

Induction of infection in *Sesbania exaltata* by *Colletotrichum gloeosporioides* f. sp. *aeschynomene* formulated in an invert emulsion

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Abstract In greenhouse experiments, an invert emulsion (MSG 8.25) was tested with spores of the mycoherbicide fungus *Colletotrichum gloeosporioides* f. sp. *aeschynomene*, a highly virulent pathogen of the leguminous weed *Aeschynomene virginica* (northern jointvetch), but considered “immune” against another leguminous weed, *Sesbania exaltata* (hemp sesbania). A 1:1 (v/v) fungus/invert emulsion mixture resulted in 100% infection and mortality of inoculated hemp sesbania seedlings over a 21 day period. Microscopic examinations revealed that the fungus proliferated within the cells of hemp sesbania and produced anthracnose lesions containing acervuli on infected stems. The fungus was reisolated and found to infect and kill northern jointvetch seedlings, thus fulfilling Koch’s postulates for disease identification. These results suggest that this invert emulsion expands the host range of *C. gloeosporioides* f. sp. *aeschynomene*, and possibly improves the bioherbicide potential of this pathogen.

Keywords Bioherbicide · Biological weed control · *Colletotrichum gloeosporioides* f. sp. *aeschynomene* · Invert emulsion · Mycoherbicide · *Sesbania exaltata*

Introduction

A formulation of a strain of the fungus, *Colletotrichum gloeosporioides* f. sp. *aeschynomene* (Penz) Sacc. (CGA; ATCC No. 20358), for controlling the weed *Aeschynomene virginica* (L.) B.S.P. (northern jointvetch) received U.S.

Environmental Protection Agency (US-EPA) registration in 1982 as a commercial biological herbicide under the trade-name Collego® (Templeton et al. 1989). It was re-registered with US-EPA in 1997 and more recently, this fungus was newly registered under the trade-name Lock-down™ for the control of northern jointvetch (Cartwright et al. 2008). The fungus induces anthracnose lesions on northern jointvetch that increase in severity over a several week period under field conditions, eventually killing infected weeds as the lesions formed by the fungus girdle the stem (Sandrin et al. 2003).

The fungus effectively (and selectively) controls northern jointvetch in rice and irrigated soybean fields (TeBeest 1985; Templeton et al. 1989). Host range tests originally indicated that this strain of CGA was highly virulent against northern jointvetch, while several other crop and weed species were considered “immune” to infection by CGA (Daniel et al. 1973). However, TeBeest (1988) later found that CGA was also pathogenic, with varying degrees of virulence, to seven of thirteen *Aeschynomene* spp., as well as several other leguminous spp., such as some cvs. of *Lathyrus*, *Lupinus*, *Pisum*, and *Vicia*. No weed species other than *Aeschynomene* spp. were included in this report.

Although narrow host specificity of a bioherbicide fungus may be beneficial from both biological and perhaps US-EPA registration perspectives, this trait may restrict bioherbicide agents from practical and commercial standpoints (Cartwright 2008, personal communication). Research has shown that it is possible to alter the host ranges of some fungal pathogens through formulation-based approaches using an invert emulsion formulation (Amsellem et al. 1990, 1991; Boyette and Abbas 1994). The purpose of the present research was to determine if the host range of CGA could be expanded through a formulation-based approach. More specifically, an invert

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emulsion formulation [designated MSG 8.25] (Quimby et al. 1989; Boyette et al. 1993) was selected for testing, based on preliminary results (Boyette et al. 1991). Those experiments showed that that hemp sesbania [*Sesbania exaltata* (Rydb.) ex. A.W. Hill], a troublesome leguminous weed in Southern U.S. rice fields, originally reported as immune to infection by CGA (Daniel et al. 1973), could be controlled by CGA when spores were formulated in an invert emulsion.

Materials and methods

Accession, culture, and maintenance of the fungus

The CGA culture used in the present studies was obtained from Cartwright, Agricultural Research Initiatives, Fayetteville, AR, USA. The fungus 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 $\sim 200 \mu\text{mol}/\text{m}^2/\text{s}$ photosynthetically active radiation (PAR) as measured with a light meter (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.

Greenhouse experiments

Hemp sesbania seeds (Azlin Seed Co., Leland, MS, USA) and northern jointvetch seeds (collected near Stuttgart, AR, USA) were surface-sterilized in 0.05% NaOCl for 5 min, rinsed with sterile distilled water, and germinated on moistened filter paper. After the seeds germinated (~ 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) fertilizer (Osmocote; Grace Sierra Horticultural Products, Milpitas, CA, USA). The plants were placed in subirrigated trays that were mounted on greenhouse benches. Greenhouse temperatures ranged from 25 to 30°C with 40–90% relative humidity (RH). The photoperiod was ~ 14 h, with 1800 PAR as measured at midday with a light meter.

The treatments were: (1) CGA spores in water suspension; (2) a 1:1 (v:v) aqueous spore suspension/invert emulsion; (3) an invert emulsion control, (4) wound-inoculated, and (5) an untreated control. 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). The oil phase of the invert emulsion consisted of a paraffinic oil (Orchex 797; Exxon Corp., Baytown, TX, USA; 777.5 g l^{-1}), a monoglyceride emulsifier (Myverol 18-99; Eastman Chem. Prod., Inc., Kingsport, TN, USA; 14.5 g l^{-1}), household paraffin wax (Strohmeier & Arpe Co., Inc., Short Hills, NJ, USA; 74.25 g l^{-1}), and lanolin (93 g l^{-1}). A stable invert emulsion was formed when equal parts of the oil phase and water phase were combined and stirred briskly by hand for 2–3 min. Inoculum densities for all treatments containing the fungal component were adjusted to 5.0×10^7 spores ml^{-1} with the aid of a hemacytometer. Spray application rates were $\sim 100 \text{ l ha}^{-1}$, and were made with a pressurized backpack sprayer (Spray Doc, Model 101P; Gilmour Mfg., Somerset, PA, USA).

Plants that received wound-inoculations were treated by scratching and injecting CGA spores (ca. 1 μl , at a concentration of 5×10^7 spores ml^{-1}) in sterile distilled water into hemp sesbania stems using a sterile syringe (25 G \times 5/8, Terumo Medical Corp., Somerset, NJ, USA). Control plants were wound-inoculated as described with sterile distilled water only. Following treatments, seedlings were placed in darkened dew chambers (Model I-36 DL; Percival Sci. Ind., Perry, IA, USA) at 28°C, 100 RH for 12 h, and then placed on greenhouse benches. Plants were monitored at 3 day intervals for disease kinetic studies over a 21-day period.

A subjective 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 = complete plant death. Percent control, plant height and biomass reductions were determined after 21 days. 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 (Steele et al. 1997). A randomized complete block experimental design was utilized. The mean percentage of plant mortalities, plant height reductions, and biomass 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. 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 kinetic studies, data were analyzed using standard mean errors and best-fit regression analysis.

Light microscopy and immunocytochemistry

Visual examination indicated that stems of hemp sesbania seedlings (treated 9 days earlier with CGA/invert emulsion) were cut into thin slices with a razor blade and fixed in 3% (v/v) glutaraldehyde in 0.05 M PIPES buffer (pH 7.4) for 2 h at room temperature (22–23°C). Stem pieces were then washed twice (15 min each) in cold PIPES buffer and dehydrated in a graded series of ethanol. After reaching 100% ethanol, the samples were transferred to –20°C. LR White resin (Sigma–Aldrich Co., St. Louis, MO, USA) was added in 25% increments, with a 1 day interval at each step, to finally attain 100% resin. Specimens were then transferred to a rocking shaker at room temperature for an additional 24 h. Individual pieces of infiltrated material were then transferred to flat-bottomed BEEM capsules and polymerized at 55°C for 2 h.

Tissue sections were obtained with a Delaware Histo-knife (Delaware Diamond Knives, Inc., Lancaster, PA, USA) on a Reichert Ultracut E ultramicrotome (Reichert Inc., Depew, NY, USA) set at 0.35 µm, and dried onto chrome-alum-coated slides on a slide-warming tray. Sections for light microscopic examination were stained for 10–30 sec with 1% (w/v) toluidine blue in 1% (w/v) sodium borate on the warming tray, washed with distilled water, mounted with Permount (Fisher Sci. Inc., Pittsburg, PA, USA), and observed with a Zeiss photomicroscope (Carl Zeiss Inc., Oberkochen, Germany). Images were collected using an Olympus Q-Color 3 digital camera (Olympus America, Inc., Melville, NY, USA).

Results and discussion

Visual examinations indicated that stem lesions began to occur on hemp sesbania plants that had been inoculated with the CGA/invert emulsion formulations 3–6 days after treatment (DAT). Samples of infected stems were collected 9 DAT, and preserved for microscopic analyses as described previously, while the remaining samples were collected for testing, according to Koch's Postulates (1893) for disease confirmation (Tuite 1969), and for disease severity as described previously. In the disease kinetic studies, only data from the CGA/invert treatment are presented. A polynomial regression curve provided the best fit, with an R^2 value of 0.98. Hemp sesbania seedlings treated

with CGA/invert emulsion were severely injured 9 DAT (≥ 3.5 disease rating), and the disease continued to progress until complete mortality (5.0 disease rating) occurred in all plants inoculated with the CGA/invert emulsion treatment 21 DAT (Fig. 1).

The fungus produced typical anthracnose lesions on hemp sesbania stems, with acervuli scattered throughout the lesions. The acervuli were glabrous (non-setose) and were slightly sunken in the host tissue (Fig. 2a). Masses of spores accumulated on the upper surface of the acervulus, breaking the epidermal layer and cuticle (Fig. 2b, c, e). The spores were possessed characteristics (size and shape) of CGA spores (Daniel et al. 1973). The disease symptomatology was similar to that occurring on northern jointvetch infected by *C. gloeosporioides* f. sp. *aeschynomene* (Daniel et al. 1973; Sandrin et al. 2003). Stem tissues of untreated hemp sesbania are shown in Fig. 2d.

The fungus was readily re-isolated from diseased hemp sesbania tissue, and it sporulated abundantly when diseased samples (~ 5 cm²) were surface sterilized (0.5% NaOCl), rinsed in sterile distilled water, and placed on potato-dextrose agar (PDA, Difco Laboratories, Detroit, MI, USA) containing the antibiotics streptomycin (50 µg ml⁻¹) and chloramphenicol (50 µg ml⁻¹). When re-inoculated onto healthy northern jointvetch seedlings, the fungus was highly virulent, causing 100% mortality of northern jointvetch plants within 7 days, while the un-inoculated control plants remained healthy (data not shown). Koch's postulates (1893) maintain, that for disease causation to be

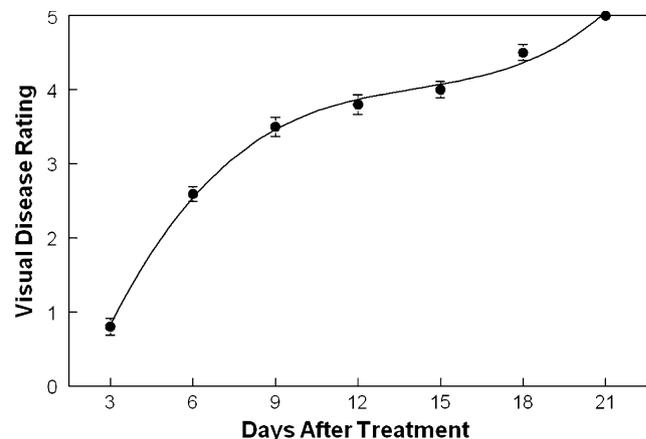


Fig. 1 Disease progression of *Colletotrichum gloeosporioides* f. sp. *aeschynomene* infecting *S. exaltata*. A subjective visual disease severity rating scale (Sandrin et al. 2003) was used to estimate disease progression where: 0 no disease, 1 1–25% disease, 2 26–50% disease, 3 51–75% disease, 4 76–99% disease, and 5 complete plant death. Symptomatology was considered “severe” at ratings of 3.5–5.0. Inoculum densities for all treatments containing the fungal component were adjusted to 5.0×10^7 spores ml⁻¹ using a hemacytometer. The relationship is best described by the equation $Y = -1.99 + 3.47 - 0.700 + 0.050$, $R^2 = 0.98$. Error bars represent ± 1 SEM

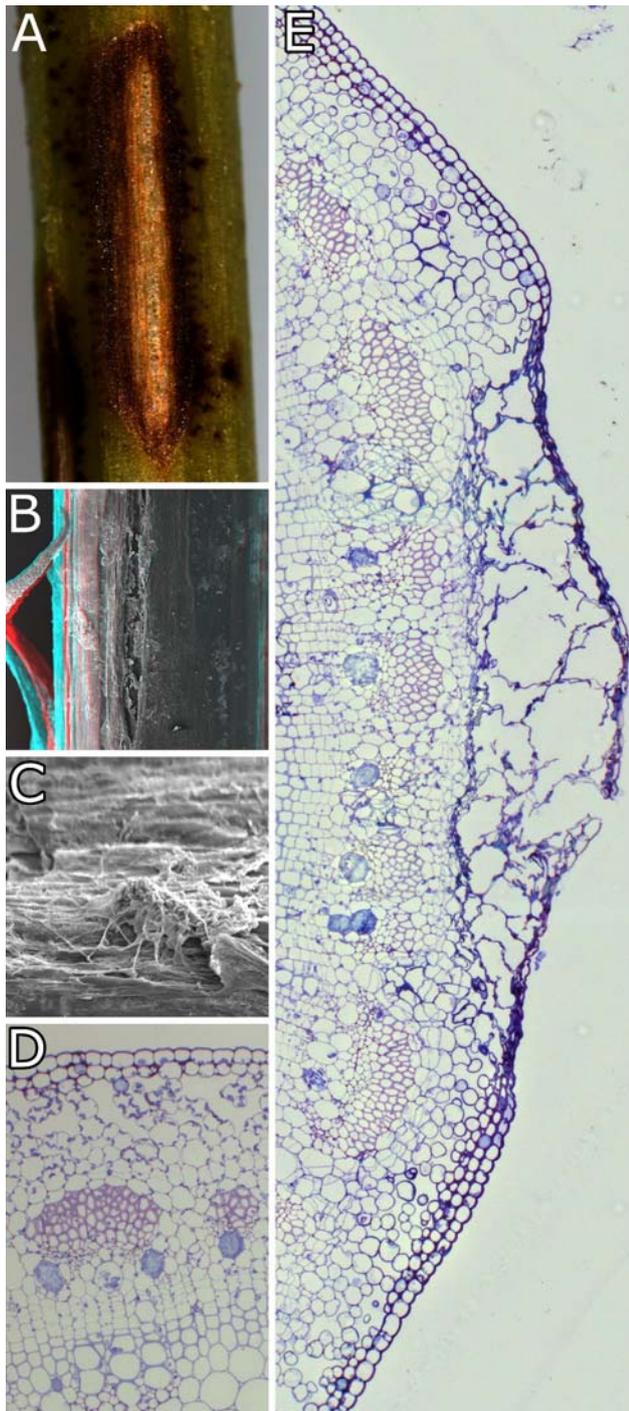


Fig. 2 Infection of hemp sesbania stem incited by *Colletotrichum gloeosporioides* f. sp. *aeschyromene* formulated in an invert emulsion. **a** sunken anthracnose lesion surrounded by masses of CGA spores; **b, c** scanning electron microscopic images of anthracnose lesions on hemp sesbania stems; **d** light microscopic image of untreated cross-section of hemp sesbania stem showing intact epidermis; **e** light microscopic image of anthracnose lesion on hemp sesbania stem showing disrupted epidermal cells and intercellular hyphae

established, it is necessary to (1) isolate the microbe from an organism that has become infected with the disease, (2) re-inoculate the causal agent to a healthy host where it incites the same disease symptomatology, and (3) the causal agent must be isolated again. Since Koch's postulates were fulfilled in the studies in this report, we conclude that CGA is pathogenic to hemp sesbania.

Hemp sesbania seedlings were controlled 100% 21 DAT when spores were formulated in the invert emulsion (Table 1). Plant biomass (dry weights) and plant heights were also greatly reduced by the CGA/invert emulsion treatments, with only slight reductions occurring with the invert emulsion alone treatment (Table 1). No mortality, biomass, or plant height reduction occurred on hemp sesbania seedlings that were inoculated either with the fungus in water, or in wound-inoculated plants (Table 1).

Research has shown that the virulence of several plant pathogenic fungi can be enhanced through formulation-based approaches. For example, several vegetable oils have enhanced the biological weed control efficacy of various weed: pathosystems (Mintz et al. 1992; Auld 1993; Boyette 1994; Yang and Jong 1995; Yang et al. 1998; Sandrin et al. 2003; Boyette et al. 2007). Womack and Burge (1993) suggested that vegetable oils may increase spore germination, germ-tube elongation, and appressorium formation, and protect the spores and germ tubes from adverse environmental conditions, as well as possibly reducing host resistance factors. Similarly, invert emulsions have also been utilized to improve bioherbicide efficacy and performance of several mycoherbicidal fungi. (Quimby et al. 1989; Boyette et al. 2007). Amsellem et al. (1990) found that the host specificities of *Alternaria cassiae* Juriar. & Khan and *A. crassa* (Sacc.) Rands were expanded, and that a saprophytic *Cephalosporium* species became pathogenic

Table 1 Effect of Formulation on Biological Control of *Sesbania exaltata* with *Colletotrichum gloeosporioides* f. s. *aeschyromene*

Treatment ^a	Mortality (%) ^b	Plant Height Reduction (%)	Dry Weight Reduction (%)
CGA/INV	100 a	100 a	100 a
CGA/H ₂ O	0 c	0 c	0 c
CGA/wound	0 c	0 c	0 c
INV	0 c	5 bc	8 b
H ₂ O	0 c	0 c	0 c
Untreated	0 c	0 c	0 c

^a Values followed by the same letter are not significantly different ($p = 0.05$) according to Fisher's least significant difference

^b *Sesbania exaltata* seedlings averaged 10 cm in height at time of treatment; plant mortality, plant height reduction, and dry weight reduction were measured at 21 DAT

to sicklepod [*Senna obtusifolia* (L.) Irwin & Barneby] when formulated in an invert emulsion (Amsellem et al. 1991).

We have previously shown that it is possible to alter the host selectivity of *A. crassa*, a mycoherbicide for jimsonweed (*Datura stramonium* L.), by the addition of water-soluble filtrates of jimsonweed or dilute fruit pectin to spore suspensions (Boyette and Abbas 1994). Several plant species, including hemp sesbania, that were either immune to, or exhibited a hypersensitive reaction to the fungus alone exhibited various degrees of susceptibility following these amendments (Boyette and Abbas 1994).

Plant pathogenic fungi have been classified as either biotrophic, necrotrophic, or hemibiotrophic (Vanderplank 1982). Biotrophic fungi (such as rust fungi), are often highly specific, but rarely incite rapid plant tissue damage and death. Necrotrophic fungi (such as *Rhizoctonia* spp.), usually have much broader host ranges than biotrophs, and are often highly virulent, resulting in relatively rapid and often extremely severe plant tissue necrosis. Many *Colletotrichum* spp. (including *C. gloeosporioides*) are considered by some mycologists and epidemiologists to be hemibiotrophic fungi, having an initial biotrophic phase (presumed to convey narrow host specificity), followed by a necrotrophic phase (inciting extensive tissue death; Vanderplank 1982; Dodd et al. 1992; Wei et al. 1997). This combination of desirable plant disease traits may account for the fact that *Colletotrichum* spp. are commonly found on lists of promising mycoherbicides, and that several are in various stages of development (Watson et al. 2000; Charudattan 2005; Weaver et al. 2007).

In addition to hemibiotrophs, some species, such as *C. capsici* display ‘subcuticular, intramural’ necrotrophy, in which the fungal hyphae initially grow within the cell walls of the host epidermal cells, before proliferating rapidly through the tissue that is killed (O’Connell et al. 2000). *Colletotrichum gloeosporioides* follows both strategies, depending on the host plant, while other *Colletotrichum* spp. have been shown to form long-term biotrophic, or quiescent relationships with their host plants (Perfect et al. 1999).

In the infection process of *Colletotrichum gloeosporioides* f. sp. *malvae* (Penz.) Penz. & Sacc. in Penz., a biological control agent of round-leaved mallow (*Malva pusilla* Sm.; Makowski and Mortensen 1992) there are indications that fungal genes coding for pectinase, arylsulfatase, and catalase (involved in nutrition and degradation of host materials) as well as protein kinase, which is involved in the regulation of fungal metabolism, may play a role in fungal-plant disease interactions. Additionally, plant genes encoding actin and glutathione *S*-transferase may also be involved in host reactions to the invading

fungus. These genes exhibit different patterns of expression during penetration, biotrophy, and necrotrophy, demonstrating the complexity of the infection process (Goodwin 2001).

In the present report, CGA, when formulated in an invert emulsion, behaves as described above, as a biotrophic plant pathogen (possibly exhibiting subcuticular, intramural necrotrophy) during its various infection stages in hemp sesbania. Further histological studies are currently underway that will further elucidate the infection process of hemp sesbania by CGA.

The inoculum densities (5×10^7 spores ml⁻¹) used in these experiments were much higher as compared to the inoculum densities in the original host range evaluation (1×10^6 spores ml⁻¹; Daniel et al. 1973; Sandrin et al. 2003). However, because CGA spores in water had no effect on inoculated hemp sesbania seedlings (Table 1), it appears that the high inoculum density is not responsible for this host range alteration. Future research will examine CGA spore germination on host and non-host plants as related to infectivity, disease progression, and spore concentration.

A possible explanation for this host range alteration phenomenon, is that the invert emulsion [or component(s)] could incite phytotoxin(s) production by the fungus, and/or promote entry and translocation of phytotoxins in this host. Another plausible explanation is that hydrolytic enzymes (e.g., proteolytic enzymes) are produced by CGA in response to the invert emulsion [or a component(s)] that break down plant tissues and promote infection. This is a focus of ongoing and future research. A better understanding of the biochemical and ultrastructural events as they relate to disease promotion and infection processes may provide insight into developing biological control agents with improved traits, such as expanded host range and higher virulence for optimized weed control. Future research will be needed to evaluate closely related plant species to assure that host range expansion does not result in injury to desirable plant species.

References

- Amsellem Z, Sharon A, Gressel J, Quimby PC (1990) Complete abolition of high threshold of two mycoherbicides (*Alternaria cassiae* and *Alternaria crassa*) when applied in invert emulsion. *Phytopathology* 80:925–929
- 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* 81:985–988
- Auld BA (1993) Vegetable oil suspension emulsions reduce dew dependence of a mycoherbicide. *Crop Protect* 12:477–479
- Boyette CD (1994) Unrefined corn oil improves the mycoherbicide activity of *Colletotrichum truncatum* for hemp sesbania (*Sesbania exaltata*) control. *Weed Technol* 8:526–528

- Boyette CD, Abbas HK (1994) Host range alteration of the bioherbicidal fungus *Alternaria crassa* with fruit pectin and plant filtrates. *Weed Sci* 42:487–491
- Boyette CD, Abbas HK, Smith RJ Jr (1991) Invert emulsions alter host specificity of biocontrol fungi. *Proc Am Phytopathol Soc* 18:126
- 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 Sci* 41:497–500
- 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 Biol & Manage* 7:70–76
- Cartwright KD, Boyette CD, Scott R, Cartwright RD, Hoagland RE, Stetina KC, Weaver MA (2008) Return of the mycoherbicide Collego to mid-south rice fields: Lockdown 2008. *Abstr Weed Sci Soc Am* 51:136
- Charudattan R (2005) Ecological, practical, and political inputs into selection of weed targets: what makes a good biological control target? *Biolog Control* 35:183–196
- Daniel JT, Templeton GE, Smith RJ Jr, Fox WT (1973) Biological control of northern jointvetch in rice with an endemic fungal disease. *Weed Sci* 21:303–307
- Dodd JC, Estrada A, Jeger MJ (1992) Epidemiology of *Colletotrichum gloeosporioides* in the tropics. In: Bailey JA, Jeger MJ (eds) *Colletotrichum: biology, pathology and control*. CAB International, Wallingford, pp 308–325
- Goodwin PH (2001) A molecular weed—mycoherbicide interaction: *Colletotrichum gloeosporioides* f. sp. malvae and round-leaved mallow, *Malva pusilla*. *Can J Plant Pathol* 23:28–35
- Koch R (1893) “Über den augenblicklichen Stand der bakteriologischen Choleradiagnose” (in German). *Zeit Hygiene Infektionskrankheiten* 14:319–333
- Makowski RMD, Mortensen K (1992) The first mycoherbicide in Canada: *Colletotrichum gloeosporioides* f.sp. malvae for round-leaved mallow control. In: *Proceedings of 1st international weed control conference weed science society of Victoria*, vol 2, pp 298–300
- Mintz AS, Heiny DK, Weidemann GJ (1992) Factors influencing the biocontrol of tumble pigweed (*Amaranthus albus*) with *Aposphaeria amarantii*. *Plant Dis* 76:267–269
- O’Connell RJ, Perfect S, Hughes B, Carzaniga R, Bailey J, Green J (2000) Dissecting the cell biology of *Colletotrichum* infection processes. In: Prusky D, Freeman S, Dickman MB (eds) *Colletotrichum—host specificity, pathology and host–pathogen interactions*. APS Press, St. Paul, pp 57–77
- Perfect SE, Hughes B, O’Connell RJ, Green JR (1999) *Colletotrichum*—a model genus for studies on pathology and fungal-plant interactions. *Fungal Gen Biol* 27:186–198
- 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 (eds) *Pesticide formulations and application systems*. American Society for Testing Materials, West Conshohocken, pp 267–270
- Sandrin TR, TeBeest DO, Weidemann GJ (2003) Soybean and sunflower oils increase the infectivity of *Colletotrichum gloeosporioides* f.sp. *aeschynomene* to northern jointvetch. *Biolog Control* 26:244–252
- Steele RGD, Torrey JH, Dickey DA (1997) *Multiple comparisons. Principles and procedures of statistics—a biometrical approach*. McGraw Hill, New York, 365 pp
- TeBeest DO (1985) Techniques for testing and evaluating plant pathogens for weed control. *J Agric Entomol* 2:123–129
- TeBeest DO (1988) Additions to the host range of *Colletotrichum gloeosporioides* f. sp. *aeschynomene*. *Plant Dis* 72:16–18
- Templeton GE, Smith RJ Jr, TeBeest DO (1989) Perspectives on mycoherbicides two decades after discovery of the Collego pathogen. In: Delfosse ES (ed) *Proceedings of the VII international symposium on the biological control of weeds 6–11 March 1988, Rome*. Ist Sper Patol Veg (MAF), Rome, pp 553–558
- Tuite J (1969) *Plant pathological methods: fungi and bacteria*. Burgess Press, Minneapolis, 239 pp
- Vanderplank JE (1982) *Host-pathogen interactions in plant diseases*. American phytopathological society, 1984. Academic Press, New York 207 pp
- Watson AK, Gressel J, Sharon A, Dinoor A (2000) *Colletotrichum* strains for biological control. In: Prusky D, Freeman S, Dickman MB (eds) *Colletotrichum: host specificity, pathology and host-pathogen interactions*. American Phytopathological Society Press, St. Paul, pp 245–265
- Weaver MA, Lyn ME, Boyette CD, Hoagland RE (2007) Bioherbicides for weed control. In: Updhyaya MK, Blackshaw RE (eds) *Non-chemical weed management*. CABI, Internat, Cambridge, pp 93–110
- Wei YD, Byer KN, Goodwin PH (1997) Hemibiotrophic infection of round-leaved mallow by *Colletotrichum gloeosporioides* f. sp. malvae in relation to leaf senescence and reducing reagents. *Mycol Res* 101:357–364
- Womack GJ, Burge MN (1993) Mycoherbicide formulation and the potential for bracken control. *Pest Sci* 37:337–341
- Yang S, Jong SC (1995) Factors influencing pathogenicity of *Myrothecium verrucaria* isolated from *Euphorbia esula* on species of *Euphorbia*. *Plant Dis* 79:998–1002
- Yang S, Dowler WM, Schaad NW, Connick WJ Jr (1998) Method for the control of weeds with weakly virulent or non-virulent plant pathogens. US Patent No. 5,795,845