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Source: Weed Technology, 24(4):551-556. 2010.
Published By: Weed Science Society of America
DOI: http://dx.doi.org/10.1614/WT-08-154.1
URL: http://www.bioone.org/doi/full/10.1614/WT-08-154.1

Weed Technology 2010 24:551–556

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.

Nomenclature: *Colletotrichum gloeosporioides* (Penz.) Penz and Sacc.; *Colletotrichum truncatum* (Schw.) Andrews and Moore; hemp sesbania, *Sesbania exaltata* (Rydb.) ex A.W. Hill SEBEX; sicklepod, *Senna obtusifolia* (L.) Irwin and Barneby CASOB; soybean, *Glycine max* (L.) Merr.

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

En experimentos de campo y de invernadero conducidos durante 3 años, las mezclas de esporas de los hongos *Colletotrichum truncatum* y *C. gloeosporioides* , formuladas en aceite de maíz no refinado y surfactante Silwet L-77, fueron evaluadas como una mezcla mico-herbicida para el control simultáneo de *Sesbania exaltata* y *Senna obtusifolia*, respectivamente. En las pruebas de invernadero, se logró el 100% de mortalidad y la reducción del peso seco de *Sesbania exaltata* 6 días después del tratamiento (DAT), mientras que se requirieron 15 días para alcanzar el 100% de mortalidad y la reducción del peso seco de *Senna obtusifolia*. En los experimentos de campo llevados al cabo en parcelas experimentales de soya sembradas en surcos estrechos (51cm), una sola aplicación de la mezcla de hongos, formulada como se describió anteriormente, controló a *Sesbania exaltata* y *Senna obtusifolia* en 94 y 88% respectivamente a los 28 DAT. Ninguno de los hongos, aplicados como suspensiones acuosas conidiales, controló su respectiva maleza hospedera. Estos resultados indican que las mezclas de estos patógenos causantes de la antracnosis pueden controlar con efectividad a *Sesbania exaltata* y *Senna obtusifolia*, con una sola aplicación.

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 mycoherbicidal 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

DOI: 10.1614/WT-08-154.1

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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 strains[1] 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 incubator[3] 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 hemacytometers[4] 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 days 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 juice[5] (Daniel et al. 1973), either in shaken Erlenmeyer flasks (25 C and 125 rpm), or in a laboratory fermentor[6] 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 (1×10^7 spores ml^{-1}). In experiments that included unrefined CO[7] in a 1 : 1 ratio with sterile, distilled water containing 0.20% (v/v) SW,[8] spores of each pathogen were prepared at 2×10^7 spores ml^{-1} so as to achieve a final formulation containing 1×10^7 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 seed[10] in a commercial potting mix[11] 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 μmol 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 1×10^7 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 chambers[15] (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 DG5160RR[17] 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 seed 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) glyphosate[18] (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 sprayers[19] at spray rates of ~ 200 L ha^{-1}. The inoculum concentrations

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.

	Air[b]			Soil[c]			Relative humidity[d]		
	Year								
Event[a]	2003	2004	2005	2003	2004	2005	2003	2004	2005
Planting	27/19	29/20	33/23	26/21	32/21	41/27	95/63	95/56	84/41
Application	29/18	31/21	33/20	36/19	37/22	39/26	79/36	94/44	89/34
7-d rating	24/17	33/26	34/23	32/20	39/27	42/28	85/56	87/38	91/38
14-d rating	31/18	32/22	33/23	36/32	33/23	36/22	96/82	94/58	94/54
21-d rating	31/2	28/22	36/23	35/24	36/24	39/24	96/59	95/71	92/48
28-d rating	34/22	28/22	34/23	41/27	31/23	39/27	88/55	95/76	93/50

[a] Dates of each event presented in Materials and Methods section.

[b] Maximum/minimum temperature (C) at 1 m above soil surface.

[c] Maximum/minimum temperature (C) at 5.1 cm below soil surface.

[d] Maximum/minimum relative humidity at 1 m above soil surface.

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 equaling plant mortality. Symptomatology was considered 'severe' at ratings of 0.8 to 1.0.

Experimental Design. The experiments were arranged as randomized complete block designs with four replications. Data over the 3-yr testing period were examined for homogeneity of variance (Steele et al. 1997), combined, and analyzed using ANOVA. In both the greenhouse and field experiments, data received arcsin transformation. When significant differences were detected by the *F* test, means were separated with Fisher's Protected LSD test at the 0.05 level of probability. In the weed control evaluation study in the greenhouse and the disease progression studies in the field, data were analyzed using standard mean errors and linear and best-fit regression analysis, respectively.

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).

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

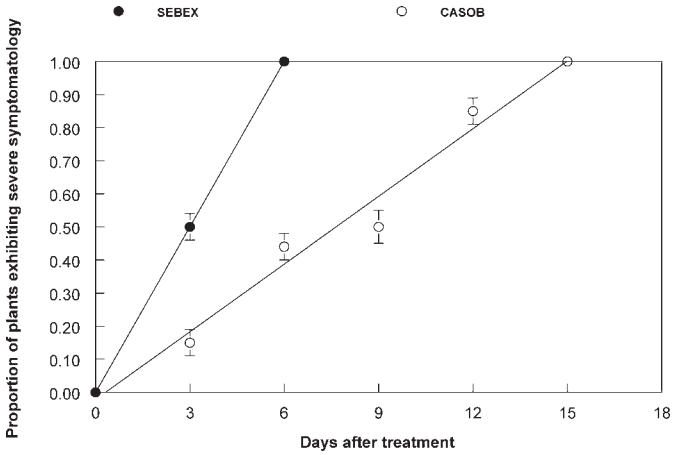

Figure 1. Disease progression of *Colletotrichum truncatum* (CT) infecting hemp sesbania and *Colletotrichum gloeosporioides* (CG) infecting sicklepod under greenhouse conditions. Diseased plant ratings were based on a modified Horsfall–Barratt (1945) rating scale as described in the Materials and Methods. 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: $Y = 0 + 16.67\ X$; $R^2 = 1.0$. For disease progression of CG on sicklepod, the relationship is best described by the following equation: $Y = -2.1 + 6.82X$; $R^2 = 0.98$.

Table 2. Percentage of control and dry weight reduction of hemp sesbania and sicklepod 15 d after inoculation with *Colletotrichum truncatum* and *Colletotrichum gloeosporioides* under greenhouse conditions.

Treatment[a]	Weed control		Dry weight reduction	
	SEBEX	CASOB	SEBEX	CASOB
	%			
CO/SW emulsion control	0	0	0	0
Distilled water control	0	0	0	0
CT in distilled water	96 a[b]	0	96 a	0
CG in distilled water	0	0	0	0
CT + CG in distilled water	96 a	0	98 a	0
CT in CO/SW emulsion	100 a	0	100 a	15 c
CG in CO/SW emulsion	0	100 a	40 b	100 a
CT + CG in CO/SW emulsion	100 a	100 a	100 a	100 a

[a] Abbreviations: SEBEX, hemp sesbania; CASOB, sicklepod; CO, unrefined corn oil; SW, Silwet L-77 surfactant; CT, *Colletotrichum truncatum*; CG, *Colletotrichum gloeosporioides*.

[b] Means followed by the same letter within columns are not significantly different at P = 0.05 according to Fisher's LSD.

Table 3. Percentage of control of hemp sesbania and sicklepod, and soybean yield 28 d after inoculation with mixtures of *Colletotrichum truncatum* and *Colletotrichum gloeosporioides* under field conditions in Stoneville, MS.

Treatment[a]	Weed control		Soybean yield (kg ha^{-1})
	SEBEX	CASOB	
	%		
UNT	0	0	2994 c
CO/SW emulsion control	0	0	2303 c
Weed-free control	100 a[b]	100 a	4703 a
GLY	95 ab	95 ab	4602 a
CT in CO/SW	94 ab	88 b	3342 b
CG in CO/SW	0	88 b	3393 b
CT + CG in CO/SW	94 ab	88 b	4556 a

[a] Abbreviations: SEBEX, hemp sesbania; CASOB, sicklepod; UNT, untreated weedy control, CO, unrefined corn oil; SW, Silwet L-77 surfactant; GLY, glyphosate; CT, *Colletotrichum truncatum*; CG, *Colletotrichum gloeosporioides*.

[b] Means followed by the same letter within columns are not significantly different at P = 0.05 according to Fisher's LSD.

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 *Alternaria cassiae* Juriar. & Khan (a pathogen of sicklepod) and *Alternaria crassa* Sacc. Rands (a pathogen of jimsonweed, *Datura stramonium* L.) were expanded, and that saprophytic fungi [*Aspergillus nidulans* (Eidam) G. Winter and *Trichoderma harzianum* Rifai] became pathogenic when formulated in an invert emulsion (Amsellem et al. 1991). More recently, greenhouse studies indicated that spores of *Colletotrichum gloeosporioides* f. sp. *aeschynomene* (virulent pathogen of the leguminous weed northern jointvetch [*Aeschynomene virginica* (L.) B.S.P.], but considered nonvirulent against another leguminous weed, hemp sesbania), formulated in an invert emulsion, could infect and cause mortality of hemp sesbania seedlings (Boyette et al. 2010). Because sicklepod was considered ''immune'' to an aqueous CG formulation (Boyette and McAlpine 1996), and CG infects and kills sicklepod only when formulated in either an invert or an unrefined CO emulsion (Boyette 2006; Boyette et al. 2007b), the findings in this present report add further support to the hypothesis that fungal host ranges can be altered by formulation.

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 (Table 3). Comparison of all of these treatments showed that the weed-free, glyphosate, and pathogen-mixture treatments provided the highest soybean yields.

The feasibility of controlling mixed weed populations with mixtures of pathogens has been previously demonstrated (Boyette et al. 1979; Chandramohan et al. 2002; Chandramohan 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

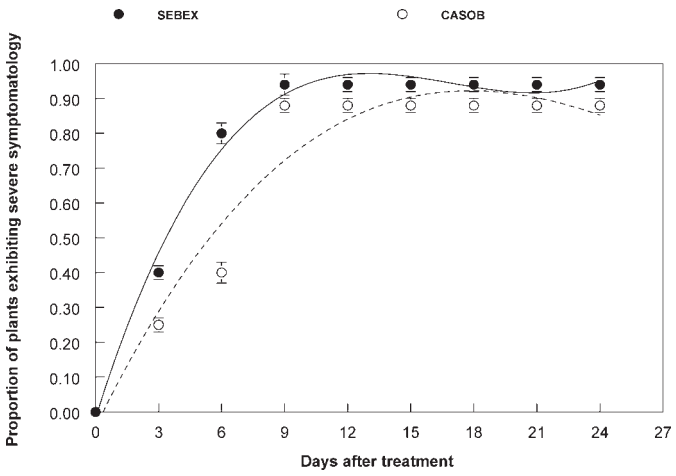

Figure 2. Disease progression of *Colletotrichum truncatum* (CT) infecting hemp sesbania and *Colletotrichum gloeosporioides* (CG) infecting sicklepod under field conditions. Diseased plant ratings were based on a modified Horsfall–Barratt (1945) rating scale as described in the Materials and Methods. 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 hand-held sprayers. For disease progression of CT on hemp sesbania, the relationship is best described by the following equation: $Y = -0.02 + 0.19X - 0.01X^2 + 0.01X^3$; $R^2 = 0.99$. For disease progression of CG on sicklepod, the relationship is best described by the following equation: $Y = -0.04 + 0.13X - 0.09X^2 + 0.01X^3$; $R^2 = 0.94$.

requirements to incite an epiphytotic 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-35LLVL, Percival Scientific, Perry, IA 50220.

[4] Hemacytometer, Improved Neubauer, no. 4040, Thermo Fisher Scientific, Waltham, MA 02451.

[5] V-8 vegetable juice, Campbell Soup Co., Camden, NJ 08103.

[6] Model 10-E; New Brunswick Scientific Co. Inc., Edison, NJ 08817.

[7] Unrefined corn oil, Spectrum Naturals, Petaluma, CA 94952.

[8] Silwet L-77 surfactant, Osi Specialties, Greeley, CO 80632.

[9] Hand-held mixer, Vortex Genie, Scientific Industries, Inc., Bohemia, NY 11716.

[10] Azlin Seed Co., Leland, MS 38756.

[11] Jiffy-mix, Jiffy Products of America, Inc., Batavia, IL 60510.

[12] Osmocote, Grace Sierra Horticultural Products, Milpitas, CA 95035.

[13] LI-COR, Model LI-185B, Inc., Lincoln, NE 38504.

[14] Spra-tool Power Pack, Aervoe Industries, Inc., Gardnersville, NV 89410.

[15] Model I 35-D, Percival Scientific Mfg., Boone, IA 50036.

[16] Garmin, Model Oregon 300, Garmin Ltd., Olathe, KS 66062.

[17] Delta and Pine Land Company, Scott, MS 38772.

[18] Glyphosate, Roundup ULTRA, Monsanto Company, St. Louis, MO 63167.

[19] Spray Doc, Model 101P, Gilmour Mfg., Somerset, PA 15501.

Literature Cited

Amsellem, Z., A. Sharon, and J. Gressel. 1991. Abolition of selectivity of two mycoherbicidal organisms and enhanced virulence of avirulent fungi by an invert emulsion. Phytopathology 81:985–988.

Amsellem, Z., A. Sharon, J. Gressel, and P. C. Quimby, Jr. 1990. Complete abolition of high threshold of two mycoherbicides (*Alternaria cassiae*) and *Alternaria crassa*) when applied in invert emulsion. Phytopathology 80:925–929.

Bakerspigel, A. 1953. Soils as a storage medium for fungi. Mycologia 45:596–604.

Banks, P. A., T. N. Tripp, J. W. Wells, and J. E. Hammel. 1985. Effects of tillage on sicklepod (*Cassia obtusifolia*) interference with soybeans (*Glycine max*) and soil water use. Weed Sci. 34:143–149.

Boyette, C. D. 2006. Adjuvants enhance the biological control potential of an isolate of *Colletotrichum gloeosporioides* for biological control of sicklepod (*Senna obtusifolia*). Biocontrol Sci. Technol. 16:1057–1066.

Boyette, C. D., A. J. Bowling, K. C. Vaughn, R. E. Hoagland, and K. C. Stetina. 2010. Induction of infection in *Sesbania exaltata* by *Colletotrichum gloeosporioides* f. sp. *aeschynomene* formulated in an invert emulsion. World J. Microbiol. Biotechnol. 26:951–956.

Boyette, C. D., R. E. Hoagland, and M. A. Weaver. 2007a. Biocontrol efficacy of *Colletotrichum truncatum* for hemp sesbania (*Sesbania exaltata*) control is enhanced with unrefined corn oil and surfactants. Weed Biol. Manag. 7:70–76.

Boyette, C. D., R. E. Hoagland, and M. A. Weaver. 2007b. Effect of row spacing on biological control of sicklepod (*Senna obtusifolia*) with *Colletotrichum gloeosporioides*. Biocontrol Sci. Technol. 17:957–967.

Boyette, C. D. and J. R. McAlpine. 1996. Herbicidal control of sicklepod and coffee senna with *Colletotrichum gloeosporioides*. U.S. Patent No. 5,529,773.

Boyette, C. D., P. C. Quimby Jr., C. T. Bryson, G. H. Egley, and F. E. Fulgham. 1993. Biological control of hemp sesbania (*Sesbania exaltata*) under field conditions with *Colletotrichum truncatum* formulated in an invert emulsion. Weed Sci. 41:497–500.

Boyette, C. D., G. E. Templeton, and R. J. Smith, Jr. 1979. Control of winged waterprimrose (*Jussiaea decurrens*) and northern jointvetch (*Aeschynomene virginica*) with fungal pathogens. Weed Sci. 27:497–501.

Bridges, D. C. and R. H. Walker. 1987. Economics of sicklepod (*Cassia obtusifolia*) management. Weed Sci. 35:594–598.

Buchanan, G. A., R. H. Crowley, J. E. Street, and J. A. McGuire. 1980. Competition of sicklepod (*Cassia obtusifolia*) and redroot pigweed (*Amaranthus retroflexus*) with cotton (*Gossypium hirstum*). Weed Sci. 28:258–262.

Chandramohan, S. and R. Charudattan. 2003. A multiple-pathogen system for bioherbicidal control of several weeds. Biocontrol Sci. Technol. 13:199–205.

Chandramohan, S., R. Charudattan, R. M. Sonoda, and M. Singh. 2002. Field evaluation of a fungal mixture for the control of seven weedy grasses. Weed Sci. 50:204–213.

Charudattan, R. 2001. Biological control of weeds by means of plant pathogens: significance for integrated weed management in modern agroecology. BioControl 46:229–260.

Charudattan, R. 2005. Ecological, practical, and political inputs into selection of weed targets: what makes a good biological control target? Biol. Control 35:183–196.
Cook, R. J. and K. F. Baker. 1983. The Nature and Practice of Biological Control of Plant Pathogens. St. Paul, MN: American Phytopathological Society. 368 p.
Crawford, L., G. M. McDonald, and M. Friedman. 1990. Composition of sicklepod (*Cassia obtusifolia*) toxic seeds. J. Agric. Food Chem. 38:2169–2175.
Daniel, J. T., G. E. Templeton, R. J. Smith, Jr., and W. T. Fox. 1973. Biological control of northern jointvetch in rice with an endemic fungal disease. Weed Sci. 21:303–307.
Dowler, C. C. 1992. Weed survey—southern states. Proc. South. Weed Sci. Soc. 45:392–407.
Hallett, S. G. 2005. Where are the bioherbicides? Weed Sci. 53:404–415.
Harry-O'kuru, R. E., Y. V. Wu, R. Evangelista, S. F. Vaughn, W. Rayford, W., and R. F. Wilson. 2005. Sicklepod (*Senna obtusifolia*) seed processing and potential utilization. J. Agric. Food Chem. 53:4784–4787.
Horsfall, J. G. and R. W. Barratt. 1945. An improved grading system for measuring diseases. Phytopathology 35:655.
Jackson, M. A. and D. A. Schisler. 1992. The composition and attributes of *Colletotrichum truncatum* spores are altered by the nutritional environment. Appl. Environ. Microbiol. 58:2260–2265.
McWhorter, C. G. and J. M. Anderson. 1979. Hemp sesbania (*Sesbania exaltata*) competition in soybeans (*Glycine max*). Weed Sci. 27:58–64.
Nelson, K. A. and K. A. Renner. 1999. Weed management in wide- and narrow-row glyphosate resistant soybean. J. Prod. Agric. 12:460–465.
Norris, R. F. 2007. Weed fecundity: current status and future needs. Crop Prot. 26:182–188.
Norsworthy, J. K. and L. R. Oliver. 2000. Hemp sesbania interference in drill-seeded glyphosate-resistant soybean. Weed Sci. 50:34–41.
Pal, K. K. and B. McSpadden Gardener. 2006. Biological control of plant pathogens. Plant Health Instructor. DOI:10.1094/PHI-A-2006-1117-02
Powell, R. G., R. D. Plattner, and M. Suffness. 1990. Occurrence of sesbanimide in seeds of toxic sesbania species. Weed Sci. 38:148–152.
Putnam, M. R., T. Boosinger, J. Spano, J. Wright, A. Wiggins, and G. D'Andrea. 1988. Evaluation of *Cassia obtusifolia* (sicklepod) seed consumption in Holstein calves. Vet. Hum. Toxicol. 30:316–318.
Reddy, K. N. 2002. Weed control and economic comparisons in soybeans planting systems. J. Sustain. Agric. 21:21–35.
Sandrin, T. R., D. O. TeBeest, and G. J. Weidemann. 2003. Soybean and sunflower oils increase the infectivity of *Colletotrichum gloeosporioides* f. sp. *aeschynomene* to northern jointvetch. Biol. Control 26:244–252.
Steele, R. G. D., J. H. Torrey, and D. A. Dickeys. 1997. Multiple Comparisons. Principles and Procedures of Statistics—A Biometrical Approach. New York: McGraw Hill. 666 p.
Watson, A. K. 1991. The classical approach with plant pathogens., Pages 3–23. *in* D. O. TeBeest, ed. Microbial Control of Weeds. New York: Chapman and Hall.
Weaver, M. A., M. E. Lyn, C. D. Boyette, and R. E. Hoagland. 2007. Bioherbicides for weed control., Pages 93–110. *in* M. K. Upadhyaya and R. E. Blackshaw, eds. Non-Chemical Weed Management. Cambridge, MA: CABI.
Webster, T. M. and H. D. Coble, H.D.. 1997. Changes in the weed species composition of the southern United States: 1974 to 1995. Weed Technol. 11:308–317.
Yagi, S. M., S. El Tigani, and S.E.I. Adam. 1998. Toxicity of *Senna obtusifolia* fresh and fermented leaves (kawal), *Senna alata* leaves and some products from *Senna alata* on rats. Phytother. Res. 12:324–330.

Received March 13, 2010, and approved July 19, 2010.