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Biological Control of Hemp sesbania (*Sesbania exaltata*) and Sicklepod (*Senna obtusifolia*) in Soybean with Anthracnose Pathogen Mixtures

C. Douglas Boyette and Robert E. Hoagland*

In greenhouse and field experiments conducted over 3 yr, tank mixtures of spores of the fungi *Colletotrichum truncatum* and *Colletotrichum gloeosporioides*, formulated in unrefined corn oil and Silwet L-77 surfactant, were evaluated as a mycoherbicide mixture for simultaneous control of hemp sesbania and sicklepod, respectively. In greenhouse tests, 100% mortality and dry weight reduction of hemp sesbania occurred 6 d after treatment (DAT), whereas 15 d were required to achieve 100% mortality and dry weight reduction of sicklepod. In field experiments conducted in narrow-row (51-cm) soybean test plots, a single application of the fungal mixture formulated as described controlled hemp sesbania and sicklepod 94% and 88%, respectively, 28 DAT. Neither fungus, applied as aqueous conidial suspensions, provided control of their respective weed hosts. These results indicate that tank mixtures of these anthracnose-forming pathogens can effectively control hemp sesbania and sicklepod with a single application.


**Key words:** Biocontrol, bioherbicide, biocontrol agent, narrow-row soybeans, pathogen mixtures, unrefined corn oil, surfactant.

Hemp sesbania and sicklepod are problematic leguminous annual weeds in many regions of the southern United States, particularly in soybean production areas (Buchanan et al. 1980; Dowler 1992; Webster and Coble 1997). Both weeds often occur with great fecundity in the same field (Norris 2007). Prior to the introduction and widespread usage of glyphosate-resistant soybean cultivars, satisfactory herbicidal control of these weeds was particularly difficult (Norsworthy and Oliver 2000). Sicklepod has been reported to reduce the yield of soybeans up to 50% (Banks et al. 1985) and result in substantial annual monetary losses ($175 ha$−1) (Bridges and Walker 1987). In addition, sicklepod foliage and seeds produce several compounds that are toxic to mammals (Crawford et al. 1990; Harry-O’kuru et al. 2005; Putnam et al. 1988; Yagi et al. 1998). Hemp sesbania can also be detrimental to soybean yield. For example, populations of 13 hemp sesbania plants m$^{−2}$ emerging with soybean reduced yield by 80% when allowed to compete throughout the growing season (McWhorter and Anderson 1979). Hemp sesbania seed also produces metabolites (saponins) that are toxic to livestock and humans (Powell et al. 1990).

The need to develop novel weed management tools and strategies is becoming increasingly more acute due in large part to environmental concerns, rising petroleum costs, and the development of herbicide-resistant weeds, including those with resistance to glyphosate. One alternative could be the use of bioherbicides that could provide weed control, as summarized in several reviews (Charudattan 2001, 2005; Hallett 2005; Weaver et al. 2007). Many research projects have evaluated various *Colletotrichum* spp. as bioherbicides for several different weeds (Charudattan 2001, 2005; Watson 1991) due to their high virulence and the ability to induce epiphytotic disease spread (Sandrin et al. 2003). We have previously shown, in separate field trials, that the mycoherbicial fungi, *Colletotrichum truncatum* (CT) (NRRL no. 18434) and *Colletotrichum gloeosporioides* (CG) (NRRL no. 21046), effectively control hemp sesbania (Boyette et al. 2007a) and sicklepod (Boyette et al. 2007b), respectively, in narrow-row (51 cm) soybeans. In both of these studies, fungal spores were formulated in unrefined corn oil (CO) and Silwet L-77 surfactant (SW). Neither fungus effectively controlled their respective weed host when applied only in aqueous
formulation (Boyette et al. 2007a,b). Other research on bioherbicides has indicated that application of formulations containing a mixture of pathogens can be useful to provide control of a broader range of weeds than that bestowed by each individual pathogen applied alone (Boyette et al. 1979; Chandramohan et al. 2002; Chandramohan and Charudattan 2003). The research objective of the present report was to evaluate the feasibility of applying mixtures of CT and CG spores formulated in unrefined CO and SW for simultaneous control of hemp sesbania and sicklepod under greenhouse and field environments.

Materials and Methods
Pathogen Culture and Maintenance. Single strains1 of CT and CG were used in all experiments. The fungi were preserved (separately) in screw-capped tubes containing sterilized soil (Bakerspigel 1953). The cultures were grown for 5 to 7 d on potato dextrose agar (PDA)2 in 10-cm plastic petri dishes that were incubated on the open-mesh wire shelves of an incubator3 at 25 C under cool, white fluorescent lighting (12-h photoperiod). The spores were harvested by rinsing the cultures with water and straining through double-layered cheesecloth. The spore densities were determined with hemacytometers4 and dilutions were made to give the desired inoculum concentrations.

Inoculum Production. Because CT sporulates sparingly in submerged liquid culture (Jackson and Schisler 1992), a solid-substrate production technique was utilized to mass-produce this fungus. The cultures were grown for 5 to 7 d on PDA in 10-cm plastic petri dishes that were incubated on open-mesh wire shelves of an incubator as described above. CG (21046) has been shown to sporulate prolifically in submerged liquid culture (Boyette 2006), therefore, spores were produced (after 96 h) on modified Richard’s medium containing vegetable juice5 (Daniel et al. 1973), either in shaken Erlenmeyer flasks (25 C and 125 rpm), or in a laboratory fermentor6 under similar conditions. CT spores produced in solid state and CG spores produced in broth were separated from the mycelium by filtering through double-layered cheesecloth, which retained the mycelium and unspent solid media. The spore densities of each fungus were determined using hemacytometers, and dilutions were made with distilled water to provide the desired inoculum concentrations (1 \times 10^7 spores ml^{-1}). In experiments that included unrefined CO7 in a 1 : 1 ratio with sterile, distilled water containing 0.20% (v/v) SW,8 spores of each pathogen were prepared at 2 \times 10^7 spores ml^{-1} so as to achieve a final formulation containing 1 \times 10^7 spores ml^{-1} of each organism. The emulsion formulations were prepared by adding unrefined CO to water containing SW. The mixtures were thoroughly mixed using a vortex mixer.9

Greenhouse Experiments. Test Plant Propagation. Hemp sesbania and sicklepod plants were grown from seed10 in a commercial potting mix11 contained in peat strips. Each strip contained 12 plants of each weed species. The potting mix was supplemented with a controlled-release (14–14–14, N–P–K) fertilizer.12 The plants were placed in subirrigated trays that were mounted on greenhouse benches. Greenhouse temperatures ranged from 25 to 30 C with 40 to 90% relative humidity. The photoperiod was 12 h with 1,650 \mu mol m^{-2} s^{-1} photosynthetically active radiation measured at midday with a light meter.13

The treatments utilized in greenhouse studies were as follows: (1) CT spores in distilled water suspension, (2) CG spores in distilled water suspension, (3) CT + CG in distilled water suspension, (4) CT spores in CO/SW emulsion, (5) CG spores in CO/SW emulsion, (6) CT + CG spores in CO/SW emulsion, (7) CO/SW emulsion control, and (8) distilled water control. Inoculum densities were 1 \times 10^7 spores ml^{-1} for treatments containing each fungus alone or for each fungal component when formulated as a mixture. Spray applications were made with Spra-tool Power Pack hand-held sprayers.14 New aerosol canisters were used to deliver high and equal pressure (and volume delivery) to all treatment sets. Plants were sprayed at a rate of approximately 200 L ha^{-1}. Following spray treatments, groups of seedlings were placed in darkened dew chambers15 (28 C, 100% relative humidity) for 12 h, then placed on greenhouse benches. Plants were monitored 15 d for disease development, and percentage of control was determined. Surviving plants were excised at the soil line, oven-dried for 48 h at 85 C and weighed; percentage of biomass reduction was determined. Treatments were replicated four times, for a total of 48 individual plants. The experiment was repeated over time, and data were averaged following Bartlett’s test for homogeneity of variance (Steele et al. 1997).

Field Experiments. Narrow-row soybean production can result in greater weed control and increased yields (Nelson and Renner 1999; Reddy 2002). Previous research has shown that individual applications of CT or CG will effectively control their respective weed hosts, hemp sesbania and sicklepod, in narrow-row (51-cm) soybean plots (Boyette et al. 2007a,b). Therefore, only narrow-row soybean plots were utilized in these experiments. The field experiments were conducted on a Dundee, very fine sandy loam (Aeric Ochraqualf; 24% clay, 29% sand, 47% silt, 1.2% organic matter content, pH = 6.3) from 2003 to 2005 at the Southern Weed Science Research Unit Experimental Farm, Stoneville, MS, at 33°26’N, 90°53’W, as determined using a global positioning device.16 The test plots consisted of eight rows of Delta Grow DG5160RR17 glyphosate-resistant soybeans, 12.2 m long and 0.5 m apart, with the four center rows receiving treatment. All rows were planted with mechanically scarified hemp sesbania and sicklepod seeded at a density of ~ 100 seeds m^{-1} of row. Emergence and stand densities of ~ 90% of both weeds were attained. The following field treatments were used: (1) CT spores formulated in unrefined CO/SW; 2) CG in CO/SW; 3) CT + CG in CO/SW; 4) CO/SW control; 5) untreated, weedy control; 6) glyphosate18 (1 kg ai ha^{-1}); and 7) hand-weeded, weed-free control. Treatments of either fungus alone in distilled water were not used, because they do not provide control of their respective weed hosts under field conditions when applied in water only (Boyette et al. 1993, 2008). All spray applications were made with hand-held pump sprayers19 at spray rates of ~ 200 L ha^{-1}. The inoculum concentrations
were 1.0 × 10^7 conidia ml^-1 in those treatments receiving a fungal component. The planting dates were May 15, 2003; May 20, 2004; and June 8, 2005. Environmental conditions (air temperature, soil temperature, and relative humidity) at planting, application, and rating dates are summarized in Table 1. The treatments were made when weed seedlings of each species were in the first-to-third true leaf stages (~2 wk after planting). The percentage of control of each weed species was determined in randomly selected 3.0 by 0.23-m areas at 7-d intervals for 28 d. Two such areas were rated in each replicated treatment. The weed mortality/injury rating scale (0 to 100%) reflected the number of plants that were killed or severely damaged within each quadrant. The extent of disease progression was based on a modified Horsfall and Barratt (1945) rating scale, assigning symptom expression from 0 to 1.0, with 0 being unaffected, and 0.2, 0.4, 0.6, 0.8 equal to 20, 40, 60, and 80% leaf and stem lesion coverage/injury, respectively, and 1.0 equating plant mortality. Symptomatology was considered ‘severe’ at ratings of 0.8 to 1.0. For CG control of sicklepod was also linear (R^2 = 0.98) (Figure 1). Complete mortality (100%) of hemp sesbania occurred 6 d after treatment (DAT) when CT was formulated and applied in CO/SW, whereas 15 d were required to achieve similar mortality of sicklepod (Figure 1). Dry weight reductions (Table 2) generally mirrored the extent of injury or mortality caused by these treatments. Hemp sesbania was

Greenhouse Experiments. In greenhouse tests, CT exhibited a much more rapid and severe symptomatology on hemp sesbania than CG exhibited on sicklepod, with a disease rating of 1.0 occurring at 6 DAT whereas 15 d were required to achieve a 1.0 disease rating for CG on sicklepod (Figure 1). For CT control of hemp sesbania, a linear relationship of weed control vs. time was exhibited (R^2 = 1.0); the response for CG control of sicklepod was also linear (R^2 = 0.98) (Figure 1). Complete mortality (100%) of hemp sesbania occurred 6 d after treatment (DAT) when CT was formulated and applied in CO/SW, whereas 15 d were required to achieve similar mortality of sicklepod (Figure 1). Dry weight reductions (Table 2) generally mirrored the extent of injury or mortality caused by these treatments. Hemp sesbania was

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**Table 1. Environmental conditions of biological control field studies on hemp sesbania (Sesbania exaltata) and sicklepod (Senna obtusifolia) conducted at Stoneville, MS, from 2003 to 2005.**

<table>
<thead>
<tr>
<th>Event</th>
<th>Air</th>
<th>Soil</th>
<th>Relative humidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Planting</td>
<td>27/19</td>
<td>29/20</td>
<td>33/23</td>
</tr>
<tr>
<td>Application</td>
<td>29/18</td>
<td>31/21</td>
<td>35/20</td>
</tr>
<tr>
<td>7-d rating</td>
<td>31/22</td>
<td>32/26</td>
<td>34/23</td>
</tr>
<tr>
<td>14-d rating</td>
<td>31/18</td>
<td>32/22</td>
<td>33/23</td>
</tr>
<tr>
<td>21-d rating</td>
<td>31/2</td>
<td>31/22</td>
<td>36/23</td>
</tr>
<tr>
<td>28-d rating</td>
<td>34/22</td>
<td>32/28</td>
<td>34/23</td>
</tr>
</tbody>
</table>

*Dates of each event presented in Materials and Methods section.

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Results and Discussion

**Isolation and Culture.** CT sporulated prolifically on PDA after 7 d. Spores rinsed from agar plates routinely yielded 1.5 × 10^8 to 2.0 × 10^8 spores ml^-1. CG sporulated prolifically in liquid culture in modified Richard’s medium with spore yields averaging 3.0 × 10^8 conidia ml^-1 after 7 d (data not shown).

Figure 1. Disease progression of Colletotrichum truncatum (CT) infecting hemp sesbania and Colletotrichum gloeosporioides (CG) infecting sicklepod under greenhouse conditions. Diseased plant ratings were based on a modified Horsfall–Barratt (1945) rating scale as described in the Materials and Methods. Symptomatology was considered severe at ratings of 0.8 to 1.0. Fungal spores (density of 1 × 10^7 spores ml^-1 of each fungus) were formulated in 20% unrefined corn oil and 0.2% Silwet L-77 surfactant and sprayed at a volume of 200 L ha^-1 using Spra-tool Power Pack 14 hand-held sprayers. For disease progression of CT on hemp sesbania, the relationship is best described by the following equation: Y = 0 + 16.67 X; R^2 = 0.98. For disease progression of CG on sicklepod, the relationship is best described by the following equation: Y = −2.1 + 6.82X; R^2 = 0.98.
controlled 96 and 100% with CT in distilled water and CT in CO/SW, respectively, whereas sicklepod was unaffected (weed control or dry weight reduction) by CG in distilled water treatments (Table 2).

Although no mortality occurred to either weed species treated with the nonpathogenic fungus, significant dry weight reductions of 40 and 15% were observed on hemp sesbania treated with CG formulated in the CO/SW emulsion, and on sicklepod treated with CT formulated in the CO/SW emulsion, respectively (Table 2). Similar phenomenology has been reported with other bioherbicidal fungi formulated in invert emulsions. For example, Amsellem et al. (1990) reported that the host specificities of \( \text{Colletotrichum gloeosporioides} \) and \( \text{Colletotrichum truncatum} \) for hemp sesbania, and 0.94 for CG-infected sicklepod (Figure 2). In sicklepod, the best fits, with \( R^2 \) values of 0.98 for CT-infected hemp sesbania, and 0.94 for CG-infected sicklepod (Figure 2). In these field results, CT had no effect on sicklepod, and CG had no effect on hemp sesbania, which is in contrast to results in greenhouse tests (see Table 2 and Table 3).

The symptomatology of both diseases was somewhat similar during the first several DAT, with scattered anthracnose lesions forming on stems of both species. Lesions began to enlarge and coalesce on infected hemp sesbania 3 DAT. Disease progressed more rapidly on hemp sesbania infected by CT than on sicklepod infected with CG during the first 6 DAT (Figure 2). However, disease development began to increase rapidly on CG-infected sicklepod 6 to 9 DAT, becoming only slightly less severe than CT-infected hemp sesbania 9 DAT (Figure 2). By 9 DAT, most of the entire leaf and stem areas were completely blighted (severe necrosis or widespread plant mortality) by both pathogens on their respective hosts (Figure 2). With both pathogen–weed systems, third degree polynomial regression curves provided the best fits, with \( R^2 \) values of 0.98 for CT-infected hemp sesbania, and 0.94 for CG-infected sicklepod (Figure 2). In test plots where effective biological control of both hemp sesbania and sicklepod control was achieved (CT in CO/SW treatments, and CG in CO/SW treatments), soybean yields were not significantly different from the yields recorded from hand-weeded plots, or in plots treated with glyphosate. Furthermore, control of sicklepod by CG, and the control of hemp sesbania (92%) achieved with CT were also not significantly different (Table 3). In these field results, the difference at \( P = 0.05 \) according to Fisher’s LSD.

### Table 2. Percentage of control of hemp sesbania and sicklepod 15 d after inoculation with \( \text{Colletotrichum truncatum} \) and \( \text{Colletotrichum gloeosporioides} \) under greenhouse conditions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weed control</th>
<th>Dry weight reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SEBEX</td>
<td>CASOB</td>
</tr>
<tr>
<td>CO/SW emulsion control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Distilled water control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CT in distilled water</td>
<td>96 a</td>
<td>96 a</td>
</tr>
<tr>
<td>CG in distilled water</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CT + CG in distilled water</td>
<td>96 a</td>
<td>98 a</td>
</tr>
<tr>
<td>CG in CO/SW emulsion</td>
<td>100 a</td>
<td>100 a</td>
</tr>
<tr>
<td>CT + CG in CO/SW emulsion</td>
<td>100 a</td>
<td>100 a</td>
</tr>
</tbody>
</table>

*a Abbreviations: SEBEX, hemp sesbania; CASOB, sicklepod; UNT, untreated.*

### Table 3. Percentage of control of hemp sesbania and sicklepod, and soybean yield 28 d after inoculation with mixtures of \( \text{Colletotrichum truncatum} \) and \( \text{Colletotrichum gloeosporioides} \) under field conditions in Stoneville, MS.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weed control</th>
<th>Soybean yield (kg ha(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SEBEX</td>
<td>CASOB</td>
</tr>
<tr>
<td>UNT</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CO/SW emulsion control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Weed-free control</td>
<td>100 a</td>
<td>100 a</td>
</tr>
<tr>
<td>GLY</td>
<td>95 ab</td>
<td>95 ab</td>
</tr>
<tr>
<td>CT in CO/SW</td>
<td>94 ab</td>
<td>88 b</td>
</tr>
<tr>
<td>CG in CO/SW</td>
<td>0</td>
<td>88 b</td>
</tr>
<tr>
<td>CT + CG in CO/SW</td>
<td>94 ab</td>
<td>88 b</td>
</tr>
</tbody>
</table>

*a Abbreviations: SEBEX, hemp sesbania; CASOB, sicklepod; UNT, untreated.*

### Field Experiments

In field experiments, a single application of the fungal mixture formulated as described controlled hemp sesbania and sicklepod 94 and 88%, respectively, 28 DAT (Table 3). The control of hemp sesbania that was achieved with CT was similar to that caused by glyphosate (95%) (Table 3). Control of sicklepod (88%) with CG tended to be lower than control caused by CT on hemp sesbania, but was not significantly less than the control caused by glyphosate. Furthermore, control of sicklepod by CG, and the control of hemp sesbania (92%) achieved with CT were also not significantly different (Table 3). In these field results, CT had no effect on sicklepod, and CG had no effect on hemp sesbania, which is in contrast to results in greenhouse tests (see Table 2 and Table 3).

The symptomatology of both diseases was somewhat similar during the first several DAT, with scattered anthracnose lesions forming on stems of both species. Lesions began to enlarge and coalesce on infected hemp sesbania 3 DAT. Disease progressed more rapidly on hemp sesbania infected by CT than on sicklepod infected with CG during the first 6 DAT (Figure 2). However, disease development began to increase rapidly on CG-infected sicklepod 6 to 9 DAT, becoming only slightly less severe than CT-infected hemp sesbania 9 DAT (Figure 2). By 9 DAT, most of the entire leaf and stem areas were completely blighted (severe necrosis or widespread plant mortality) by both pathogens on their respective hosts (Figure 2). With both pathogen–weed systems, third degree polynomial regression curves provided the best fits, with \( R^2 \) values of 0.98 for CT-infected hemp sesbania, and 0.94 for CG-infected sicklepod (Figure 2). In test plots where effective biological control of both hemp sesbania and sicklepod control was achieved (CT in CO/SW treatments, and CG in CO/SW treatments), soybean yields were not significantly different from the yields recorded from hand-weeded plots, or in plots treated with glyphosate. Furthermore, control of sicklepod by CG, and the control of hemp sesbania (92%) achieved with CT were also not significantly different (Table 3). In these field results, CT had no effect on sicklepod, and CG had no effect on hemp sesbania, which is in contrast to results in greenhouse tests (see Table 2 and Table 3).

The feasibility of controlling mixed weed populations with mixtures of pathogens has been previously demonstrated (Boyette et al. 1979; Chandramohan et al. 2002; Chandra-mohan and Charudattan 2003). The concept of applying pathogen mixtures to mixed weed populations appears to be a simple one, but mixing microbes or microbial species could result in antagonistic interactions with regard to propagule germination, growth, infectivity, and efficacy. The fungi used in this report have similar growth and environmental
requirements to incite an epiphytic event (Boyette et al. 1993; Boyette 2006). In addition, there appears to be no antagonism between the fungi under these treatment and application conditions. However, because antagonism can occur between some fungi (Cook and Baker 1983; Pal and McSpadden Gardener 2006), especially if the fungi belong to different fungal genera, and if optimal environmental conditions required for maximum weed control vary greatly between host and parasite, it is unlikely that a mixed-pathogen approach to expand the host range of host-specific bioherbicidal pathogens would be feasible in all situations. The ability to apply mixtures of host-specific bioherbicides should improve their marketability, if such mixtures were to be commercialized. We will continue to investigate this approach of expanding the host ranges of bioherbicidal plant pathogens by using pathogen combinations.

**Sources of Materials**

1. NRRL 18434 and 21046, Agricultural Research Service Patent Culture Collection, Peoria, IL 61604.
2. Potato dextrose agar, Difco Laboratories, Detroit, MI 48201.
3. Environmental chamber, Model I-35LVL, Percival Scientific, Perry, IA 50220.
5. V-8 vegetable juice, Campbell Soup Co., Camden, NJ 08103.
7. Unrefined corn oil, Spectrum Naturals, Petaluma, CA 94952.
8. Silwet L-77 surfactant, Osi Specialties, Greeley, CO 80632.
10. Adin Seed Co., Leland, MS 38756.
12. Osmocote, Grace Sierra Horticultural Products, Milipitas, CA 95035.
15. Model I 35-D, Percival Scientific Mfg., Boone, IA 50036.
18. Glyphosate, Roundup ULTRA, Monsanto Company, St. Louis, MO 63167.

**Literature Cited**


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