

SHORT COMMUNICATION



## Extending the host range of the bioherbicidal fungus *Colletotrichum gloeosporioides* f. sp. *aeschynomene*

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### ABSTRACT

Spore formulations of the bioherbicidal fungus *Colletotrichum gloeosporioides* f. sp. *aeschynomene* (ATCC No. 20358) (CGA) were evaluated for control of three weed species: northern jointvetch (*Aeschynomene virginica*), Indian jointvetch (*A. indica*), and hemp sesbania (*Sesbania exaltata*) in greenhouse experiments. Mortality, dry weight reduction, and plant height reduction of *A. virginica* seedlings ranged from 98% to 100%, 15 days after inoculation with CGA in water, in an invert emulsion, or in Silwet L-77 surfactant. However, CGA in water caused no effects of these parameters on *S. exaltata*, and only slight effects on *A. indica*. *A. indica* and *S. exaltata* were also severely injured (mortality, dry weight and plant height reduction, 98–100%) by CGA in the invert emulsion or in Silwet L-77. The CGA in Silwet formulation incited severe disease development more rapidly than the invert emulsion or water formulations of CGA in all species. These results suggest that the host range of CGA can be expanded through formulation modification to enable this bioherbicide to control multiple weeds, thus making this product more economically acceptable.

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Northern jointvetch [*Aeschynomene virginica* (L.) B.S.P], Indian jointvetch (*A. indica* L.), and hemp sesbania [*Sesbania exaltata* (Raf.) Rydb. ex A.W. Hill] are three of the most economically important weeds in current southern U.S. rice production (Shivrain, Burgos, Moldenhauer, McNew, & Baldwin, 2006). These weeds also occur in other crops in the southern U.S. (Dowler, 1992). Herbicides are necessary to provide effective weed control in crops, such as rice. However, in situations where certain weeds are not controlled, they can become major problems. For example, the Clearfield® rice production system has become the predominant rice production system in the mid-south rice producing states of Arkansas, Louisiana, Mississippi, and Missouri (Shivrain et al., 2006). This system uses natural rice mutants with tolerance to imazethapyr [2-(4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl)-5-ethyl-3-pyridinecarboxylic acid] (Newpath®). This herbicide controls many broadleaf and grassy weeds (including red rice) but has little-or-no activity on northern jointvetch, Indian jointvetch, or hemp sesbania, which can result in massive infestations of these weeds if other weed control

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measures are not utilised (Scott, Meins, & Smith, 2005). This nearly complete lack of control of these weeds creates a significant need for effective weed control measures for these troublesome weeds.

A formulation of a strain of the fungus, *Colletotrichum gloeosporioides* f. sp. *aeschynomene* (Penz) Sacc. (ATCC No. 20358; CGA) received U.S. Environmental Protection Agency (US-EPA) registration in 1982 as a commercial biological herbicide (Collego®) for controlling the leguminous weed, northern jointvetch (Templeton, Smith, & TeBeest, 1989). CGA effectively controls northern jointvetch in rice and irrigated soybean fields by inducing anthracnose lesions on plant stems that girdle the stem, eventually killing infected plants (Sandrin, TeBeest, & Weidemann, 2003; Templeton et al., 1989). Host range tests indicated that while CGA was highly virulent to northern jointvetch, only slight, non-lethal infection occurred to Indian jointvetch, a closely related species also problematic in southern U.S. rice fields. Several other crop and weed species were considered 'immune' to infection by CGA (Daniel, Templeton, Smith, & Fox, 1973). However, subsequent research revealed that CGA was also pathogenic, with varying degrees of virulence, toward several *Aeschynomene* spp. (including *A. indica*), as well as some leguminous crop species (TeBeest, 1988). Although narrow host specificity of a bioherbicidal fungus can be beneficial from biological and perhaps US-EPA registration perspectives, this trait may restrict bioherbicidal agents from practical and commercial perspectives.

Previous research has shown that invert (water – in – oil) emulsions provide a method to retard evaporation and trap water in a spray mixture, thereby decreasing the amount of additional free – moisture required for spore germination and infection to occur (Amsellem, Sharon, & Gressel, 1991; Boyette, Hoagland, & Stetina, 2018; Boyette, Quimby, Bryson, Egley, & Fulgham, 1993; Quimby, Fulgham, Boyette, & Connick, 1989). We also found that this emulsion formulation infected and controlled hemp sesbania under greenhouse and field conditions, (Boyette, Bowling, Vaughn, Hoagland, & Stetina, 2010; Boyette, Gealy, Hoagland, Vaughn, & Bowling, 2011). Although the research on bioherbicides formulated in invert emulsions revealed that they were effective in controlling various weeds, some required specialised spray equipment and were restricted to ground application. Therefore, we sought to develop a CGA formulation that required no specialised spray equipment and that could perhaps be applied aerially to these three problematic weeds in rice.

Preliminary research indicated that Indian jointvetch and hemp sesbania were infected and killed by CGA (ATCC No. 20358) formulated in the surfactant Silwet L-77 {nonoxynol (0–10 POE) [ $\alpha$ -(p-nonylphenyl)- $\omega$ -hydroxypoly(oxyethylene)]} (OSi Specialties Inc., Danbury, CT, USA). Lesion formation and disease manifestation on these species were similar to that observed on northern jointvetch inoculated with CGA spores formulated in water (Boyette, unpublished). Tests of Koch's postulates (Koch, 1893) revealed that CGA was responsible for the infection. The purpose of the research reported herein was to determine if the host range of CGA could be expanded to control other weeds, specifically Indian jointvetch and hemp sesbania through the use of a CGA + Silwet L-77 surfactant formulation, and to compare formulations of CGA in water, invert emulsion, and Silwet L-77 for various weed control parameters, specifically plant mortality, plant biomass reduction, and plant height reduction.

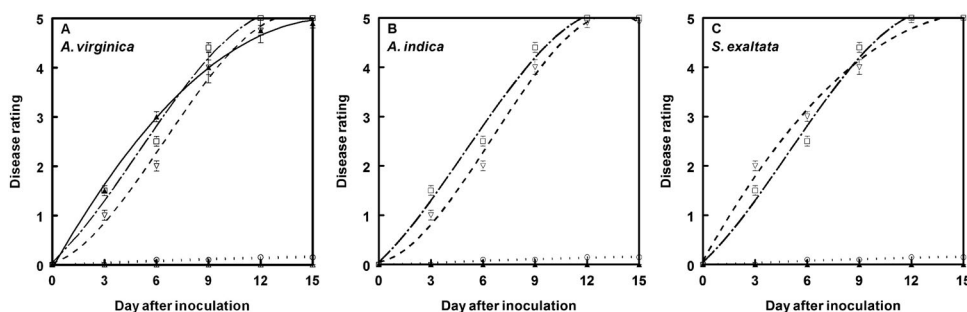
The CGA culture (ATCC No. 20358; the same CGA strain in Lockdown™ bioherbicide) used in the present studies was obtained from D. K. Cartwright, Agricultural Research Initiatives, Fayetteville, AR, USA. CGA was maintained on Emerson's YpSs agar (Difco Laboratories, Detroit, MI, USA) at 28°C on open-mesh wire shelves of an incubator (Precision Scientific Inc., Chicago, IL, USA). Twelve-hour photoperiods were provided by two 20-W, cool-white fluorescent lamps positioned in the incubator door. Light intensity at dish level was  $\sim 200 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  photosynthetically active radiation (PAR) as measured with a light metre (LI-COR Inc., Lincoln, NE, USA). Cultures were maintained by transferring to Emerson's YpSs agar every 5–7 days. Inoculum for all experiments was produced in liquid culture in modified Richards' medium containing V-8 vegetable juice (Campbell Soup Co., Camden, NJ, USA; Daniel et al., 1973), either in shaken Erlenmeyer flasks at 28°C and 125 RPM, or in laboratory fermenters (Models 214-E and 230-C; New Brunswick Scientific Co. Inc., Talmadge, NJ, USA) under similar temperature and agitation regimes.

Hemp sesbania seeds were obtained from Azlin Seed Co., Leland, MS, USA; northern jointvetch and Indian jointvetch seeds were collected near Stuttgart, AR. All seeds were surface-sterilized in 0.05% NaOCl for 5 min, rinsed with sterile distilled water, and germinated on moistened filter paper in petri dishes. After the seeds germinated ( $\sim 48$  h) they were planted in a commercial potting mix (Jiffy-mix; Jiffy Products of America, Batavia, IL, USA) contained in peat strips. Each strip contained 12 plants. The potting mix was supplemented with a controlled-release (14:14:14, NPK) fertiliser (Osmocote; Grace Sierra Horticultural Products, Milpitas, CA, USA). The plants were placed in sub-irrigated trays that were mounted on greenhouse benches. Greenhouse temperatures ranged from 25°C to 30°C with 40–90% relative humidity (RH). The photoperiod was approximately 14 h, with 1600–1700  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  PAR as measured at midday with a light metre.

The treatments utilised were: (1) untreated control; (2) water control; (3) invert control; (4) Silwet L-77 surfactant control; (5) CGA in water suspension; (6) a 1:1 (v:v) aqueous CGA suspension/invert emulsion; and (7) CGA in 0.20% (V:V) Silwet L-77 surfactant:distilled water. CGA spore concentrations were  $2.0 \times 10^6$  spores  $\text{ml}^{-1}$ . The composition of the invert emulsion was identical to that used previously to investigate control of hemp sesbania with the bioherbicidal fungus *Colletotrichum truncatum* (Schw.) Andrus & Moore (Boyette et al., 1993). All components of the invert emulsion were newly purchased and used to prepare fresh invert emulsions for each experiment. Spray application rates ( $\sim 100 \text{ L ha}^{-1}$ ) were made with a pressurised backpack sprayer (Spray Doc, Model 101P; Gilmour Mfg., Somerset, PA, USA). Following treatments, seedlings were placed in dew chambers (Model I-36 DL; Percival Scientific Inc., Perry, IA, USA) at 28°C, 100 RH for 12 h in darkness, and then placed on greenhouse benches. Photoperiods were 14 h with 820–840  $\mu\text{E m}^{-2} \text{ s}^{-1}$  PAR with a RH of 65%. Plants were monitored at three-day intervals for disease progression studies over a 15-day period after treatment. A visual disease severity rating scale (per plant basis) (Sandrin et al., 2003) was used to estimate disease progression, where 0 = no disease, 1 = 1–25% disease, 2 = 26–50% disease, 3 = 51–75% disease, 4 = 76–99% disease, and 5 = plant death. Disease ratings of 4–5 were considered 'severe'. Percent mortality, dry weight reductions, and plant height reductions were determined 15 days after treatment (DAT). Surviving plants were excised at the soil line, oven-dried for 48 h at 85°C, weighed, and the percent biomass reduction was determined. Treatments were replicated four times, for a total of 48 individual plants per treatment. The

experiment was repeated over time, and data were averaged following Bartlett's test for homogeneity of variance. A randomised complete block experimental design was utilised. The mean percentage of plant mortalities, biomass and plant height reductions were calculated for each treatment and were subjected to Arcsin transformation. The transformed data were statistically compared using analysis of variance (ANOVA) at the 5% probability level. Values are presented as the means of replicated experiments. Data were analysed via the PROC MIXED function of SAS v9.3 (SAS Institute, Cary, NC, USA) using a least significant difference of 0.05. In the disease progression studies, data were analysed using standard mean errors (SEM) and best-fit regression analysis.

No visual injury symptoms were observed on plants of the three species in the untreated, water or surfactant treatments during a 15-day time course (Figure 1 A, B and C). The invert alone treatment caused only minor injury (<0.20 disease rating) on each of the three species. The CGA in Silwet L-77 formulation incited severe disease development more rapidly (9 DAT) than the CGA in water or invert emulsion (12 DAT) (Figure 1). No plant re-growth occurred in any of these weed species that were severely infected. Mortality, dry weight reduction and plant height reduction of *A. virginica* seedlings ranged from 98% to 100% when treated with CGA in water or in the invert emulsion, while 100% mortality occurred in all disease parameters with CGA in Silwet L-77 surfactant, 15 DAT (Table 1). Mortality, dry weight and plant height reduction (98–100%) of *A. indica* and *S. exaltata* was achieved with CGA in the invert emulsion or in Silwet L-77. However, CGA in water caused no effects on these parameters on *S. exaltata*, and only slight effects (0% mortality, 5% dry weight reduction, and 8% plant height reduction) on *A. indica*. This confirms the findings of other research, that CGA formulated in water incites only minor disease on *A. indica* (Daniel et al., 1973; TeBeest, 1988). No injury resulted on any of the plant species sprayed with either water or Silwet L-77. Some injury (2–3% mortality, 5–7% dry weight reduction, and 5–7% plant height



**Figure 1.** Disease progression of CGA infecting: (A) *A. virginica*; (B) *A. indica*; (C) *S. exaltata* over a 15-day period after spray application of fungal spores prepared in various formulations, under greenhouse conditions. Error bars represent  $\pm 1$  SEM.

Note: Regression equations with  $R^2$  values are as follows: (A)  $\circ$ : Invert,  $Y = -0.01 + 0.02X$ ,  $R^2 = 0.97$ ;  $\square$ : CGA + Silwet,  $Y = 0.05 + 0.33X + 0.04X^2$ ,  $R^2 = 0.98$ ;  $\Delta$ :  $H_2O$ ,  $Y = 0$ ,  $R^2 = 1.00$ ;  $\nabla$ : CGA + Invert,  $Y = 0.03 + 0.11X + 0.06X^2$ ,  $R^2 = 0.98$ ;  $\blacktriangle$ : CGA +  $H_2O$ ,  $Y = -0.07 + 0.63X - 0.02X^2$ ,  $R^2 = 0.98$ ;  $\bullet$ : untreated,  $Y = 0$ ,  $R^2 = 1.00$ ;  $\blacktriangledown$ : Silwet,  $Y = 0$ ,  $R^2 = 1.00$ . (B)  $\circ$ : Invert,  $Y = -0.01 + 0.02X$ ,  $R^2 = 0.96$ ;  $\square$ : CGA + Silwet,  $Y = 0.06 + 0.33X + 0.04X^2$ ,  $R^2 = 0.98$ ;  $\Delta$ :  $H_2O$ ,  $Y = 0$ ,  $R^2 = 1.00$ ;  $\nabla$ : CGA + Invert,  $Y = 0.05 + 0.07X + 0.07X^2$ ,  $R^2 = 0.98$ ;  $\blacktriangle$ : CGA +  $H_2O$ ,  $Y = 0$ ,  $R^2 = 1.00$ ;  $\bullet$ : untreated,  $Y = 0$ ,  $R^2 = 1.00$ ;  $\blacktriangledown$ : Silwet,  $Y = 0$ ,  $R^2 = 1.00$ . (C)  $\circ$ : Invert,  $Y = -0.01 + 0.02X$ ,  $R^2 = 0.96$ ;  $\square$ : CGA + Silwet,  $Y = 0.05 + 0.33X + 0.04X^2$ ,  $R^2 = 0.99$ ;  $\Delta$ :  $H_2O$ ,  $Y = 0$ ,  $R^2 = 1.00$ ;  $\nabla$ : CGA + Invert,  $Y = 0.07 + 0.63X - 0.02X^2$ ,  $R^2 = 0.98$ ;  $\blacktriangle$ : CGA +  $H_2O$ ,  $Y = 0$ ,  $R^2 = 1.00$ ;  $\bullet$ : untreated,  $Y = 0$ ,  $R^2 = 1.00$ ;  $\blacktriangledown$ : Silwet,  $Y = 0$ ,  $R^2 = 1.00$ .

**Table 1.** Effect of various adjuvants on biological control of three leguminous weeds with *Colletotrichum gloeosporioides* f. sp. *aeschynomene* (CGA).

Treatment	Weed Species								
	<i>A. virginica</i>			<i>A. indica</i>			<i>S. exaltata</i>		
	Mortality (%) <sup>a</sup>	Dry weight reduction (%)	Plant height reduction (%)	Mortality (%)	Dry weight reduction (%)	Plant height reduction (%)	Mortality (%)	Dry weight reduction (%)	Plant height reduction (%)
Untreated	0 c	0 c	0 c	0 c	0 c	0 c	0 c	0 c	0 c
H <sub>2</sub> O	0 c	0 c	0 c	0 c	0 c	0 c	0 c	0 c	0 c
Invert	3 c	5 c	7 bc	3 c	7 bc	8 bc	2 c	5 c	8 bc
Silwet L-77	0 c	0 c	0 c	0 c	0 c	0 c	0 c	0 c	0 c
CGA/H <sub>2</sub> O	98 a	99 a	99 a	0 c	5 c	8 bc	0 c	0 c	0 c
CGA/Invert	100 a	100 a	100 a	98 a	98 a	98 a	100 a	100 a	100 a
CGA/Silwet L-77	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a

<sup>a</sup>Values followed by the same letter do not differ significantly at  $P=0.05$  using Fisher's Least Significant Difference.

reduction) was observed on plants treated with the invert emulsion alone. Similar findings indicating that the invert emulsion alone can result in slight injury to various plant species has been reported (Boyette et al., 2018).

Increasing the pathogenicity of a bioherbicide fungus, should theoretically increase the effectiveness of the pathogen (Yang & TeBeest, 1993). CGA provides excellent control of northern jointvetch (Sandrin et al., 2003; Yang & TeBeest, 1993). Although the registration of this effective bioherbicide (Collego®) expired, it was re-registered with US-EPA in 1997, and more recently, this fungus was newly registered as Lockdown® and Lockdown Retro® for the control of northern jointvetch (Cartwright, Boyette, & Roberts, 2010). The results from our findings in this report indicate that it may also be possible to also control Indian jointvetch and hemp sesbania with this bioherbicide fungus, using a simplified formulation consisting of CGA in a surfactant (e.g. Silwet L-77). Because northern jointvetch, Indian jointvetch, and hemp sesbania emerge at similar rates, and infection by CGA manifests at similar rates, the need for multiple bioherbicide applications may not be necessary. Since this formulation would allow for control of all three of these troublesome weeds, this product may become more economically acceptable to rice producers. This is the first report of the alteration of the host range of a fungus using a surfactant-based formulation. Further research will focus on testing other adjuvants (surfactants) and evaluation of these CGA formulations for control of these three weeds under field conditions. The effects of novel formulations of CGA will also be examined on some crops, to assure safety.

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## Disclosure statement

No potential conflict of interest was reported by the authors.

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