

Infection and Control of Hemp Sesbania by *Colletotrichum gloeosporioides* f. sp. *aeshynomene* Formulated in an Invert Emulsion.



C. D. Boyette, A. J. Bowling, K. C. Vaughn, R. E. Hoagland, and K. C. Stetina
USDA-ARS, Southern Weed Science Research Unit, Stoneville, Mississippi.



ABSTRACT

Colletotrichum gloeosporioides f. sp. *aeshynomene* (CGA) is a highly virulent pathogen of the leguminous weed northern jointvetch [*Aeschynomene virginica* (L.) B.S.P.], but another leguminous weed, hemp sesbania [*Sesbania exaltata* (Raf.) Rydb. ex A.W. Hill], is considered immune to this fungus. Research has shown that the host range of some fungi can be altered through formulation. In greenhouse studies, we found that a 1:1 (v/v) CGA:invert emulsion formulation resulted in 100% infection and mortality of 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 re-isolated from infected hemp sesbania tissues, and found to infect and kill northern jointvetch seedlings, thus fulfilling Koch's postulates for disease identification. These results, and preliminary results in field trials suggest that this invert emulsion expands the host range of *C. gloeosporioides* f. sp. *aeshynomene*, and improves the bioherbicide potential of this pathogen.

INTRODUCTION

The fungus *Colletotrichum gloeosporioides* f. sp. *aeshynomene* (Collego™) for controlling northern jointvetch received US-EPA registration in 1982 as a commercial biological (1), and recently registered under the trade-name Lockdown™ for controlling northern jointvetch (2). The fungus induces anthracnose lesions on northern jointvetch that increase in severity, eventually killing infected weeds as the lesions girdle the stem.

Early host range tests indicated that CGA was highly virulent against northern jointvetch, but several other crop and weed species were considered "immune" to infection by CGA (3). Tebeest et al. (4) later found that CGA also infected several other *Aeschynomene* spp., as well as several other leguminous spp. No weeds 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 may restrict biological control agents from practical and commercial standpoints. Research has shown that it is possible to alter the host ranges of some fungal pathogens through formulation-based approaches using invert emulsion formulations (5, 6). 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 emulsion formulation [designated MSG 8.25] (7, 8) was selected based on preliminary results (9) indicating that hemp sesbania, originally reported as immune to infection by CGA (3), could be infected and controlled by CGA when formulated in an invert emulsion.

MATERIALS AND METHODS

Culture and inoculum mass-production

The CGA culture used in the present studies was obtained from K. Cartwright, Agricultural Research Initiatives, Fayetteville, AR, USA. The fungus was maintained on Emerson's YpSs agar at 28°C. Inoculum for all experiments was produced in liquid culture in Richards' V-8 medium, either in shake flasks at 28°C and 125 RPM, or in laboratory fermenters under similar temperature and agitation regimes.

Greenhouse experiments

Hemp sesbania and northern jointvetch seed were surface-sterilized in 0.05% NaOCl for 5 min, rinsed with sterile distilled water, germinated on moistened filter paper, and planted in a commercial potting mix. The plants were placed in sub-irrigated trays mounted on greenhouse benches. Greenhouse temperatures ranged from 25 to 30°C with 40-90% RH. The treatments were as follows: 1) CGA spore/water suspension; 2) 1:1 aqueous spore suspension/invert emulsion; 3) invert emulsion control, 4) wound-inoculated, and 5) untreated control. The oil phase of the invert emulsion consisted of a paraffinic oil (Orchex 797; 777.5 g/L), monoglyceride emulsifier (Myverol 18-99; 14.5 g/L), paraffin (74.25 g/L), and lanolin (93g/L). A stable invert emulsion was formed when a 1:1 (v:v) oil phase:water phase were combined and stirred briskly 2-3 min. Inoculum densities for all treatments were adjusted to 5.0 x 10⁷ spores/ml. Spray rates were ~100 L/ha and made with a pressurized sprayer. Wound-inoculated plants were treated by scratching and injecting CGA spores (1 µl, 5 x 10⁷ spores/ml) in sterile distilled water into hemp sesbania stems using a sterile syringe. Control plants were inoculated as described with sterile distilled water only. Following treatments, seedlings were placed in dew chambers (28° C, 100 RH, 12 h), then placed on greenhouse benches, and monitored at three day intervals for disease progression for 21 d. A subjective visual disease severity rating scale (per plant basis) was used to estimate disease progression in which 0 = no disease, 1 = 1-25% disease, 2 = 26-50% disease, 3 = 51-75% disease, 4 = 76-99% disease, and 5 = complete plant death. Data were analyzed using standard mean errors and best-fit regression analysis.

Light microscopy and immunocytochemistry

Stems of hemp sesbania seedlings (treated 9 days earlier with CGA/invert) 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 was added in 25% increments, with a one day interval at each step, to finally attain 100% resin. Specimens were then transferred to a 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 Histoknife on a Reichert Ultracut E ultramicrotome 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 Permunt, and observed with a Zeiss photomicroscope. Images were collected using an Olympus Q-Color 3 digital camera.

RESULTS AND DISCUSSION

Stem lesions began to occur on hemp sesbania plants that had been inoculated with the CGA/invert formulations 3-6 DAT. Samples of infected stems were collected 9 DAT, and preserved for microscopic analyses as described previously, while the remaining samples were collected for submission to Koch's Postulates for disease confirmation (Tuite 1969) and for disease measurements 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 R² 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 to all plants inoculated with the CGA/invert emulsion treatment 21 DAT (Fig. 1).

The organism produced typical anthracnose lesions on hemp sesbania stems, with acervuli scattered throughout the lesions (Fig. 2a). 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). The spores were characteristic of CGA spores (Fig. 3a & b). The disease symptomatology was similar to that occurring on northern jointvetch infected by *C. gloeosporioides* f. sp. *aeshynomene* (Fig. 4a & b).

The fungus was readily isolated from diseased tissues and sporulated abundantly on PDA containing antibiotics. When re-inoculated onto healthy northern jointvetch seedlings, the fungus was highly virulent and killed plants within 5 days, while the un-inoculated controls were unaffected (data not shown). The fungus, subsequently isolated from these infected northern jointvetch seedlings, was identical in appearance and in growth habit to the original CGA inoculum. Since Koch's postulates (10) were fulfilled in this report, it appears that hemp sesbania is not 'immune' to infection by CGA as originally reported, but rather is 'weakly' susceptible to CGA. Future research will examine CGA spore germination on host and non-host plants as related to infectivity, disease progression, and spore concentration. It is also possible that the invert emulsion [or component(s)] could incite phytotoxin(s)

production by CGA. Another explanation is that hydrolytic enzymes (e.g., proteolytic enzymes) are produced by CGA in response to the invert emulsion that break down plant tissues and promote infection. This is a focus of ongoing and future research. A better understanding of the biochemical and ultra-structural 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.

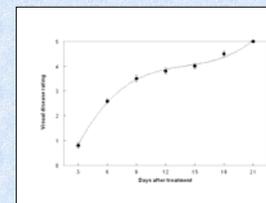


Figure 1. Disease progression of CGA infecting *S. exaltata*: 0 = no disease, 1 = 1-25% disease, 2 = 26-50% disease, 3 = 51-75% disease, 4 = 76-99% disease, and 5 = complete plant death. Symptomatology was considered "severe" at ratings of 3.5 - 5.0.

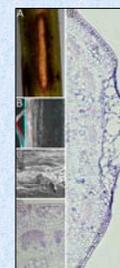


Figure 2. Infection of hemp sesbania stem incited by CGA formulated in an invert emulsion. A = sunken anthracnose lesion surrounded by masses of CGA spores; B & C = scanning electron microscopic images of anthracnose lesions on hemp sesbania stems; D = transmission electron microscopic image of untreated cross-section of hemp sesbania stem showing intact epidermis; E = transmission electron microscopic images of anthracnose lesion on hemp sesbania stem showing disrupted epidermal cells and intercellular hyphae.



Figure 3A. CGA colony of YpSs agar plate.

Figure 3B. CGA spores.



Figure 4A. Hemp sesbania stem infected and killed by CGA.

Figure 4B. Northern jointvetch lesion incited by CGA.

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