

Effect of row spacing on biological control of sicklepod (*Senna obtusifolia*) with *Colletotrichum gloeosporioides*

C. DOUGLAS BOYETTE, ROBERT E. HOAGLAND, & MARK A. WEAVER

USDA–Agricultural Research Service, Southern Weed Science Research Unit, Stoneville, MS, USA

(Received 11 May 2007; returned 4 July 2007; accepted 4 July 2007)

Abstract

In field experiments conducted over 3 years, the mycoherbicide fungus *Colletotrichum gloeosporioides*, formulated either in 20% (v/v) unrefined corn oil and 0.2% Silwet L-77 surfactant or with an invert emulsion, provided season-long control of *Senna obtusifolia* in narrow (51 cm) rows of soybean. However, in wide (102 cm) rows, one application of either formulation failed to provide season-long control of *S. obtusifolia*, and two applications were required to achieve season-long weed control. In narrow (51 cm) rows, one application of the fungus either in unrefined corn oil or an invert emulsion controlled *S. obtusifolia* an average of >90%, and a second application was not required for season long weed control. Soybean yields in wide-row plots treated with two applications of either the fungus/corn oil or fungus/invert emulsion, or with a single application of the fungal treatments in narrow-row soybean plots, were not significantly different from weed-free control plots, or from plots treated with the herbicide chlorimuron. These results suggest that row spacing can affect mycoherbicide efficacy of this fungus for controlling *S. obtusifolia*.

Keywords: *Colletotrichum gloeosporioides*, *Senna obtusifolia*, *Senna occidentalis*, bioherbicide, microbial herbicide, mycoherbicide

Introduction

Senna obtusifolia (L.) Barnaby (sicklepod), a non-nodulating weedy legume, is a problematic weed in much of the southeastern US (Elmore 1989). It is an especially important weed in soybean [*Glycine max* (L.) Merr.] and is considered one of the most difficult weeds to control in that crop (Buchanan et al. 1980; Dowler 1992; Rankins et al. 2006). Heavy infestations of this weed can reduce soybean yields by 70% or more (Anonymous 1999) and can also cause difficulty with harvest, and reduce seed quality (Brown & Bridges 1989). This species also produces a toxic chemical agent(s) of unknown etiology, that can cause illness or death of cattle if large amounts of foliage are consumed (Anonymous 2000). Because of its fecundity, continual emergence

Correspondence: C. Douglas Boyette, USDA-ARS, Southern Weed Science Research Unit, P.O. Box 350, Stoneville, MS 38776, USA. Tel: +1 662 686 5217. Fax: +1 662 686 5422. E-mail: doug.boyette@ars.usda.gov

throughout the growing season, and high tolerance to many commonly used herbicides, adequate control is difficult (Brown & Bridges 1989; Elmore 1989).

Although a candidate bioherbicide may be highly efficacious to target weeds under controlled environments, its activity under field conditions may be variable or very low. Thus field evaluation of weed control efficacy is perhaps arguably the single most important component of a pathogen's bioherbicidal potential. To improve bioherbicidal efficacy in the field or to help overcome certain detrimental field environmental factors, formulation-based approaches, i.e. vegetable oils and other adjuvants for plant pathogenic fungi have been successful (Mintz et al. 1992; Boyette 1994, 2006; Auld et al. 2003; Sandrin et al. 2003). Invert emulsions can also improve bioherbicide efficacy of several other mycoherbicidal fungi (Quimby et al. 1989; Amsellem et al. 1991; Boyette et al. 1993; Yang & Jong 1995; Millhollon et al. 2003).

Crop management systems may also influence the success of a given bioherbicide under field conditions. For example, a practice gaining some popularity in US and Brazilian soybean production is an alteration from wide rows to more narrow rows (Marking 1997; Johnson et al. 1998; Leibold et al. 2001). Concurrent with decreased row spacing is an increase in crop plant population (Buehring et al. 2002). Plant canopy closure occurs quicker in narrow-row soybean, resulting in 90–95% light interception earlier than in wide rows. Because yield is a function of canopy light interception during vegetative and early reproductive stages (Board et al. 1992), and narrow-row soybeans make better use of available light, higher yields are usually obtained in narrow versus wide row plantings (Costa et al. 1980; Ethredge et al. 1989; Ablett et al. 1991; Board et al. 1992).

Narrow row spacing in soybean also reduces weed competition (Legere & Schreiber 1989). The emergence of sicklepod has been shown to be reduced by 68% by soybean canopy closure (Norsworthy 2004). Nice et al. (2001) showed that by reducing soybean row spacing, coupled with increased seed planting density, sicklepod was reduced up to 80%, but shading increased sicklepod height.

Contrary to the potential for better yields, narrow row spacing may foster more optimal micro-climates for the spread of soybean diseases. Narrow row soybean planting can increase *Sclerotinia* stem rot disease development, whereas wide row plantings resulted in decreased disease severity (Grau & Rake 1984). Stem rot disease severity was also lower when seed planting densities were reduced (Lee et al. 2005). More air movement and less soil/plant surface moisture within the canopy may be the causes of reduced disease in certain soybean cultivars (Kim et al. 1999).

Deleterious effects of UV radiation have been reported in several genera of entomopathogenic fungi (Fargues et al. 1996; Braga et al. 2002). Increased UV inhibited, not only the pathogenic fungus *Botrytis cinerea*, but also *Trichoderma harzianum*, a fungal disease biocontrol agent of this pathogen (Paul et al. 2005). UV interactions can also limit the effectiveness of bioherbicides (Leathers et al. 1993). The rapid closure of narrow row planting could help shield or shade bioherbicidal propagules from UV radiation that might aid in promoting secondary infection beneath the canopy.

Based on these reports, we hypothesized that the higher density of soybean plants and the more rapid canopy closure in narrow-row as compared with wide-row soybean might provide a more favorable micro-environment for bioherbicidal disease development. Furthermore, *Colletotrichum gloeosporioides* (Penz.) Penz & Sacc. [NRRL No. 21046], originally isolated from *Senna occidentalis* L. (coffee senna) (Boyette &

McAlpine 1996), provided high bioherbicidal efficacy against *S. obtusifolia* seedlings when applied as invert emulsions under controlled environmental conditions (Boyette 2006). Thus, the objectives of the present studies were to determine if a single application of *C. gloeosporioides*, formulated either in unrefined corn oil or in an invert emulsion, could more effectively control *S. obtusifolia* in narrow-row versus wide row soybean field test plots (Figure 1).

Materials and methods

Isolation and culture

The strain of *C. gloeosporioides* (NRRL 21046) used throughout these studies was originally isolated from diseased *S. occidentalis* tissue by surface sterilizing sections of diseased plant tissues in 0.05% NaOCl for 1 min, rinsing with distilled water, and then placing the sections on potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI, USA) that was amended with the antibiotics chloramphenicol (0.75 mg mL^{-1}) and streptomycin sulfate (1.25 mg mL^{-1}). The plates were incubated for 48 h at 25°C . Advancing edges of fungal colonies were transferred to PDA plates and incubated for 5 days at 25°C under an alternating 12/12-h light/dark regime, provided by cool-white fluorescent lights (average of $275 \text{ mol m}^{-2} \text{ s}^{-1}$). The fungus was subcultured on PDA without antibiotics, and preserved at 4°C in sterilized sandy loam soil (25% water holding capacity) (Tuite 1969), and on sterile silica gel containing skim milk (Windels et al. 1988). Inoculum for all experiments was produced in liquid culture after 96 h growth on modified Richard's medium containing vegetable juice (V-8 vegetable juice, Campbell Soup Co., Camden, NJ, USA) (Daniel et al. 1973) either in shaken Erlenmeyer flasks at 25°C and 125 rpm, or in a laboratory fermentor (Model 10-E; New Brunswick Scientific Co. Inc., Edison, NJ, USA), under similar conditions. Spores produced in the broth were separated from the mycelium by



Figure 1. Biological control of *Senna obtusifolia* with a single application of *Colletotrichum gloeosporioides* formulated in an invert emulsion in narrow-row soybean field test plots.

filtering through double layered cheesecloth which retained the mycelium. Conidial concentrations in these filtrates were determined and adjusted using hemacytometers.

Adjuvants and spray mixture preparation

The adjuvants used in these studies consisted of: (1) an invert emulsion (Quimby et al. 1989; Boyette et al. 1993); (2) a silicone-polyether copolymer surfactant (Silwet L-77; Loveland Industries, Greeley, CO, USA) (Boyette 1994); and (3) unrefined corn oil (Spectrum's Natural, Petaluma, CA, USA) (Boyette 1994). All of these adjuvants have previously been shown to increase biocontrol efficacy of various bioherbicidal pathogens (Quimby et al. 1989; Zidak et al. 1992; Boyette et al. 1993, 2007; Boyette 2006).

Field experiments

Field experiments were conducted in 1999–2001 at the Southern Weed Science Experimental Farm, Stoneville, Mississippi, USA, on a Dundee very fine sandy loam (Aeric Ochraqualf). Both wide-row (102 cm row width) and narrow-row (51 cm row width) were utilized. For wide row soybeans, test plots consisted of four rows of 'Centennial' cv. soybeans, 12.2 m long and 1 m apart, with the two center rows receiving treatment. For narrow row soybeans, test plots were as described, with the exception that test plots consisted of eight soybean rows with the four center rows receiving treatment. All rows were planted with mechanically scarified *S. obtusifolia* seeds (Azlin Seed Co, Leland, MS, USA) at a density of approximately 100 seeds m^{-1} of row. Treatments consisted of: (1) fungus (conidia) in 0.2% (v/v) Silwet L-77 only (one application); (2) conidia in Silwet L-77 only (two applications, with the second application 7 days after the first application); (3) conidia in 20% (v/v) unrefined corn oil (one application); (4) conidia in unrefined corn oil (two applications, with the second application 7 days after the first application); (5) conidia in unrefined corn oil and Silwet L-77 (one application); (6) conidia in unrefined corn oil and Silwet L-77 (two applications, with the second application 7 days after the first application); (7) conidia in invert emulsion (one application); (8) conidia in invert emulsion (two applications, with the second application 7 days after the first application); (9) unrefined corn oil (one application); (10) unrefined corn oil (two applications, with the second application 7 days after the first application); (11) unrefined corn oil and Silwet L-77 (one application); (12) unrefined corn oil and Silwet L-77 (2 applications, with the second application 7 days after the first application); (13) invert emulsion only; (14) chlorimuron (Classic; E.I. DuPont de Nemours and Co., Inc., Wilmington, DE, USA) (applied post-emergence at 0.4 kg ha^{-1}); (15) hand-weeded control; and (16) untreated control. Inoculum (conidial) concentrations were adjusted to 1.0×10^7 conidia mL^{-1} in treatments receiving a fungal component. All spray applications were made with back-pack sprayers at spray volumes of 200 L ha^{-1} . Planting dates were 21 May 1999, 20 May 2000 and 30 May 2001, and bioherbicide treatments were applied on 4, 2 and 14 June of each respective year when soybeans were in the V-2 stage of growth. Plots that received two inoculations of bioherbicide were treated 7 days after initial treatments, i.e. 9, 11 and 21 June of each respective year. Environmental data (air temperature, soil temperature, relative humidity) at planting and inoculation dates are summarized in Table I. Percentages of weed control and disease progression of *S. obtusifolia* were determined

Table I. Environmental conditions of biological control studies on *S. obtusifolia* conducted at Stoneville, Mississippi, USA during 1999–2001.

Event ⁴	Air ¹			Soil ²			RH ³		
	Year								
	1999	2000	2001	1999	2000	2001	1999	2000	2001
Planting	31/20	27/17	29/19	36/21	28/21	34/23	83/31	100/74	90/38
1st application	33/23	34/21	34/24	38/26	39/24	41/28	95/51	92/81	99/46
7 day rating	31/19	31/18	33/22	39/27	36/18	41/26	97/38	96/33	100/41
14 day rating	28/15	33/23	30/18	36/23	38/26	35/23	87/40	94/50	100/55
21 day rating	33/22	32/23	33/24	36/26	32/23	41/27	99/61	100/60	100/48
28 day rating	33/24	27/20	36/24	37/27	29/23	43/30	97/61	100/72	100/86
2nd application	31/19	31/18	33/22	39/27	36/18	41/26	97/38	96/33	100/41
7 day rating	28/15	33/23	30/18	36/23	38/26	35/23	87/40	94/50	100/55
14 day rating	33/22	32/23	33/24	36/26	32/23	41/27	99/61	100/60	100/48
21 day rating	33/24	27/20	36/24	37/27	29/23	43/30	97/61	100/72	100/86
28 day rating	32/25	30/23	34/25	39/25	33/25	45/34	86/66	95/70	98/86

¹Maximum/minimum temperature (°C) at 1 m above soil surface. ²Maximum/minimum temperature (°C) at 5.1 cm below soil surface. ³Maximum/minimum relative humidity (RH) 1 m above soil surface. ⁴Dates of each event presented in Materials and methods section.

in randomly selected 3.0 × 0.46-m areas at 3, 7, 14, 21, and 28 days after inoculation. Data in Tables II and III were values taken at 28 days after inoculation. 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 = 20, 40, 60, and 80% leaf and stem lesion coverage/injury, respectively, and 1.0 = plant mortality. Symptomatology was considered 'severe' at ratings of 0.8–1.0.

Experimental design

The experiments were arranged as randomized complete block designs with four replications. Data over the 3-year testing period were examined for homogeneity of variance (Steele et al. 1997), combined, and analyzed using ANOVA. Because arcsine and square-root transformation of data did not alter interpretation of data, non-transformed data are presented. 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 disease progression studies, data were analyzed using standard mean errors and best-fit regression analysis.

Results and discussion

Isolation and culture

The fungus sporulated prolifically in liquid culture in modified Richard's medium and on PDA. Conidial yields averaged 3.0 × 10⁸ conidia mL⁻¹ after 7 days at 25°C and 250 rpm. This yield compares favorably to conidial densities produced by isolates of *C. gloeosporioides formae speciales* that have been evaluated as bioherbicides against other weeds (Daniel et al. 1973; Boyette et al. 1979).

Table II. Effects of adjuvants and soybean row spacing on biological control of *Senna obtusifolia* with *Colletotrichum gloeosporioides*, 28 days after inoculation^a.

Treatment ^{b,c,d,e,f}	<i>S. obtusifolia</i> Control (%)	
	Row width (cm)	
	51	102
Fungus/Silwet L-77 (1 application)	8 d	5 d
Fungus/Silwet L-77 (2 applications)	5 d	5 d
Fungus/unrefined corn oil (1 application)	88 b	40 c
Fungus/unrefined corn oil (2 applications)	90 ab	85 b
Fungus/unrefined corn oil/Silwet L-77 (1 application)	90 ab	42 c
Fungus/unrefined corn oil/Silwet L-77 (2 applications)	93 a	95 a
Fungus/invert (1 application)	90 ab	36 c
Fungus/invert (2 applications)	92 ab	94 ab
Unrefined corn oil (1 application)	0 e	0 e
Unrefined corn oil (2 applications)	5 d	5 d
Unrefined corn oil/Silwet L-77 (1 application)	6 d	7 d
Unrefined corn oil/Silwet L-77 (2 applications)	8 d	5 d
Invert (1 application)	7 d	8 d
Invert (2 applications)	9 d	7 d
Chlorimuron	100 a	100 a
Weed-free check	100 a	100 a
Untreated	0 e	0 e

^aLetters followed by the same letter are not significantly different at $P = 0.05$ using Fisher's Protected LSD.

^bData were collected at 3, 7, 14, 21, and 28 days after inoculations. For those plots receiving two treatments, the second applications were made 7 days after the initial application. ^cInoculum rate: 1×10^7 conidia mL^{-1} . ^dUnrefined corn oil was added to make a 1:1 (v/v) emulsion. ^eSilwet L-77 was added to make a 0.2% solution. ^fSilwet L-77 was added to a 1:1 unrefined corn oil/aqueous conidia emulsion to make a 0.2% solution.

Field experiments

Wide row versus narrow row. Effective *S. obtusifolia* control was achieved in test plots receiving the fungus formulated in either the invert emulsion, or in unrefined corn oil (with or without surfactant) (Table II). Weeds in wide-row plots were controlled 88–90% within 9 days with a single application of either the fungus/corn oil, fungus/corn oil/surfactant, or fungus/invert emulsion formulation. However, *S. obtusifolia* seedlings emerging between the 7-day rating period through the duration of the tests (28 days), were not controlled by a single application of any of the formulations containing the fungus, resulting in overall poor weed control (40, 42, and 36%, respectively), for the fungus/unrefined corn oil, fungus/unrefined corn oil/surfactant, or fungus/invert formulation after 4 weeks (Table II). In contrast, two applications of these formulations were required to control *S. obtusifolia* seedlings at an 85–95% control level in wide row spacing (Table II). However, in narrow-row plots, *S. obtusifolia* seedlings were effectively controlled over a 4-week period by a single application of either the fungus in invert or in unrefined corn oil emulsions (88–90%), with or without surfactant. A second application of any of these treatments did not significantly increase *S. obtusifolia* control (Table II). Maximum weed control levels achieved, either with two applications in wide rows, or with a single application in

Table III. Effects of *C. gloeosporioides* adjuvants and row spacing on soybean yield^a.

Treatment ^{ab,c,d,e}	Soybean Yield (kg ha ⁻¹)	
	Row width (cm)	
	51	102
Fungus/Silwet L-77 (1 application)	2342 b	1340 c
Fungus/Silwet L-77 (2 applications)	2499 b	1366 c
Fungus/unrefined corn oil (1 application)	4268 a	1995 b
Fungus/unrefined corn oil (2 applications)	4318 a	2810 a
Fungus/unrefined corn oil/Silwet L-77 (1 application)	4387 a	1967 b
Fungus/unrefined corn oil/Silwet L-77 (2 applications)	4317 a	2750 a
Fungus/invert (1 application)	4344 a	1950 b
Fungus/invert (2 applications)	4366 a	2900 a
Unrefined corn oil (1 application)	2120 c	1278 d
Unrefined corn oil (2 applications)	2400 b	1380 c
Unrefined corn oil/Silwet L-77 (1 application)	2220 c	1274 d
Unrefined corn oil/Silwet L-77 (2 applications)	2318 b	1318 c
Invert (1 application)	2309 b	1266 c
Invert (2 applications)	2455 b	1369 c
Chlorimuron	4367 a	2911 a
Untreated	2314 b	1355 c
Weed-free check	4389 a	2845 a

^aLetters within columns followed by the same letter are not significantly different at $P = 0.05$ using Fisher's Protected LSD. ^bInoculum rate: 1×10^7 conidia Ml^{-1} . ^cUnrefined corn oil was added to make a 1:1 (v/v) emulsion. ^dSilwet L-77 was added to make a 0.2% solution. ^eSilwet L-77 was added to a 1:1 unrefined corn oil/aqueous conidia emulsion to make a 0.2% solution.

narrow rows, were not significantly different from the level of *S. obtusifolia* control provided by the herbicide chlorimuron (Table II).

Disease progression

Senna obtusifolia seedlings treated with fungal spores in surfactant alone exhibited only small (<1 mm) leaf spots within 48 h, covering less than 10% of cotyledon surfaces. No disease was observed on expanded true leaves, and no stem lesions developed. However, on plants treated with the fungus/invert or fungus/corn oil (with or without surfactant), disease symptoms (leaf spotting) occurred, both on inoculated cotyledons and true leaves, and stem lesions were visible within 72 h after inoculation. The lesions coalesced, and by 7 days after inoculation, the entire leaf and stem areas were completely blighted (severe necrosis and/or plant mortality). In the disease progression studies, only data from plots receiving the fungus-invert treatments are presented, because there were no significant differences between the most effective treatment effects as compared to corn oil with or without surfactant (Figure 2). In all cases, a polynomial regression curve provided the best fit, with R^2 values ranging from 0.94 to 0.98. Disease progression with the fungus/corn oil and fungus-invert formulation was similar in both wide- and narrow-row plots for the first 3–7 days following inoculation. However, seedlings continued to emerge after 7 days in wide-row plots, resulting in overall poor *S. obtusifolia* control after 4 weeks. In contrast, maximal disease occurred after 7 days in narrow-row plots, and emerging sicklepod seedlings were either killed, severely injured, or reduced in number and vigor by shading and crop plant density

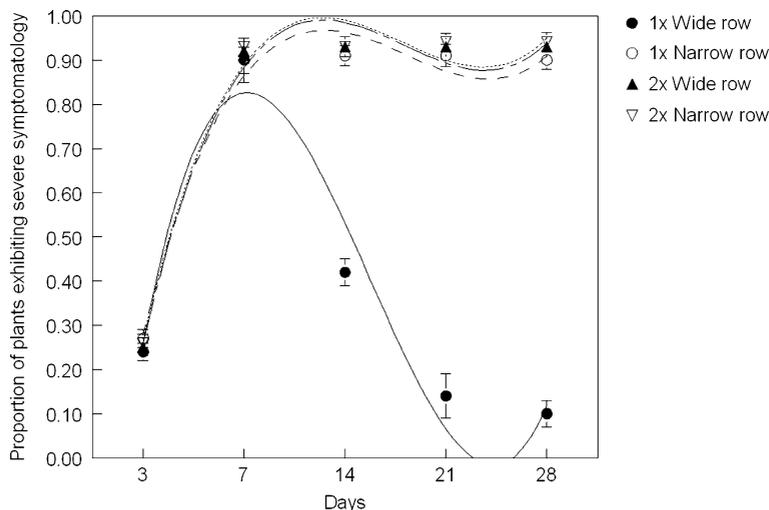


Figure 2. Disease progression of *Colletotrichum gloeosporioides* infecting *Senna obtusifolia*. Diseased plant ratings were based on a modified Horsfall–Barratt rating scale as described in the Materials and methods. Symptomatology was considered ‘severe’ at ratings of 0.8–1.0. Fungal spores (density of 1×10^7 spores mL^{-1}) were suspended in an invert emulsion and sprayed at a volume of 200 L ha^{-1} using hand-held sprayers. For wide-row plots receiving a single application (1 ×, wide-row), the relationship is best described by the equation $Y = -1.86 + 3.12X - 1.12X^2 + 0.12X^3$, $R^2 = 0.94$. For wide-row plots receiving two applications (second application 7 days after initial application) (2 ×, wide-row), the relationship is best described by the equation: $Y = -1.1 + 1.88X - 0.55 + 0.051X^3$, X^2 , $R^2 = 0.98$. For narrow-row plots receiving a single application (1 ×, narrow-row), the relationship is best described by the equation: $Y = -1.22 + 2.04X - 0.591X^2 + 0.055X^3$, $R^2 = 0.98$. For narrow-row plots receiving two applications (second application 7 days after initial application) (2 ×, narrow-row), the relationship is best described by the equation: $Y = -1.2X + 2.01X^2 - 0.59X^2 + 0.55X^3$, where Y = plants exhibiting severe disease or mortality, and X = days after treatment.

(Nice et al. 2001; Norsworthy 2004). resulting in dramatically greater weed control over the 4-week period (Figure 2). This suggests that the bioherbicide continued to infect newly emerging seedlings within the environment produced by narrow row planting. However, since adjuvant is required by this bioherbicide to infect *S. obtusifolia* (Boyette 2006), sufficient quantities of the formulation adjuvants and/or residual inoculum would be required for this scenario. Further research should address the residual effects of these formulation adjuvants. Soybean yields were higher in narrow-row plots as compared to wide-row plots, regardless of treatment (Table III). Soybean yields in wide- or narrow-row plots where effective *S. obtusifolia* control was achieved by *C. gloeosporioides* formulations were not significantly different from the yields from hand-weeded plots or in plots treated with chlorimuron.

The inability for single applications of this mycoherbicide to control emerging *S. obtusifolia* seedlings in wide-row plots indicates that either this fungus does not possess the capability to incite adequate epiphytotic disease spread necessary for season long *S. obtusifolia* control, or that the fungus is very sensitive to restrictive environmental conditions (e.g. moisture limitations, high temperatures, UV light, and possibly others). Previous research has also shown that two applications of the mycoherbicide *Alternaria cassiae*, resulted in significantly greater control of *S. obtusifolia* than a single application (Walker & Boyette 1985). In the present studies (although not measured), it is possible that earlier canopy closure and denser plant

populations in narrow-row soybean plots, as compared to wide-row plots, may have provided a more favorable environment for disease development on emerging *S. obtusifolia* over the 4-week period. Soybean canopy closure alone is effective in reducing *S. obtusifolia* emergence (Nice et al. 2001; Norsworthy 2004), but since canopy closure had not occurred over most of this test period in our studies, disease-promoting factors other than total canopy closure may be operative. Further studies will be needed to evaluate the effects of parameters such as crop and weed densities, UV effects, canopy shading effects, and strain selection upon disease spread and weed control.

The addition of Silwet L-77 to *C. gloeosporioides* conidial suspensions in unrefined corn oil resulted in slightly increased mortality to *S. obtusifolia* in the present studies. However, in other bioherbicide:weed systems, the effect of the addition of this surfactant was much more pronounced (Zidak et al. 1992; Walker & Tilley 1997; Boyette et al. 2002). A possible explanation for the increased mortality to *S. obtusifolia* when formulated with these adjuvants is the stimulation of conidia germination, appressoria formation and germination induced by the adjuvants, or their components. Other research has shown that germination and appressorial formation of *C. gloeosporioides* f. sp. *aeschynomene* conidia for *Aeschynomene virginica* (L.) B.S.P. (northern jointvetch) control (Sandrin et al. 2003) and *C. truncatum* conidia for *S. exaltata* control (Boyette et al. 2007) are stimulated by some of the adjuvants used in the research presented herein. It is also possible that the unrefined corn oil and the invert emulsion helped to maintain viability of the conidia on the plant surfaces during moisture-limited periods during hot, dry field conditions since conidia of this organism requires free moisture or dew periods of 8–12 h (Boyette 2006). This formulation may have also protected the conidia from desiccation by substituting for, or supplementing the extracellular matrix normally produced by the conidia. Pathogenicity and the extracellular matrix can be altered by media composition supplied to *Colletotrichum orbiculare*, a pathogen of spiny cocklebur (*Xanthium spinosum*) (McRae & Stevens 1990). The results in this report indicate that, when properly formulated, a single application of *C. gloeosporioides* NRRL No. 21046 can be a very effective bioherbicide for controlling sicklepod in narrow-row soybeans.

Acknowledgements

The authors thank J.R. McAlpine and T.A. Newton for valuable technical assistance.

References

- Ablett GR, Beversdorf WD, Dirks VA. 1991. Row width and seeding rate performance of indeterminate, semi-determinate, and determinate soybean. *Journal of Production Agriculture* 4:391–395.
- Anonymous. 1999. Soybean weeds. North Carolina Cooperative Extension Service. North Carolina State University. p 16.
- Anonymous. 2000. Texas toxic plants. Available: <http://texnat.tamu.edu/cmplants/sicklepod-senna.html>
- Amsellem Z, Sharon A, Gressel J. 1991. Abolition of selectivity of two mycoherbicide organisms and enhanced virulence of avirulent fungi by an invert emulsion. *Phytopathology* 81:925–929.
- Auld BA, Hetherington SD, Smith HE. 2003. Advances in bioherbicide formulation. *Weed Biology and Management* 3:61–67.
- Board JE, Kamal M, Harville BG. 1992. Temporal importance of greater light interception to increased yield in narrow-row soybean. *Agronomy Journal* 84:575–579.
- Boyette CD. 1994. Unrefined corn oil improves the mycoherbicide activity of *Colletotrichum truncatum* for hemp sesbania (*Sesbania exaltata*) control. *Weed Technology* 8:526–529.

- Boyette CD. 2006. Adjuvants enhance the biological control potential of an isolate of *Colletotrichum gloeosporioides* for biological control of sicklepod (*Senna obtusifolia*). *Biocontrol Science and Technology* 16:1057–1066.
- Boyette CD, McAlpine JR. 1996. Herbicidal control of sicklepod and coffee senna with *Colletotrichum gloeosporioides*. US Patent No. 5,529,773.
- Boyette CD, Templeton GE, Smith RJ Jr. 1979. Control of winged waterprimrose (*Jussiaea decurrens*) and northern jointvetch (*Aeschynomene virginica*) with fungal pathogens. *Weed Science* 27:497–501.
- Boyette CD, Quimby PC Jr, Bryson CT, Egley GH, Fulgham FE. 1993. Biological control of hemp sesbania (*Sesbania exaltata*) under field conditions with *Colletotrichum truncatum* formulated in an invert emulsion. *Weed Science* 41:497–500.
- Boyette CD, Walker HL, Abbas HK. 2002. Biological control of kudzu (*Pueraria lobata*) with an isolate of *Myrothecium verrucaria*. *Biocontrol Science and Technology* 11:75–82.
- Boyette CD, Hoagland RE, Weaver MA. 2007. Biocontrol efficacy of *Colletotrichum truncatum* for hemp sesbania (*Sesbania exaltata*) is enhanced with unrefined corn oil and surfactant. *Weed Biology and Management* 7:70–76.
- Braga GUL, Rangel DEN, Flint SD, Miller CD, Anderson AJ, Roberts DW. 2002. Damage and recovery from UV-B exposure in conidia of the entomopathogens *Vorticillium lecanii* and *Aphanocladium album*. *Mycologia* 94:912–920.
- Brown SM, Bridges DC. 1989. Comparative biology and control of sicklepod and coffee senna. *Proceedings of the Southern Weed Science Society* 42:11.
- Buchanan GA, Crowley RH, Street JE, McGuire JA. 1980. Competition of sicklepod (*Cassia obtusifolia*) and redroot pigweed (*Amaranthus retroflexus*) with cotton (*Gossypium hirsutum*). *Weed Science* 28:258–262.
- Buehring NW, Nice GR, Shaw DR. 2002. Sicklepod (*Senna obtusifolia*) control and soybean (*Glycine max*) response to soybean row spacing and population in three weed management systems. *Weed Technology* 16:131–141.
- Costa JA, Oplinger ES, Pendleton JW. 1980. Response of soybean cultivars to planting patterns. *Agronomy Journal* 72:153–156.
- Daniel JT, Templeton GE, Smith RJ Jr, Fox WT. 1973. Biological control of northern jointvetch in rice with an endemic fungal disease. *Weed Science* 21:303–307.
- Dowler CC. 1992. Weed survey-southern states. *Proceedings of the Southern Weed Science Society* 45:392–407.
- Elmore CD. 1989. Weed survey: southern states. *Proceedings of the Southern Weed Science Society* 42:408–420.
- Ethredge WJ, Ashley DA, Woodruff JM. 1989. Row spacing and plant population effects on yield components of soybean. *Agronomy Journal* 81:947–951.
- Fargues J, Goettel MS, Smits N, Ouedraogo A, Vidal C, Lacey LA, Lomer CJ, Rougier M. 1996. Variability in susceptibility to simulated sunlight of conidia among isolates of entomopathogenic Hyphomycetes. *Mycopathologia* 135:171–181.
- Grau CR, Radke VL. 1984. Effects of cultivars and cultural practices on *Sclerotinia* stem rot of soybean. *Plant Disease* 68:56–58.
- Horsfall JG, Barratt RW. 1945. An improved grading system for measuring diseases. *Phytopathology* 35:655.
- Johnson WG, Dilbeck JS, DeFelice MS, Kendig JA. 1998. Weed control with reduced rates of imazaquin and imazethapyr in no-till narrow-row soybean (*Glycine max*). *Weed Science* 46:105–110.
- Kim HS, Sneller CH, Diers BW. 1999. Evaluation of soybean cultivars for resistance to *Sclerotinia* stem rot in field environments. *Crop Science* 39:64–68.
- Leathers TD, Gupta SC, Alexander NJ. 1993. Mycopesticides: status, challenges and potential. *Journal of Industrial Microbiology and Biotechnology* 12:69–75.
- Lee CD, Renner KA, Penner D, Hammerschmidt R, Kelly JD. 2005. Glyphosate-resistant soybean management system effect on *Sclerotinia* stem rot. *Weed Technology* 19:580–588.
- Legere A, Schreiber MM. 1989. Competition and canopy architecture as affected by soybean (*Glycine max*) row width and density of redroot pigweed (*Amaranthus retroflexus*). *Weed Science* 37:84–92.
- Leibold K, Baumel P, Wisner B, McVey M. 2001. Brazil's soybean production—production inputs. *AgDecision Maker* 5:1–3.
- Marking S. 1997. All-out effort to cut costs is under way. *Soybean Digest* February: 20–22.
- McRae CF, Stevens GR. 1990. Role of matrix of *Colletotrichum orbiculare* in pathogenesis of *Xanthium spinosum*. *Mycological Research* 94:890–896.

- Millhollon RW, Berner DK, Paxson LK, Jarvis BB, Bean GW. 2003. *Myrothecium verrucaria* for control of annual morningglories in sugarcane. *Weed Technology* 17:276–283.
- Mintz AS, Heiny DK, Weidemann GJ. 1992. Factors influencing the biocontrol of tumble pigweed (*Amaranthus albus*) with *Aposphaeria amaranthi*. *Plant Disease* 76:267–269.
- Norsworthy JK. 2004. Soybean canopy formation effects on pitted morningglory (*Ipomoea*), common cocklebur (*Xanthium strumarium*), and sicklepod (*Senna obtusifolia*) emergence. *Weed Science* 52:954–960.
- Nice GRW, Buehring NW, Shaw DR. 2001. Sicklepod (*Senna obtusifolia*) response to shading, soybean (*Glycine max*) row spacing, and population in three management systems. *Weed Technology* 15:155–162.
- Paul ND, Jacobson RJ, Taylor A, Wargent JJ, More JP. 2005. The use of wavelength-selective plastic cladding materials in horticulture: understanding of crop and fungal responses through the assessment of biological spectral weighting functions. *Photochemistry and Photobiology* 81:1052–1060.
- Quimby PC Jr, Fulgham FE, Boyette CD, Connick WJ Jr. 1989. An invert emulsion replaces dew in biocontrol of sicklepod – a preliminary study. In: Hovde D, Beestman GB, editors. *Pesticide formulations and application systems*. West Conshohocken, PA: American Society for Testing Materials. pp 267–270.
- Rankins A Jr., Byrd JD, Blaine A, Poston D, Shaw DR. 2006. Soybean management strategies for sicklepod. Available: <http://msucares.com/pubs/infosheets/is1024.pdf#search='sicklepod%20rankins'>
- Sandrin TR, TeBeest DO, Weidemann GJ. 2003. Soybean and sunflower oils increase the infectivity of *Colletotrichum gloeosporioides* f.sp. *aeschynomene* to northern jointvetch. *Biological Control* 26:244–252.
- Steele RGD, Torrey JH, Dickey DA. 1997. Multiple comparisons. *Principles and procedures of statistics—a biometrical approach*. New York: McGraw-Hill.
- Tuite J. 1969. *Plant pathological methods: fungi and bacteria*. Minneapolis, MN: Burgess Press. p 239.
- Walker HL, Boyette CD. 1985. Biocontrol of sicklepod (*Cassia obtusifolia*) in soybeans (*Glycine max*) with *Alternaria cassiae*. *Weed Science* 33:212–215.
- Walker HL, Tilley AM. 1997. *Myrothecium verrucaria* from sicklepod (*Senna obtusifolia*) as a potential mycoherbicide agent. *Biological Control* 10:104–112.
- Windels CE, Burnes PM, Kommendahl T. 1988. Five-year preservation of *Fusarium* species in silica gel and soil. *Phytopathology* 78:107–109.
- Yang SM, Jong SC. 1995. Factors influencing pathogenicity of *Myrothecium verrucaria* isolated from *Euphorbia esula* on species of *Euphorbia*. *Plant Disease* 79:998–1002.
- Zidak NK, Backman PA, Shaw JJ. 1992. Promotion of bacteria infection of leaves by an organosilicone surfactant: implications for biological weed control. *Biological Control* 2:111–117.