

**Apparent Systemic Effect of *Colletotrichum truncatum* and *C. lagenarium*
on the Interaction Between Soybean and *C. truncatum***

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ABSTRACT

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Laboratory studies were conducted to determine whether the interaction between soybean cotyledons and *Colletotrichum truncatum* (causal agent of soybean anthracnose), *C. lagenarium* (a cucumber pathogen), and heat-killed *C. lagenarium* spore suspensions interfered with the interaction between the soybean epicotyl and *C. truncatum*. Cotyledon treatments, injection with spore suspensions of *C. truncatum*, *C. lagenarium*, and heat-killed *C. lagenarium*, affected the size of lesions that developed on epicotyls inoculated with *C. truncatum*. Epicotyl lesions were

significantly smaller than the control when the cotyledons were injected 24–96 hr before epicotyl inoculation with *C. truncatum*. Epicotyl lesions were not significantly different in size when the cotyledon treatments and epicotyl inoculation occurred simultaneously. Lesions rarely developed on epicotyls inoculated with *C. truncatum* when cotyledons had been previously treated with heat-killed *C. lagenarium*. The cotyledon treatments protected the epicotyl against *C. truncatum*.

Tiffany (17) reported that in greenhouse studies soybean (*Glycine max* (L.) Merr.) cotyledons injected with *Colletotrichum truncatum* (Schwein.) Andrus & W.D. Moore became chlorotic in 4 days, shriveled, and subsequently defoliated prematurely. The fungus was isolated from the cotyledonary node a few days after the inoculation. It remained there until 7–10 days before flowering. During the time *C. truncatum* was localized in the soybean cotyledonary node, the host-pathogen interaction may have affected the physiology of the plant. This interaction may have altered the response of other plant parts to the pathogen, i.e., other plant parts may have been protected against *C. truncatum*.

This phenomenon, which is commonly referred to as cross-protection, systemic protection, or induced systemic protection, has been observed in several host-pathogen systems (8). For example, when *C. lagenarium* (Pass.) Ellis & Halst. interacted with the first leaf of a cucumber plant, the size and number of lesions that developed on the second leaf, because of a second inoculation, were reduced (9). Thus, the infection of the first leaf protected the second leaf against *C. lagenarium*. Other

investigators have reported the protection of plants against a fungal pathogen by the same pathogen (2,3,10,14), various nonpathogens (6,7), and fungal components or metabolites (12,13,18).

We had three objectives for these experiments. Our first was to determine whether *C. truncatum* injected into soybean cotyledons would colonize the cotyledon node area and remain localized there. The second was to determine whether this host-pathogen interaction altered the response of other plant parts to *C. truncatum*, i.e., whether this host-pathogen interaction protected other plant parts against the pathogen. The third objective was to determine whether *C. lagenarium*, a nonpathogen of soybean, and a heat-killed preparation of that fungus injected into cotyledons would protect other plant parts against *C. truncatum*. Thus, we could determine whether the host cotyledon interaction with a pathogen or nonpathogen protected other plant parts against *C. truncatum* and whether fungal components or metabolites might elicit the same response.

MATERIALS AND METHODS

Seeds of the soybean cultivars Essex, Evans, and McCall were supplied by Dr. H. Minor of the University of Missouri. Seeds

were germinated in vertical rolled towels (1) at 26 C and 16 hr of light per day in a growth chamber (Pyraonic Industries, San Diego, CA) illuminated with cool white fluorescent bulbs (100 $\mu\text{mol sec}^{-1}\text{m}^{-2}$). Three-day-old seedlings with 5-mm radicles were transplanted into a medium of steamed, sandy loam soil (heated with steam to 82 C for 4 hr); vermiculite; and peat (1:1:1, v/v) in 6- \times 8-cm plastic containers. These containers were incubated as described above, unless noted otherwise.

A culture of *C. truncatum*, obtained from naturally infected soybeans, was maintained on oat agar at 26 C and 16 hr of light per day. Spore suspensions were prepared from 7-day-old cultures and adjusted to 5×10^3 , 5×10^4 , and 5×10^5 spores per milliliter of sterile deionized water.

A culture of *C. lagenarium*, supplied by Dr. J. Kuc of the University of Kentucky, was maintained on green bean agar at 26 C in the dark. Spore suspensions were prepared from 7-day-old cultures and adjusted to 5×10^4 spores per milliliter of sterile deionized water.

The cotyledons of 7-day-old Essex, Evans, and McCall plants were injected hypodermically with 4 μl of water, a suspension of *C. truncatum* spores, *C. lagenarium* spores, or heat-killed *C. lagenarium* spores (adjusted to 5×10^4 spores per milliliter). The shoots from six plants of each variety were collected 3, 5, and 7 days after inoculation and at weekly intervals thereafter until pod fill. A 1-cm segment of stem at each node, approximately 0.5 cm above and below the node, was surface-sterilized by immersion in 10% NaOCl for 5 min, rinsed in sterile water, and plated on potato-dextrose agar. Stem tissues were incubated at 26 C for 4 days and then examined for *C. truncatum* and *C. lagenarium* growth.

The cotyledons of 7-day-old Essex seedlings were injected hypodermically with 4 μl of sterile water, a suspension of *C. truncatum* spores, *C. lagenarium* spores, or heat-killed *C. lagenarium* spores. These seedlings were inoculated with *C. truncatum* 0, 48, or 96 hr later by placing a 2- μl drop of the spore suspension (5×10^4 spores per milliliter) on the epicotyl 2.5 cm above the cotyledonary node. These plants were then incubated in a moist chamber at 26 C and 16 hr of light per day. During this time, the 2- μl drop remained on the stem without runoff. Four days later, the epicotyl lesions that had developed at the site of inoculation were measured.

Treatments were replicated six times, six plants per treatment, in each experiment. Plants were placed randomly in the incubator. Each experiment was repeated once except where noted, and the data were pooled for analysis. Data were analyzed by conventional analysis of variance, and treatment means were compared against LSD (15).

RESULTS

Cotyledons of 7-day-old soybean seedlings injected with 4 μl of a *C. truncatum* spore suspension (5×10^4 spores per milliliter) became water-soaked 3 days after injection and subsequently shriveled and defoliated within 10 days of injection. The plants were then about 17 days old. Cotyledons injected with water appeared normal and defoliated when the plants were about 28 days old. *C. truncatum* was isolated from the cotyledonary node

TABLE 1. Lesion area (mm^2) on Essex soybean epicotyls 4 days after inoculation with *Colletotrichum truncatum* when plant cotyledons were treated with *C. truncatum*, *C. lagenarium*, or heat-killed *C. lagenarium* spore suspensions (5×10^4 spores per milliliter)

Cotyledon treatment	Lesion area at hr from cotyledon to epicotyl inoculation			
	0	24	48	96
Control, water injected	8.5	9.0	10.2	9.9
<i>C. truncatum</i>	7.0	3.8	3.3	3.0
<i>C. lagenarium</i>	6.7	2.9	2.6	2.3
Heat-killed <i>C. lagenarium</i>	5.6	1.0	0.2	0.3
LSD ($P = 0.05$)	NS	1.1	0.8	1.3

7 days after it was injected into the cotyledon. The fungus was never isolated from any other node through the R6 (pod fill) stage of growth. Cotyledons that had been injected with *C. lagenarium* and heat-killed *C. lagenarium* spore suspensions resembled the controls in that they were symptomless. *C. lagenarium* was never isolated from any node.

Two days after 2 μl of a *C. truncatum* spore suspension was placed on 10-day-old soybean epicotyls, a lesion developed at the inoculation site. The lesion size slowly increased, radially and acropetally, over the next few days. On the rare occasions when it advanced basipetally from the inoculation site, it was only 0.5–1 mm.

Treatment of cotyledons with a suspension of *C. truncatum* spores, *C. lagenarium* spores, or heat-killed *C. lagenarium* spores affected the size of the lesions that developed on epicotyls inoculated with *C. truncatum* (Table 1). This interaction occurred only when the treatment of cotyledons preceded the epicotyl inoculation with *C. truncatum* by 24–96 hr. No differences were found in size of epicotyl lesions among treatments when cotyledon injection and epicotyl inoculation were simultaneous. Epicotyl lesions were smallest when cotyledons were first treated with heat-killed *C. lagenarium*. Treatment of cotyledons with various concentrations of *C. truncatum* spores did not affect the size of epicotyl lesions (Table 2).

DISCUSSION

In this laboratory study, *C. truncatum* remained localized in the cotyledonary node adjacent to cotyledons inoculated with *C. truncatum* through the R6 stage of plant growth, pod fill. Tiffany (17) showed that *C. truncatum* remained localized in the cotyledonary node area of greenhouse- and field-grown plants until 7–10 days before the R1 growth stage, flowering, and then began to move up the stem into the petioles and pods. The differences between our results and those of Tiffany could be attributable to the cultivar used.

The interaction between soybean cotyledons and *C. truncatum*, *C. lagenarium*, and heat-killed *C. lagenarium* did interfere with the interaction between a plant's epicotyl and *C. truncatum*. Since a delay between the cotyledon treatment and epicotyl inoculation is necessary for interference and the plant tissues involved are separated by 2.5 cm, the interference is probably not because of direct competition between the cotyledon and epicotyl treatments. Physiological responses incited by the cotyledon treatments are probably responsible for interfering with the epicotyl interaction with *C. truncatum*. Others have referred to similar phenomena in different hosts as systemic protection (6,7).

Soybean has been locally (inducer inoculum and challenge inoculum applied to the same site) protected against *Phytophthora* (11,16), but systemic (inducer inoculum and challenge inoculum applied to different sites) protection in soybean has not been reported. We do not know whether the interference in epicotyl lesion development incited by *C. truncatum* because of prior cotyledon treatment with *C. truncatum*, *C. lagenarium*, or heat-killed *C. lagenarium* is attributable to induced systemic resistance, as shown in other host-parasite systems (2–5).

We do not know why the treatment of cotyledons with heat-killed *C. lagenarium* was more effective in retarding *C. truncatum* lesion development on epicotyls than was treatment with living *C. lagenarium*. The difference may be attributable to the alteration

TABLE 2. Lesion area (mm^2) on epicotyls 4 days after inoculation with *Colletotrichum truncatum* when plant cotyledons were injected with 4 μl of a *C. truncatum* spore suspension 48 hr previously

Spores/ml of <i>C. truncatum</i> inoculum injected into cotyledon	Lesion area
5×10^3	3.4
5×10^4	4.0
5×10^5	4.1
LSD ($P = 0.05$)	NS

of the suspension's chemical composition by heat. It suggests that fungal components or metabolites of *C. lagenarium* can elicit systemic protection of soybean against *C. truncatum*.

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