# Incidence of *Colletotrichum* spp. on Soybeans and Weeds in Illinois and Pathogenicity of *Colletotrichum truncatum*

G. L. HARTMAN, Graduate Research Assistant, J. B. MANANDHAR, Graduate Research Assistant, and J. B. SINCLAIR, Professor, Department of Plant Pathology, University of Illinois at Urbana-Champaign, Urbana 61801

**ABSTRACT**

Hartman, G. L., Manandhar, J. B., and Sinclair, J. B. 1986. Incidence of *Colletotrichum* spp. on soybeans and weeds in Illinois and pathogenicity of *Colletotrichum truncatum*. Plant Disease 70:780-782.

*Colletotrichum destructivum*, *C. truncatum*, and *Glomerella glycines* occurred on 22% of stubble samples collected in 1984 and on 100% of samples from all but one of 50 fields of soybean (*Glycine max*) planted in 1983 in three Illinois counties. *Colletotrichum* spp. were recovered from 50% of soybean leaflets and from 48% of either stem pieces or leaf samples of 17 weeds from 18 fields. *C. truncatum* was recovered from 14 genera of weed hosts. Single-conidium isolates of *C. truncatum* from 11 weeds and soybeans varied significantly ($P = 0.05$) in pathogenicity on two soybean cultivars; one soybean isolate and four weed isolates were pathogenic on four of the weed hosts.

*Colletotrichum destructivum* O'Gara, *C. gloeosporioides* (Penz.) Penz. & Sacc., *C. truncatum* (Schw.) Andrus & Moore, and *Glomerella glycines* (Hori) Lehman & Wolf cause anthracnose on soybean (*Glycine max* (L.) Merr.) and other crops (5,7,9,10). Estimated losses to anthracnose in 1983 ranged from 0.5 to 6% in 16 southern U.S. states (8). Weeds serve as alternative hosts for *Colletotrichum* (5,9) and other soybean pathogens, including *Fusarium oxysporum* Schlecht. emend. Snyd. & Hans. (4) and *Diaporthe phaseolorum* (Cke. & Ell.) var. *sojae* Wehm. (5). As alternative hosts, weeds contribute to increased inoculum levels, allow carryover of pathogens in crop rotation systems, and provide a base for pathogenic variation.

Infection by *Colletotrichum* spp. frequently is endophytic and symptomless on soybeans and weeds during the growing season, with production of acervuli, sclerotia, and stromatic bodies occurring on dead plant parts at the end of the growing season (11,13). Overseasoning of *Colletotrichum* spp. on soybean debris has been demonstrated (6,13), but the extent of overseasoning on soybean stubble has not been reported.

We report on the overseasoning of several *Colletotrichum* spp. on soybean stubble, the occurrence of *C. truncatum* on dicotyledonous weeds, and the pathogenicity of selected isolates on inoculated soybean plants and weeds.

## MATERIALS AND METHODS

One hundred 5-cm pieces of soybean stubble were collected in May 1984 from each of 50 fields planted in 1983 in Champaign, DeKalb, and White counties in Illinois. In July 1984, 100 terminal leaflets from the fifth trifoliolate leaf were sampled from each of 18 soybean fields in the same counties. All stubble and leaf samples were washed with tap water for 8–12 hr, surface-disinfested in 0.5% NaOCl (Clorox) for 4 min, rinsed three times in sterile deionized water, then dipped in an aqueous solution of paraquat (28.1% a.i.) diluted 1:40 for 1 min (1). Plant parts were placed on moist cellulose pads (Kimpac) and incubated at about 100% relative humidity at 25 C. After 7 days, if mycelial growth covered stem surfaces, they were sprayed lightly with 95% ethanol to collapse the mycelia and then examined for evidence of fruiting structures of *Colletotrichum* spp. and *G. glycines* under a dissecting microscope. *Colletotrichum* was identified in pure culture on the basis of sporulating acervuli with setae. *C. destructivum* and *C. gloeosporioides* produced straight conidia in culture and *C. truncatum* produced curved conidia (13). *G. glycines* was identified by its glomerate clumps of perithecia and distinct allantoid ascospores ranging 24.5–29.1 × 3.3–4.0 μm (6,7).

**Incidence on weeds.** In mid-September 1983, a 5-cm stem section was collected from each of the basal portions and midstem sections of 10 species of weeds growing in a soybean field on the Agronomy-Plant Pathology South Farm, University of Illinois, Urbana-Champaign (UIUC). Symptomless leaves of 13 weed species as well as soybean leaves were collected in midseason 1984 from 18 soybean fields. The weeds sampled and assayed were velvetleaf (*Abutilon theophrasti* Medic.), rough pigweed (*Amaranthus retroflexus* L.), ragweed (*Ambrosia artemisiifolia* L.), giant ragweed (*Ambrosia trifida* L.), dogbane (*Apocynum cannabinum* L.), milkweed (*Asclepias syriaca* L.), lambsquarters (*Chenopodium album* L.), ground ivy (*Glechoma hederacea* L.), jimsonweed (*Datura stramonium* L.), Venice mallow (*Hibiscus trionum* L.), morning glory (*Ipomoea hederacea* (L.) Jacq. and *I. purpurea* (L.) Roth.), groundcherry (*Physalis heterophylla* Nees), smartweed (*Polygonum pensylvanicum* L.), ladysthumb (*Polygonum persicaria* L.), black nightshade (*Solanum nigrum* L.), horsenettle (*S. carolinense* L.), and cocklebur (*Xanthium pensylvanicum* Wallr.). In August 1984, 5-cm stem pieces from 25 soybean plants and from 11 weed species were collected from one field on the Agronomy-Plant Pathology South Farm, UIUC. Stem pieces from jimsonweed, morning glory, and foxtail (*Setaria* sp.) were also collected from an adjacent field of corn (*Zea mays* L.) along with the basal portions of cornstalks. All samples were assayed as described previously.

**Isolations.** Isolates of *Colletotrichum* spp. were selected at random from incubated diseased plant parts of weeds and soybeans. All isolates were cultured from either a single conidium or ascospore for accurate identification. A single-conidium isolate was obtained in either of two ways: 1) a sporulating acervulus from infected plant tissue was transferred onto acidified (pH 4.5) potato-dextrose agar (APDA) (Difco) and one drop (0.1 ml) of sterilized, deionized water was applied onto the acervulus to dislodge and spread conidia on the agar surface, or 2) an acervulus was held 2 cm above an APDA culture plate and a drop of water was used to dislodge conidia. To isolate ascospores, a perithecium was placed in 5 ml of sterile distilled water, the suspension was shaken, and 0.1 ml of the ascospore suspension was pipetted onto APDA. A bent-glass rod was used to spread ascospore and conidial suspensions over agar surfaces. After 12 hr, germinating conidia and ascospores were located with a dissecting microscope, and individual spores were transferred to APDA.

This study was a part of Project 68-0344 of the Agricultural Experiment Station, College of Agriculture, University of Illinois at Urbana-Champaign.

Accepted for publication 27 January 1986.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

Isolates of *Colletotrichum* spp. were maintained in the dark on PDA slants kept at 5 C.

**Pathogenicity studies.** To increase the inoculum of *C. truncatum*, five mycelial plugs were placed equidistant on plates of PDA. Two to five plates from each isolate were incubated under 12 hr of alternating dark and light (about 400 $\mu E/m^2/sec$) for 5–7 days at 28 C. Conidia were harvested by flooding the cultures with 10 ml of sterile distilled water per plate. The mycelial surface was scraped gently with a bent-glass rod and the suspension filtered through two layers of cheesecloth. Conidial concentrations were adjusted to $1–4 \times 10^4$ conidia per milliliter with a hemacytometer. Corsoy 79 and Williams 81 soybean seeds were sown in 10-cm-diameter clay pots and thinned to three plants per pot. Seedlings were inoculated at the V1 to V2 growth stage (3) by atomizing about 20 ml of conidial suspension of each isolate onto nine plants. Inoculated and water-sprayed (control) plants were kept for 72 hr in a mist chamber programmed for 15 min of mist per hour. After misting, plants were placed on a greenhouse bench. Symptoms were recorded 5 days after inoculation by the following index: 1 = no symptoms; 2 = slight veinal or leaf necrosis; 3 = veinal and petiole necrosis; 4 = veinal necrosis, petiole and stem cankering; and 5 = death of plant.

Three seedlings each of jimsonweed, lambsquarters, milkweed, and velvetleaf were grown in 10-cm-diameter clay pots. Seedlings were inoculated in the three- to four-leaf stage with a conidial suspension of either a soybean isolate or one from the respective host. Seedlings sprayed with water served as controls. Plants were rated for symptoms as described previously for soybeans.

## RESULTS

**Incidence on soybean stubble and leaves.** Acervuli and/or perithecia occurred on 22% of the 5,000 soybean stubble samples assayed. Incidence varied greatly in fields within each county, ranging from 22 to 94% in Champaign County, from 1 to 52% in DeKalb, and from 46 to 89% in White. Perithecia of *G. glycines* without acervuli were recorded on 8% of all samples examined, whereas 7% of the samples had acervuli without perithecia. Acervuli of *C. truncatum* and *C. destructivum* occurred on 50% of the 1,700 leaflet samples.

**Incidence on weeds.** *Colletotrichum* spp. occurred on 49% of the 835 weed samples collected from soybean fields (Table 1). Leaves of Venice mallow had the greatest incidence of *Colletotrichum* spp. and pigweed the lowest. No consistent trend in incidence was recorded in terms of field location, sample location on the plant, or sample year.

From a cornfield, *Colletotrichum* spp. were recovered from 76% of jimsonweed and 44% of morning glory stems but not from the stems of corn or foxtail species. Several milkweed plants showed pronounced disease symptoms including cankers and blighting of leaf veins and petioles. *C. truncatum* was isolated for two successive growing seasons from cankers on milkweed plants collected from the same field. Other weeds did not have obvious symptoms, but when lower leaves senesced or plants reached maturity, stromatic tissue typical of *Colletotrichum* spp. was observed on petioles and stems.

**Pathogenicity studies.** Some isolates of *C. truncatum* obtained from weed species were as pathogenic on soybean seedlings as an isolate from soybeans (Table 2). All 11 isolates tested caused symptoms, although veinal necrosis was not always prominent. When four selected isolates from weeds were inoculated onto their respective original hosts, each host developed veinal necrosis and leaf and stem dieback. The disease ratings for the soybean isolate, the respective host isolate, and the control, respectively, were 2.7, 3, and 1 for velvetleaf, 3.7, 1.5, and 1 for milkweed, 4.7, 2.3, and 1 for lambsquarters, and 4.6, 4.3, and 1 for jimsonweed. All ratings were significantly different ($P = 0.05$) from the control.

## DISCUSSION

Anthracnose on soybean was reported of minor economic importance in the midwestern United States (13). Symptoms in the field are not always visible on living plant parts, but stromatic bodies on dead or dying tissues lower in the canopy are visible. The presence of stromatic bodies on dead tissue of soybeans and weeds at the end of the season is indicative of its relative abundance. *C. destructivum* and *C. truncatum* are closely associated with soybean and broadleaf weeds in Illinois during the growing season. The production of fruiting structures on symptomless tissue after treatment with paraquat, on both naturally infected and inoculated plants, demonstrated that this relationship often is symptomless.

Increased weed density was positively correlated with incidence of *C. truncatum* in soybean seeds (2). Hepperly et al (5) isolated *C. gloeosporioides* and *C. truncatum* from *Abutilon theophrasti* (velvetleaf) and found that on inoculated detached soybean pods, two isolates of *C. truncatum* from velvetleaf were more virulent than two isolates from soybeans.

**Table 1.** Incidence of *Colletotrichum truncatum* on 5-cm stem pieces and leaves of weeds collected from soybean fields in Illinois

| | Incidence of *C. truncatum* | | | | |
|---|---|---|---|---|---|
| | 1983 | | 1984 | | |
| | Stems | | Leaves | | |
| Weed | % | Plants assayed (no.) | % | Plants assayed (no.) | Stems[a] % |
| Velvetleaf | 44 | 95 | 52 | 25 | 8 |
| Pigweed | –[b] | – | 0 | 3 | 8 |
| Giant ragweed | – | – | 44 | 9 | – |
| Dogbane | 71 | 21 | – | – | – |
| Milkweed | 29 | 21 | 50 | 10 | 64 |
| Lambsquarters | 94 | 100 | 9 | 11 | 24 |
| Jimsonweed | 46 | 50 | – | – | 20 |
| Venice mallow | – | – | 100 | 16 | – |
| Morning glory | 96 | 45 | 38 | 16 | 68 |
| Smartweed | 12 | 98 | 22 | 9 | 36 |
| Nightshade | 86 | 43 | 4 | 10 | – |
| Horsenettle | – | – | 39 | 13 | 40 |
| Cocklebur | – | – | 47 | 15 | 24 |

[a] Samples were collected from a single soybean field at maturity and represent 25 plants for each species.
[b] No samples collected.

**Table 2.** Mean disease severity ratings of two soybean cultivars inoculated with isolates of *Colletotrichum truncatum* obtained from soybean and 10 weed species

| Isolate source | Disease severity rating on soybean[a] |
|---|---|
| Soybean | 3.8 a |
| Milkweed | 3.8 a |
| Velvetleaf | 3.7 a |
| Jimsonweed | 3.6 ab |
| Horsenettle | 3.6 ab |
| Cocklebur | 3.6 ab |
| Lambsquarters | 3.3 b |
| Dogbane | 3.2 b |
| Morning glory | 3.2 b |
| Ground-cherry | 3.2 b |
| Smartweed | 3.2 b |
| Control | 1.0 c |

[a] Means based on two cultivars (Corsoy 79 and Williams 81), three replicates of three plants each. Disease severity ratings on a scale of 1 = no symptoms, 2 = slight veinal necrosis, 3 = veinal and petiole necrosis, 4 = veinal necrosis, petiole and stem cankering, and 5 = death of plant. Numbers followed by the same letters are not significantly different, based on FLSD ($P = 0.05$).

Roy (9) reported that the occurrence of *C. truncatum* on *X. pensylvanicum* (cocklebur) and *Cyperus rotundus* L. (purple nutsedge) was 35 and 15%, respectively, and the isolate of *C. truncatum* from each caused damping-off of soybean seedlings.

The high incidence of *Colletotrichum* spp. on both soybean and weed leaves from the early sampling dates suggested that the pathogen is present early in the growing season. *C. truncatum* was recovered from 14 weed hosts, indicating the relative abundance of this fungus on a wide range of plant species, all of which occur in soybean fields. All are new weed hosts for *C. truncatum*, except velvetleaf (5). The pathogenicity of weed and soybean isolates of *C. truncatum* varied within and among plant species, suggesting a lack of host specificity. The pathogenicity of isolates of *Colletotrichum* spp. from weeds on soybean seedlings and the cankering on milkweed in the field indicate the importance of weeds as alternative hosts for *Colletotrichum* spp.

Overseasoning of *C. destructivum* and *C. truncatum* occurred on soybean stubble samples, and both fungi were found on mature stems or leaves of soybeans and weeds. Tu (14) showed that *C. lindemuthianum* (Sacc. & Magn.) Br. & Cav. failed to overseason on infected bean (*Phaseolus* spp.) stem or stubble unless protected from water. *C. gloeosporioides* (Penz.) Sacc. f. sp. *aeschynomene* Templeton occasionally was isolated from debris of *Aeschynomene virginica* L. (northern jointvetch) buried in soil for about 2 wk but was isolated after 7 mo from host debris on the soil surface (12). With increased use of reduced tillage in Illinois soybean fields, the potential for survival of fungi that cause soybean anthracnose is increased on soybean stubble and alternative hosts.

## LITERATURE CITED


1. Cerkauskas, R. F., and Sinclair, J. B. 1980. Use of paraquat to aid detection of fungi in soybean tissues. Phytopathology 70:1036-1038.
2. Dhingra, O. D., and da Silva, J. F. 1978. Effect of weed control on the internally seedborne fungi in soybean seeds. Plant Dis. Rep. 62:513-516.
3. Fehr, W. R., Caviness, C. E., Burwood, D. T., and Pennington, J. S. 1971. Stage of development descriptions for soybean, *Glycine max* (L.) Merr. Crop Sci. 11:929-931.
4. Helbig, J. B., and Carroll, R. B. 1984. Dicotyledonous weeds as a source of *Fusarium oxysporum* pathogenic on soybean. Plant Dis. 68:694-696.
5. Hepperly, P. R., Kirkpatrick, B. L., and Sinclair, J. B. 1980. *Abutilon theophrasti*: Wild host for three fungal parasites of soybean. Phytopathology 70:307-310.
6. Lehman, S. G., and Wolf, F. A. 1926. Soybean anthracnose. J. Agric. Res. 33:381-390.
7. Manandhar, J. B., Hartman, G. L., and Sinclair, J. B. 1986. *Colletotrichum destructivum*, the anamorph of *Glomerella glycines*. Phytopathology 76: 282-285.
8. Mulrooney, R. P. 1985. Soybean disease loss estimate for southern United States in 1983. Plant Dis. 69:92.
9. Roy, K. W. 1982. Seedling diseases caused in soybean by species of *Colletotrichum* and *Glomerella*. Phytopathology 72:1093-1096.
10. Shaw, C. G. 1973. Host Fungus Index for the Pacific Northwest, II: Fungi. Wash. Agric. Exp. Sta. Bull. 766. 162 pp.
11. Sinclair, J. B., ed. 1982. Compendium of Soybean Diseases. 2nd ed. American Phytopathological Society, St. Paul, MN. 104 pp.
12. TeBeest, D. O. 1982. Survival of *Colletotrichum gloeosporioides* f. sp. *aeschynomene* in rice irrigation water and soil. Plant Dis. 66:469-472.
13. Tiffany, L. H. 1951. Delayed sporulation of *Colletotrichum* on soybean. Phytopathology 41:975-983.
14. Tu, J. C. 1983. Epidemiology of anthracnose caused by *Colletotrichum lindemuthianum* on white bean (*Phaseolus vulgaris*) in southern Ontario: Survival of the pathogen. Plant Dis. 67:402-404.