	6.2*	2.2
C. destructivum	D3	23.0*	11.9	2.0
C. gloeosporioides	GL1	93.1*	48.2*	2.8*
C. gloeosporioides	GL2	86.5*	54.7*	2.9*
C. gloeosporioides	GL3	79.0*	39.4*	2.7*
C. graminicola	GR1	25.3*	12.9	1.2
C. graminicola	GR2	4.1	5.0	1.1
C. graminicola	GR3	1.6	1.5	1.1
G. cingulata	C1	95.3*	78.2*	3.1*
G. cingulata	C2	88.5*	80.4*	3.0*
G. cingulata	C3	80.6*	67.9*	2.6*
G. glycines	G1	0.5	7.7	1.0
G. glycines	G2	1.2	2.2	1.1
G. glycines	G3	0.8	1.3	1.0
Control		3.9	7.1	

[a] Data are averages of two separate experiments conducted in the greenhouse and were obtained by evaluating a random sample of 10 surviving seedlings per replication in each experiment.
[b] By comparison with control seedlings, 1 = no stunting, 2 = slight stunting, 3 = moderate stunting, and 4 = severe stunting.
[c] For isolate T1, the number of surviving seedlings was insufficient for inclusion in the statistical analysis.
[d] An asterisk denotes a significant difference from control ($P = 0.05$). Data were analyzed by analysis of variance and means separated by Duncan's new multiple range test.

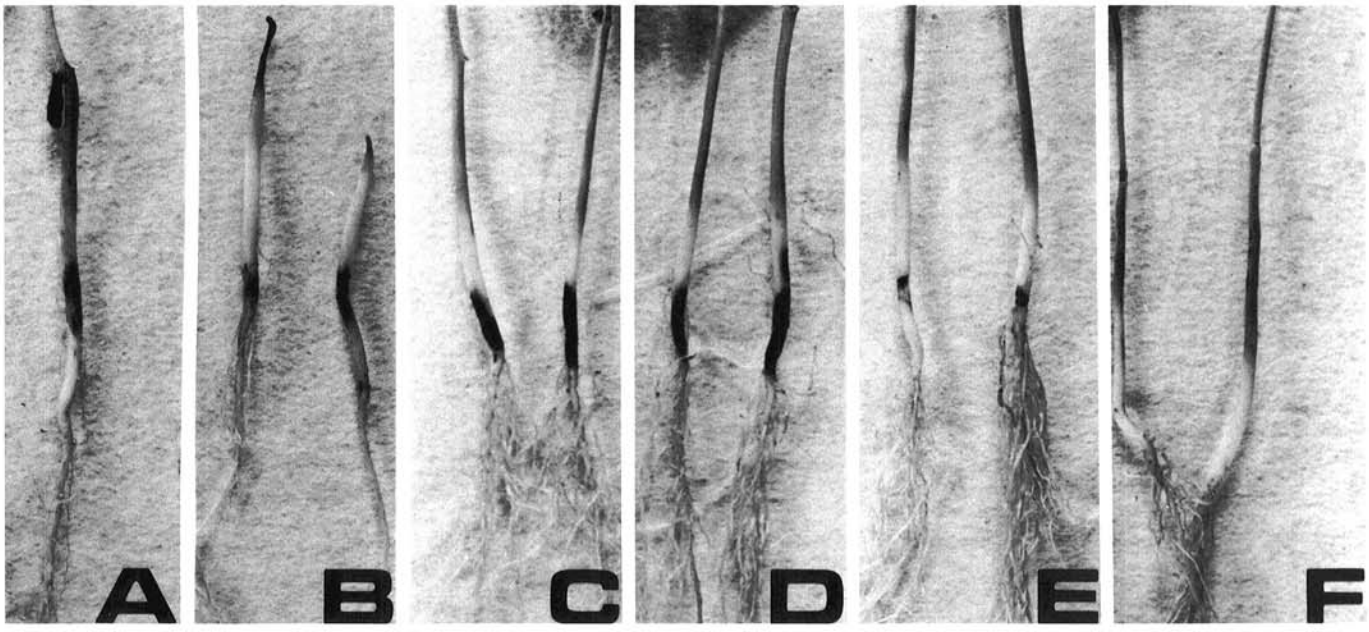

Fig. 1. Symptoms on Bragg soybean seedlings 2 wk after inoculation of seeds with spore suspensions of *Colletotrichum* or *Glomerella*: **A,** Necrosis of cotyledon and lesions on hypocotyl caused by *C. dematium* var. *truncata*; **B,** girdling of cotyledons and dieback on hypocotyls caused by *C. dematium* var. *truncata*; **C,** lesions on hypocotyls caused by *G. cingulata*; **D,** *C. gloeosporioides*; **E,** *C. destructivum*; and **F,** *C. graminicola.*

lesions on cotyledons often coalesced, resulting in a general necrosis and sometimes dehiscence of cotyledons (Fig. 1A). The fungus frequently advanced downward from the cotyledonary lesions and caused more necrosis, at times resulting in girdling of the young stem just beneath the point of attachment of cotyledons with subsequent death of seedlings. This fungus also caused lesions on hypocotyls at points above and just below the soil line which were not related to its downward movement from infected cotyledons (Fig. 1A and B). The symptoms caused on seedlings by *G. cingulata* were similar to those caused by *C. gloeosporioides*. While these two species caused high levels of cotyledonary infection, they usually did not cause stunting of cotyledons or progress downward into the hypocotyl from infected cotyledons as described for *C. dematium* var. *truncata*. The most damaging symptom caused by these two species was a cortical lesion up to 2–3 cm in length on the hypocotyl just below the soil line (Fig. 1C and D). When postemergence killing of seedlings by *G. cingulata* and *C. gloeosporioides* occurred, it was mainly due to the cortical lesions and not to necrosis or girdling of the hypocotyl above the soil line as was often the case for *C. dematium* var. *truncata*. The lesions on hypocotyls caused by *C. destructivum* and by one isolate of *C. graminicola* (Fig. 1D and E) were usually much less severe than those associated with infection by *C. dematium* var. *truncata, C. gloeosporioides,* and *G. cingulata,* and only occasionally resulted in postemergence death of seedlings.

Results of this study suggest that with respect to their potential as preemergence and postemergence pathogens of soybean seedlings in the field, *C. dematium* var. *truncata, G. cingulata,* and *C. gloeosporioides* are more significant than the other three species investigated. Because of its greater pathogenicity (Tables 2 and 3) and more frequent occurrence on soybeans (3,9), *C. dematium* var. *truncata* is probably the most important species. The recent finding (W. A. Miller and K. W. Roy, *unpublished*) that *G. cingulata* occurred much more frequently on soybean leaves, pods, and seeds than did *C. gloeosporioides* suggests that *G. cingulata* has greater potential as a pathogen of seedlings in the field than does *C. gloeosporioides*. However, natural incidence of the latter two species on seedlings needs to be determined before their potential impact on soybeans can be fully assessed.

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