



# Biocontrol of Hemp Sesbania with *Colletotrichum truncatum* Microsclerotia Formulated in 'Pesta' Granules



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## ABSTRACT

Hemp sesbania [*Sesbania exaltata* (Raf.) Rydb. ex A.W. Hill] was controlled in soybean [*Glycine max* (L.) Merr.] field test plots over a two-year testing period (1995-1996) with microsclerotia of the bioherbicide fungus, *Colletotrichum truncatum* (Schw.) Andrus & Moore, formulated in wheat gluten-kaolin granules called 'Pesta'. Weed control averaged 84 and 88%, respectively, in plots treated pre-plant incorporated (PPI) at 'Pesta' rates of 168 or 336 kg/ha, and 71 and 78%, respectively, in plots treated pre-emergence (PE) at 'Pesta' rates of 168 or 336 kg/ha over the testing period. Post-emergence (POE) control averaged 30 and 50%, respectively, for the 168 and 336 kg/ha treatments, and was significantly less effective than either PE or PPI treatments. Although pathogenesis and mortality occurred in hemp sesbania tissues, satisfactory weed control was not achieved in plots treated at rates of 17 or 84 kg/ha with any of the application methods. Soybean yields were significantly greater in test plots treated PPI or PE, as compared to yields from test plots treated either POE, with inert 'Pesta' granules, or from untreated controls. Microsclerotia formulated in 'Pesta' granules exhibited excellent shelf-life, retaining high viability after storage for 10 years at 4°C. These results suggest that microsclerotia of *C. truncatum* formulated in 'Pesta' granules offer an effective method for controlling this important weed and preserving the activity of this bioherbicide.

## INTRODUCTION

Hemp sesbania is a vigorous, nodulating leguminous weed in soybean, cotton (*Gossypium hirsutum* L.) and rice (*Oryza sativa* L.), capable of reaching heights of 3 m (Lorenzi and Jeffery, 1987). It ranks as one of the 10 most troublesome weeds in the Delta Regions of Arkansas, Louisiana, and Mississippi (Dowler, 1997), reducing crop seed yield by shading and competition (King and Purell, 1997). Hemp sesbania is a prolific seed producer, yielding up to 21,000 seed/plant (Loveless and Oliver, 2000). Populations of 0.8 to 12.9 hemp sesbania plants/m emerging with soybean reduced the yield of mid-row (1 m) soybean 10 to 80% when allowed to interfere throughout the growing season (McWhorter and Anderson, 1979). Previous research has shown that conical formulations of the fungus *C. truncatum* are effective in controlling hemp sesbania under field conditions (Boyette et al., 1993b). In addition to producing mycelium and conidia in soil and liquid culture, *C. truncatum* can also be induced to produce structures called microsclerotia; compact, dense aggregates of darkly pigmented, thick-walled hyphal cells that can remain dormant for long periods until conditions favorable for growth occur. *C. truncatum* microsclerotia can be produced in submerged liquid culture and stored in diatomaceous earth (Jackson & Schisler, 1994). Pre-emergence soil application of the dry microsclerotia/diatomaceous earth formulation infected and killed hemp sesbania seedlings in growth chamber studies. Dried microsclerotia formulated with pre-gelatinized corn flour and pre-gelatinized cornstarch prompted disease development in emerging hemp sesbania seedlings (Jackson et al., 1996). When conditions are favorable (20-30°C, moist soil), *C. truncatum* microsclerotia "germinate" and form actively growing mycelia and conidia that infect stems and root surface cells of hemp sesbania, or directly invade roots through wounds (Schisler and Jackson, 1996).

To effectively apply the fungus in the field, a formulation that is biologically effective and also stable in storage is needed. Excellent shelf-life resulted when *C. truncatum* microsclerotia were encapsulated in granules made with wheat flour and kaolin clay ('Pesta') (Connick et al., 1997). Shelf-life was at least one year at 25°C when a minimum of 7 microsclerotia/granule were encapsulated. The objective of this study was to determine if microsclerotia of *C. truncatum* formulated with 'Pesta' could effectively control hemp sesbania in soybean under field conditions.

## MATERIALS AND METHODS

**Inoculum production.** The isolate of *C. truncatum* used in this study is deposited at the NCAUR, Peoria, IL (NRRL No. 18434). The medium used for microsclerotia production (Jackson et al., 1996) contained per L: glucose, 80 g; vitamin-free casamino acids, 13.2 g; KH<sub>2</sub>PO<sub>4</sub>, 2.0 g; CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.4 g; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.3 g; CoCl<sub>2</sub>·6H<sub>2</sub>O, 37 mg; FeSO<sub>4</sub>·7H<sub>2</sub>O, 5.0 mg; MnSO<sub>4</sub>·H<sub>2</sub>O, 16 mg; ZnSO<sub>4</sub>·7H<sub>2</sub>O, 14 mg; thiamin, riboflavin, pantothenic, niacin, pyridoxamine, thiotic acid, 500 µg each; folic acid, 50 µg; thiamin B<sub>12</sub>, 50 µg each. The media had an initial pH of 5.5, and pH was not controlled during culture growth. The fermentation was conducted in a 30-L fermenter (20-L working volume) at 28°C, with an aeration rate of 10-L air/min at 250 RPM. Antifoam (HODAG FD-62) was used to control foaming as required. Microsclerotia were separated from 11-day-old shake flask cultures and 18-day-old fermenter cultures by leaving the culture broth through a 40-mesh (425 µm) screen and an 80-mesh (180 µm) screen. Microsclerotia that collected on the 80-mesh screen (180-425 µm) were used for all studies. Slaved microsclerotia were rinsed and collected in deionized water. Excess deionized water was decanted from settled microsclerotia, and 5% (w/v) diatomaceous earth (HYFLO®) was added to the microsclerotia suspension. The microsclerotia-diatomaceous earth mixture was vacuum-filtered with a Buchner funnel, and the filter cake was broken up, placed in shallow glass trays, and air-dried overnight at 22°C. The moisture content of dried microsclerotia preparations was determined with a Mark I moisture analyzer. Dried microsclerotia formulations (<5% moisture) were placed in zip-lock bags and stored at 4°C.

'Pesta'-*C. truncatum* microsclerotia granules were prepared by modifying methods described previously (Connick et al., 1991; Boyette et al., 1993b). Briefly, the dried microsclerotia formulation was suspended in

23 ml sterilized distilled water and added to an 80:20 (w:w) mixture of semolina (durum) wheat flour, and kaolin. The mixture was kneaded into a dough, pressed flat, folded 5x, and extruded through a table-mounted pasta maker. The sheets (ca. 15-25 cm wide; 1.0-1.5 mm thick) were air-dried on screens for 3 days at 28°C and 50-60% relative humidity (RH). The dried sheets were broken, milled, and sieved to obtain 14-18 mesh (10-14 mm) granules, and stored at 4°C. The final dried product contained ca. 7 microsclerotia/granule. Previous work has shown that a 'Pesta'-*C. truncatum* microsclerotia granule such as this would produce from 1.3-4.5 x 10<sup>8</sup> conidia (Connick et al., 1997).

**Greenhouse bioassay of granules.** Hemp sesbania seeds were mechanically scarified and planted in a commercial potting mixture supplemented with a controlled-release (14-14-14, N:P:K) fertilizer contained in peat strips (12, 5.5 cm<sup>2</sup> pots/strip). Seeding rates was 3 seeds/strip, to a total of 36 seeds/strip. 'Pesta'-*C. truncatum* microsclerotia granules were applied either PPI, PE or POE at rates of 1.0 g of granules/pot. POE applications were made when seedlings were in the cotyledonary-to-first leaf growth stage. Control pots were treated with 'Pesta' granules *sans* fungus. Following inoculation, the plants were incubated on sub-irrigated greenhouse trays and monitored for 14 days for disease development and mortality. Greenhouse temperatures were 28 to 32°C, with 60 to 90% RH; day lengths were 12 h with an average of 1850 µmol/m<sup>2</sup>/sec photosynthetically active radiation at midday. Surviving plants were excised at the soil line, oven dried for 48 h at 85°C, weighed, and percentages of biomass reductions were determined. Treatments were replicated three times with 36 individual seedlings in each replication. The test was repeated twice, and data were averaged. A RCB experimental design was used. Data received arc sin transformation prior to ANOVA. Significant differences were determined using Fisher's Protected Least Significant Difference (FLSD) at P=0.05.

**Field experiments.** Field experiments were conducted on a Dundee very fine sandy loam (Aerie Ochraqualf) soil in June 1995 and June 1996 at the USDA-ARS, Southern Weed Science Experimental Farm, Stoneville, MS. T-Rifluralin was applied PPI to the test area at a broadcast rate of 1.12 kg/ha to control grass weeds. Plots consisted of four rows of 'Centennial' soybeans, 12.2 m long and 1 m apart, with the two center rows treated. Scarified hemp sesbania seed was planted at a density of about 100 seeds/m of row. Treatments consisted of: 'Pesta'-*C. truncatum* microsclerotia granules applied PPI, PE, or POE at 17, 84, 168, or 336 kg/ha, a herbicide control (acifluorfen), applied POE at 1.1 kg/ha, and an untreated control. In plots receiving PPI and PE applications, treatments were made on planting dates of June 6, 1995 and June 13, 1996, respectively. PE and PPI treatments were applied with a tractor-mounted fertilizer spreader. POE applications were applied manually when hemp sesbania seedlings were in the cotyledonary-to-first leaf growth stage. Applications were made in 46-cm bands to the treated center 2 rows of each plot. Percentage data of hemp sesbania controlled and biomass reductions were determined in randomly selected 6, 1-m subplots from each treated plot 4 weeks after treatment. The experiments were arranged as RCB experimental designs with four replications. Data over the two years were averaged received arc sin transformation prior to ANOVA. Significant differences were determined using FLSD at P=0.05.

## RESULTS AND DISCUSSION

**Viability of granules.** *C. truncatum* microsclerotia, encapsulated in 'Pesta' granules, began to germinate and were discernible about 8 to 10 h after granules were placed on moistened filter paper and incubated at 25°C. Fungal growth (conidia and mycelia) was visually evident after 24 h (Figure 1) and propagule viability in these granule tests exceeded 95% (data not shown). In our present study we found that *C. truncatum* microsclerotia formulated in 'Pesta' granules were still viable, and possessed high viability even after 10 years' storage at 4°C (Figure 2).



Figure 1. Microsclerotia (dark particles) on 'Pesta' granules.

Figure 2. Conidia (crescent-shaped structures) after incubation on water agar for 72 h at 28°C; granules had been stored for 10 years at 4°C.

**Bioassay of granules.** With PE and PPI treatments, weed mortality and biomass reduction of hemp sesbania seedlings were 94 and 96%, respectively after 14 days (Table 1). Emerging seedlings were killed as a result of infection of root and hypocotyl tissues, at or below the soil line, by fungal spores produced by microsclerotia. POE treatments were significantly less effective with only 76 and 78% weed control and biomass reduction, respectively.

Table 1. Biocontrol of hemp sesbania in the greenhouse with *C. truncatum* microsclerotia 'Pesta' granules, 14 days after treatment

Application Method	Weed Mortality (%)	Biomass Reduction (%)	Soybean yield (kg/ha)
PPI	96 a	96 a	1444 c
PE	94 a	94 a	1840 c
POE	76 b	78 b	2185 b
'Pesta' alone	0 c	0 c	1405 e
Untreated	0 c	0 c	1448 e

Application rates were the equivalent of ca. 100 kg/ha. Means within a column followed by the same letter are not different according to FLSD at P=0.05.

**Field experiments.** Hemp sesbania was effectively controlled in plots treated PPI or PE, receiving the higher rates (168 and 336 kg/ha) of inoculum during both years of these experiments. Hemp sesbania control and soybean yields in plots treated PPI at 336 kg/ha were statistically equivalent to plots treated with acifluorfen (336 kg). As in greenhouse tests, emerging hemp sesbania plants were infected by *C. truncatum* conidia produced from microsclerotia (Figure 3). Lesions formed near the soil line, eventually girdling and killing infected plants (Figure 3). As in greenhouse experiments, POE applications were significantly less effective than PE or PPI during both years (Table 2).

Table 2. Biocontrol of hemp sesbania in soybean field test plots treated with *C. truncatum* microsclerotia formulated in 'Pesta' granules

Application Method	Weed Control (%)	Biomass reduction (%)	Soybean yield (kg/ha)	
PPI	87	7 h	13 f	1444 c
17	84	38 f	45 d	1840 c
168	84 bc	90 ab	90 ab	2200 b
336	88 ab	94 ab	94 ab	2385 ab
PE	17	1 h	4 fg	1400 e
84	40	4 f	44 d	1858 c
168	71 d	76 c	76 c	2042 bc
336	78 c	81 bc	81 bc	2185 b
POE	17	4 h	6 fg	1465 e
84	26 g	30 e	30 e	1785 d
168	30 g	31 e	31 e	1756 d
336	50 e	54 d	54 d	1820 c
Acifluorfen	94 ab	96 a	96 a	2495 a
Weed-free control	100 a	100 a	100 a	2555 a
Untreated	3 h	4 g	4 g	1405 e
'Pesta' alone	0 h	0 f	0 f	1448 e

PPI = pre-plant incorporated; PE = pre-emergence; POE = post-emergence. Means within a column followed by the same letter are not different according to FLSD at P=0.05.



Figure 3. Hemp sesbania infection and control in soybean field plots by *C. truncatum*.

The rates of 'Pesta' granules required for acceptable weed control probably exceed rates acceptable for practical use and for commercial development of *C. truncatum* as a bioherbicide. However, less than 0.5% of the total weight of the granules is due to the active ingredient (microsclerotia), and the remainder due to the flour and kaolin components, unspent medium, and moisture. Thus, in field treatments receiving 'Pesta' granules at 168 and 336 kg/ha, the active ingredient rate would be >0.84 and >1.68 kg/ha, respectively.

Lighter inert fillers, more critical measurements of media, and use of drier granules could reduce the total weight of the formulated product. Another and possibly better approach to reduce the granule rates is the use of smaller granules. The granules used in these experiments were 14-to-18 mesh (ca. 1.0-1.4 mm dia.). In field treatments receiving 168 and 336 kg/ha, this amounts to 0.35 and 0.70 granules/cm<sup>2</sup>, respectively. At these same rates with granules 18 to 30-mesh (0.60-1.0 mm dia.) these values are 0.94 and 1.87 granules/cm<sup>2</sup>, respectively. Previous work has shown that close proximity of mycoherbicide granules with target weeds is critical for infection and weed kill (Boyette and Walker, 1985). The use of smaller granules could improve the weed control potential via better coverage of soil and/or plant surfaces.

Contrary to this idea, there is some evidence that producing 'Pesta' granules of smaller size reduces fungal viability, possibly as a result of susceptibility to heat convection, mechanical injury to fungal propagules, or both (Connick et al., 1991). Also one report indicated reduced viability of the bioherbicide fungus *Passarula cyrenaria* formulated in similar small-sized 'Pesta' granules (Hebar et al., 1998). One solution to these problems is the use of a formulation production system that is less damaging to the bioherbicide organisms, such as that offered by extrusion technology. Research has shown that an extrusion system can produce smaller, more uniformly shaped granules that retain high fungal viability (Daigle et al., 1997).

The present study has demonstrated the successful use of soil application of microsclerotia of the bioherbicide *C. truncatum* formulated in 'Pesta' granules to effectively control hemp sesbania, one of the most widespread and troublesome weeds in the Mississippi Alluvial Plain (Dowler, 1997). Most bioherbicide research efforts to date, have been directed towards the use of pathogens that promote disease above the soil surface (Templeton and Heiny, 1990; Charudattan, 1991; TeBeest, 1991; Rosskopf et al., 1999; Charudattan, 2001). However, significant progress has been made using soilborne pathogens as bioherbicides (Boyette et al., 1984; Weidemann and Templeton, 1988; Boyette et al., 1993a, 1993b, 1993c, 1993d; Charudattan, 2000). For soilborne bioherbicides, short term fluctuations in environmental conditions, such as soil moisture and temperature, are less critical because the soil acts as a buffer to resist the rapid environmental changes often encountered above ground (Weidemann et al., 1995). The versatility of *C. truncatum* to control hemp sesbania with fungal spores in liquid carrier adjuvants, or as granules containing spores, microsclerotia, or mycelial fragments should make this weed pathogen an attractive candidate for commercial bioherbicide development.

## ACKNOWLEDGEMENTS

The authors thank Jimmy R. McAlpine, Terry A. Newton, Albert J. Tidwell, and Angela R. Payne for valuable technical assistance.

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