Formulation of Colletotrichum truncatum Microsclerotia for Improved Biocontrol of the Weed Hemp Sesbania (Sesbania exaltata)

MARK A. JACKSON,* BARUCH S. SHASHA,† AND DAVID A. SCHISLER*†

*Fermentation Biochemistry Research Unit, †Plant Polymer Research Unit, National Center for Agricultural Utilization Research, USDA, Agricultural Research Service, 1815 North University Street, Peoria, Illinois 61604

Received September 6, 1995; accepted February 27, 1996

Submerged cultures of the biocontrol fungus Colletotrichum truncatum produce high concentrations of microsclerotia (MS) when grown under specific nutritional conditions. Previous studies have shown that MS survived drying, remained viable following long-term storage at 4°C, and killed hemp sesbania seedlings when incorporated into potting soil. In this study, dried preparations of C. truncatum MS were encapsulated in wetted formulations of pregelatinized corn flour, pregelatinized cornstarch, or a mixture of the two (1:1). Germination rates of microsclerotia immediately after formulation and drying were not significantly different. After storage at 4°C for 18 months, MS germination rates were significantly lower for all formulations compared to nonformulated MS. Dried MS were capable of producing mycelia or spores upon germination when plated on water agar. After 3 days of growth on Noble agar, the MS-flour formulations produced significantly more conidia than those made with starch, starch-flour, or nonformulated MS. By the 10th day of incubation, significantly more conidia were produced in formulations containing starch-flour (~10-fold) and flour (~50-fold) than in MS-starch formulations and nonformulated MS. Bioassays showed that all MS preparations incorporated into potting soil incited disease in emerging hemp sesbania seedlings. A significantly higher incidence of disease was seen in seedlings grown in potting soil containing flour-formulated MS. These results suggest that corn flour formulations of C. truncatum microsclerotia can be used to increase the effectiveness of this bioherbicide for controlling the weed hemp sesbania and that increased conidial production may play a role in improving biocontrol efficacy.

KEY WORDS: microsclerotia; sclerotia; corn flour; cornstarch; bioherbicide; formulation; Colletotrichum truncatum, Sesbania exaltata.

INTRODUCTION

The "bioherbicide strategy" is a microbial approach being used to control weeds in agronomic crops (Templeton, 1982; Charudattan, 1991). This strategy involves treating weed-infested crops with an inundative application of microbial propagules of highly aggressive, host-specific pathogens of the target weed. Constraints to the commercial development of these agents include the lack of low-cost production methods, stable microbial formulations with extended shelf life, and consistent weed control under field situations. The development of low-cost methods for mass-producing stable bioherbicial propagules is a critical step in the commercialization of these products (Bowers, 1982). In general, submerged-culture fermentation is considered the most cost-effective method for producing microbial biocontrol agents (Stowell, 1991).

We have developed submerged-culture techniques for producing stable propagules of the bioherbicide agent Colletotrichum truncatum (Schw.) Andrus and Moore NRRL 18434, a specific fungal pathogen of the weed hemp sesbania [(Sesbania exaltata) (Raf.) Cory] Boyette, 1991a,b]. Nutritional and environmental factors have been optimized for the submerged culture production of C. truncatum conidia based on spore yield and efficacy in inciting disease on hemp sesbania (Jackson and Bothast, 1990; Schisler et al., 1991; Jackson and Schisler, 1992). Unfortunately, methods for storing C. truncatum conidia as dry preparations which retain viability have thus far eluded development (Jackson et al., 1992; Silman et al., 1993).

As an alternative to conidia, the microsclerotium (MS) of C. truncatum may be used for controlling hemp sesbania. Previous studies showed that, under specific nutritional conditions, high concentrations of C. truncatum microsclerotia were produced in liquid culture (Jackson and Bothast, 1990). When microsclerotia were mixed with diatomaceous earth, dried to 2–5% moisture, and stored at 4°C, more than 90% of 8-month-old MS germinated on water agar (Jackson and Schisler, 1995). In addition, soil-incorporated dried microsclerotia infected and killed emerging hemp sesbania seed-
lungs. Soil incorporation of *C. truncatum* MS may be advantageous because free-moisture requirement, a significant impediment to the use of foliar conidial applications, is less problematic in soil environments. Conversely, the quantity of soil-incorporated microsclerotia necessary to insure that hemp sesbania seedlings contact the pathogen and become infected may be an economic constraint.

Formulating microbial biocontrol agents with materials that provide exogenous nutrients has potential to improve biocontrol efficacy. Connick *et al.* (1991) formulated biomass from various fungal biological control agents, including *C. truncatum*, with a pasta-like formulation ("Pesta") of wheat flour (semolina) and kaolin. The Pesta process improved propagule stability and provided a granular formulation for delivery. Pesta formulated fungal biocontrol agents use the nutrient base provided by the wheat flour for growth and sporulation after application. Pregelatinized corn flour and cornstarch formulations have been shown to enhance the performance of various bioherbicides and bioinsecticides (Bothast *et al.*, 1993; McGuire and Shasha, 1990; Schisler *et al.*, 1992). Recent studies have shown that fungal biocontrol agents formulated with pregelatinized corn flour significantly reduced damping-off disease in pepper, eggplant, and zinnia caused by *Rhizoctonia solani* Kühn (J. A. Lewis, USDA-ARS, Beltsville, MD, personal communication). Pregelatinized corn flours and cornstarches are made by exposing flour or starch to steam for a short period of time, which partially gelatinizes the starch, and then drying the material. When wetted, these pregelatinized corn flours and cornstarches form gels which encapsulate (entrap) the microbial propagule, reducing exposure to high temperatures or extreme shifts in pH (McGuire and Shasha, 1992). The low cost of pregelatinized cornstarch (~$1.54/kg) and pregelatinized corn flour (~$0.44/kg) make the use of these commodities economically attractive as formulating agents.

In this study, we describe the effects of formulating *C. truncatum* microsclerotia with pregelatinized corn flour, pregelatinized cornstarch, and a starch:flour (1:1) mixture. Pregelatinized starch and pregelatinized flour differ principally in that flour contains ~10% protein and is less water soluble than starch. The influence of these different nutritional formulations was assessed in terms of MS viability after formulation, stability during storage, and biocontrol efficacy in inciting disease in emerging hemp sesbania seedlings.

**MATERIALS AND METHODS**

**Organism**

A strain of *C. truncatum* (NRRL 13737, Agricultural Research Service patent culture collection, NRRL 18434) was obtained from the USDA Agricultural Research Service, National Center for Agricultural Utilization Research Culture Collection (Peoria, IL). Stock cultures of *C. truncatum* were maintained and spore inocula were produced for liquid culturing of MS as previously described (Jackson and Bothast, 1990). Briefly, a single spore isolate of *C. truncatum* was grown and sporulated on potato dextrose agar (PDA). A 2-week-old culture was cut into 1-mm² pieces and the pieces were preserved in sterile 10% glycerol in cryovials at ~80°C. Approximately 150 cryovials were stored in this manner. Weekly, these stock cultures were used to inoculate PDA plates for spore inoculum production. PDA plates with 2-week-old *C. truncatum* cultures were rinsed with sterile deionized water to obtain spore inocula.

**Media and Culture Conditions**

The composition of the medium used for liquid-culture production of *C. truncatum* microsclerotia was glucose, 80 g/liter, and Casamino acids (vitamin-free; Difco Laboratories, Inc., Detroit, MI), 13.2 g/liter, in a basal salts medium with vitamins and trace metals, as previously described (Jackson and Bothast, 1990; Jackson and Schisler, 1995). Microsclerotia were produced in 1-liter Erlenmeyer flasks containing 100 ml of media. Sufficient conidial inoculum was added to provide submerged cultures with 5 × 10⁶ spores per milliliter. Cultures were grown at 28°C and 300 rpm in a rotary shaker–incubator (Innova 4000, New Brunswick Scientific, Edison, NJ). Flasks were hand-shaken frequently to inhibit mycelial growth on the flask wall.

**Harvesting, Drying, and Storing of Microsclerotia**

Microsclerotia were separated from 11-day-old liquid cultures of *C. truncatum* by sieving the culture broth through a 40-mesh (425-μm) screen and then through an 80-mesh (180-μm) screen. Fermentations yielded approximately 7.2 g/liter or 2 × 10⁶ MS/liter of sieved microsclerotia. Microsclerotia collected on the 180-μm screen (425–180 μm in size) were rinsed and suspended in distilled water. Excess water was removed from settled microsclerotia and diatomaceous earth (HYFLO, Celite Corp., Lompoc, CA) was added at 5% (w/v). The MS–diatomaceous earth mixture was vacuum-filtered with a Buchner funnel using Whatman No. 1 filter paper. The filtered cake was broken up, placed in shallow glass trays, and air-dried overnight in a biological containment hood. The moisture content of dried microsclerotial preparations [(wet weight − dry weight)/ wet weight × 100] was determined after drying with a moisture analyzer (Mark I, Denver Instruments, Arvada, CO). Dried microsclerotia–diatomaceous earth mixtures were placed in sealed, plastic bags and stored at 4°C.
Pregelatinized Starch and Pregelatinized Flour Formulations

Dried MS–diatomaceous earth mixtures were formulated with pregelatinized corn starch (Miragel, A. E. Staley, Decatur, IL), pregelatinized corn flour (No. 961, Illinois Cereal Mills, Paris, IL), or a pregelatinized starch:flour (1:1) mixture. To 10 g of dried MS–diatomaceous earth (1.7 x 10^6 MS/g) was added 25 ml water and 30 g of pregelatinized starch, pregelatinized flour, or starch:flour (15 g:15 g). The formulation was mixed with a mortar and pestle and air-dried overnight. Air-dried formulations were ground with a mortar and pestle and particles which passed a 20-mesh screen were collected and stored in sealed, plastic bags at 4°C. For negative controls, portions of the MS–diatomaceous earth mixtures and all the flour and starch formulations were heat-killed by incubating at 100°C for 12 h. None of the heat-killed MS preparations germinated when plated on PDA.

Analyses

Microsclerotia were observed microscopically using an inverted microscope (Olympus IM-2) at a magnification of 50×. Only microsclerotia larger than 180 µm and smaller than 425 µm were counted. During sampling, microsclerotial suspensions were constantly vortexed to ensure homogeneity. To quantify microsclerotia production, 100 µl of an appropriate dilution of the microsclerotial suspension was placed on a glass slide, overlaid with a coverslip, and the number of microsclerotia counted. Only well-formed hyphal aggregates with smooth margins were counted as microsclerotia.

Viability was assessed by spreading microsclerotiotal formulations over Noble water agar plates and incubating at 28°C for 24 h. One hundred microsclerotia were observed microscopically on water agar plates and the number of germinating microsclerotia was counted. Microsclerotia were counted only if discrete microsclerotia could be observed in the formulated granule.

Conidia production by formulated microsclerotia was determined by incubating the microsclerotial formulations, each containing 1.7 x 10^3 viable microsclerotia, on individual Noble water agar plates. After 3, 6, 10, and 14 days of incubation, duplicate water agar plates were flooded with 10 ml of sterile deionized water, scraped vigorously with a sterile, plastic inoculating loop, and swirled to produce a homogenous suspension. One milliliter of the suspension was placed on a glass slide, overlaid with a coverslip, and the number of visible microsclerotia counted. Only well-formed hyphal aggregates with smooth margins were counted as microsclerotia.

Viability was assessed by spreading microsclerotial formulations over Noble water agar plates and incubating at 28°C for 24 h. One hundred microsclerotia were observed microscopically on water agar plates and the number of germinating microsclerotia was counted. Microsclerotia were counted only if discrete microsclerotia could be observed in the formulated granule.

Conidia production by formulated microsclerotia was determined by incubating the microsclerotial formulations, each containing 1.7 x 10^3 viable microsclerotia, on individual Noble water agar plates. After 3, 6, 10, and 14 days of incubation, duplicate water agar plates were flooded with 10 ml of sterile deionized water, scraped vigorously with a sterile, plastic inoculating loop, and swirled to produce a homogenous suspension. One milliliter of the suspension was placed on a glass slide, overlaid with a coverslip, and the number of visible microsclerotia counted. Only well-formed hyphal aggregates with smooth margins were counted as microsclerotia.

Viability was assessed by spreading microsclerotial formulations over Noble water agar plates and incubating at 28°C for 24 h. One hundred microsclerotia were observed microscopically on water agar plates and the number of germinating microsclerotia was counted. Microsclerotia were counted only if discrete microsclerotia could be observed in the formulated granule.

Conidia production by formulated microsclerotia was determined by incubating the microsclerotial formulations, each containing 1.7 x 10^3 viable microsclerotia, on individual Noble water agar plates. After 3, 6, 10, and 14 days of incubation, duplicate water agar plates were flooded with 10 ml of sterile deionized water, scraped vigorously with a sterile, plastic inoculating loop, and swirled to produce a homogenous suspension. One milliliter of the suspension was placed on a glass slide, overlaid with a coverslip, and the number of visible microsclerotia counted. Only well-formed hyphal aggregates with smooth margins were counted as microsclerotia.

Viability was assessed by spreading microsclerotial formulations over Noble water agar plates and incubating at 28°C for 24 h. One hundred microsclerotia were observed microscopically on water agar plates and the number of germinating microsclerotia was counted. Microsclerotia were counted only if discrete microsclerotia could be observed in the formulated granule.

Conidia production by formulated microsclerotia was determined by incubating the microsclerotial formulations, each containing 1.7 x 10^3 viable microsclerotia, on individual Noble water agar plates. After 3, 6, 10, and 14 days of incubation, duplicate water agar plates were flooded with 10 ml of sterile deionized water, scraped vigorously with a sterile, plastic inoculating loop, and swirled to produce a homogenous suspension. One milliliter of the suspension was placed on a glass slide, overlaid with a coverslip, and the number of visible microsclerotia counted. Only well-formed hyphal aggregates with smooth margins were counted as microsclerotia.

Viability was assessed by spreading microsclerotial formulations over Noble water agar plates and incubating at 28°C for 24 h. One hundred microsclerotia were observed microscopically on water agar plates and the number of germinating microsclerotia was counted. Microsclerotia were counted only if discrete microsclerotia could be observed in the formulated granule.

Conidia production by formulated microsclerotia was determined by incubating the microsclerotial formulations, each containing 1.7 x 10^3 viable microsclerotia, on individual Noble water agar plates. After 3, 6, 10, and 14 days of incubation, duplicate water agar plates were flooded with 10 ml of sterile deionized water, scraped vigorously with a sterile, plastic inoculating loop, and swirled to produce a homogenous suspension. One milliliter of the suspension was placed on a glass slide, overlaid with a coverslip, and the number of visible microsclerotia counted. Only well-formed hyphal aggregates with smooth margins were counted as microsclerotia.

Viability was assessed by spreading microsclerotial formulations over Noble water agar plates and incubating at 28°C for 24 h. One hundred microsclerotia were observed microscopically on water agar plates and the number of germinating microsclerotia was counted. Microsclerotia were counted only if discrete microsclerotia could be observed in the formulated granule.

Conidia production by formulated microsclerotia was determined by incubating the microsclerotial formulations, each containing 1.7 x 10^3 viable microsclerotia, on individual Noble water agar plates. After 3, 6, 10, and 14 days of incubation, duplicate water agar plates were flooded with 10 ml of sterile deionized water, scraped vigorously with a sterile, plastic inoculating loop, and swirled to produce a homogenous suspension. One milliliter of the suspension was placed on a glass slide, overlaid with a coverslip, and the number of visible microsclerotia counted. Only well-formed hyphal aggregates with smooth margins were counted as microsclerotia.

Viability was assessed by spreading microsclerotial formulations over Noble water agar plates and incubating at 28°C for 24 h. One hundred microsclerotia were observed microscopically on water agar plates and the number of germinating microsclerotia was counted. Microsclerotia were counted only if discrete microsclerotia could be observed in the formulated granule.

Conidia production by formulated microsclerotia was determined by incubating the microsclerotial formulations, each containing 1.7 x 10^3 viable microsclerotia, on individual Noble water agar plates. After 3, 6, 10, and 14 days of incubation, duplicate water agar plates were flooded with 10 ml of sterile deionized water, scraped vigorously with a sterile, plastic inoculating loop, and swirled to produce a homogenous suspension. One milliliter of the suspension was placed on a glass slide, overlaid with a coverslip, and the number of visible microsclerotia counted. Only well-formed hyphal aggregates with smooth margins were counted as microsclerotia.
reduced MS viability (data not shown) and were not used in subsequent studies. After 9 and 18 months storage at 4°C, significant losses in viability were observed in all MS formulations compared to nonformulated MS (Table 1). No loss in MS viability was observed in 9-month-old MS–diatomaceous earth preparations, while only a 10% loss in viability occurred in MS formulated with pregelatinized flour. The MS–starch: flour formulation showed a significantly higher loss in MS viability after 18 months storage compared to all other formulation tested.

After 3 days of incubation on Noble water agar plates at 28°C, MS–flour formulations produced significantly more conidia per viable MS than the other formulations (Fig. 1). Measurements at Days 6, 10, and 14 showed a similar pattern where the MS–flour formulation produced significantly more conidia than the MS–starch: flour (1:1) formulation and the flour–starch formulation produced more conidia than starch or MS–diatomaceous earth preparations. Starch and diatomaceous earth preparations did not produce significantly different numbers of conidia/per viable MS (Fig. 1). Unlike other formulations, MS–starch preparations produced large numbers of secondary MS in the Noble water agar (Fig. 2).

Previous experiments showed that incorporation of dried microsclerotia at approximately 150 MS particles/cc potting mix led to over 95% mortality of hemp sesbania seedlings (Jackson and Schisler, 1995). In this study, the rate of microsclerotia incorporation was reduced to less than 30% of the previous level (≈43 MS/cc potting soil) so that changes in disease incidence and/or severity due to formulation effects on MS could be discerned. At this level of MS incorporation, 68% of the emerging hemp sesbania seedlings were healthy after 14 days using dried MS:diatomaceous earth preparations or, put another way, 32% of the seedlings exhibited disease symptoms (Table 2). The formulation of MS with pregelatinized cornstarch or starch:flour (1:1) did not significantly increase the number of diseased seedlings. The MS–flour formulation did significantly reduce seedling health, with 54% of the emerging seedlings showing symptoms of disease (Table 2). This represents a 69% increase in the number of seedlings infected with C. truncatum compared to the other MS treatments. Efficacy data based on analysis of disease severity or number of healthy seedlings showed that the MS–flour formulation was more effective in inciting disease than other formulations tested (Tables 2 and 3).

On a scale of 0–3 (0 = healthy seedling, 3 = dead seedling), dried MS–diatomaceous earth, MS–cornstarch, and MS–starch:flour (1:1) formulations all gave similar disease ratings of ~0.8/seeding (Table 3). The MS–flour formulation had a significantly higher disease rating of 1.3. After 14 days, the mortality rate for hemp sesbania seedlings grown in potting mix containing MS–diatomaceous, MS–cornstarch, or MS–starch flour formulations averaged 27%, while seedlings grown in potting mix containing the MS–flour formulation had an average mortality rate of 47%. In all cases, heat-killed MS formulations did not influence the incidence of disease or emergence of healthy seedlings compared to control seedlings grown in potting soil devoid of MS.

Differences were seen in the stability and biocontrol efficacy of C. truncatum microsclerotia when formulated with pregelatinized cornstarch or corn flour. Formulating MS with pregelatinized corn flour significantly increased disease incidence and severity in hemp sesbania seedlings compared to the other microsclerotial formulations tested. MS–starch or MS–starch: flour (1:1) mixtures were slightly less stable during formulation and storage but produced similar disease severity in emerging hemp sesbania seedlings when compared to MS:diatomaceous earth preparations. The dose of microsclerotia used was purposefully set at one-third the dose known to incite virtually 100% control of hemp sesbania seedlings (Jackson and Schisler, 1995). This enabled us to study inoculum formulation effects that would be masked if excessive inoculum doses had been utilized. Achieving 100% mortality of weeds using bioherbicides is of little consequence if this level of control requires the use of uneconomical levels of inoculum. Under the conditions of this study, we have demonstrated that pregelatinized corn flour formulations of C. truncatum microsclerotia...
FIG. 2. Photomicrographs of 8-day-old *C. truncatum* MS germinated on Noble water agar showing the MS–diatomaceous earth preparation (A) producing occasional conidial masses with setae; the MS–flour formulation (B) producing abundant conidial masses with setae; and the MS–starch formulation (C) producing numerous secondary MS structures (arrowheads) beneath the agar surface.
increased the biocontrol efficacy of these propagules (Tables 2 and 3).

Increases in stability were not possible since 93% of the dried nonformulated MS were viable after 18 months storage at 4°C. Subsequent losses in MS viability in cornstarch or flour containing formulations (Table 1) may have been partially due to the higher moisture content of the preparations or to the rehydration and subsequent drying of microsclerotia during the drying process. Slow drying in the MS–starch and MS–flour formulations may have initiated metabolic activity in MS by exposing these propagules to an extended period of free moisture and, thereby, reducing MS stability. It should be noted that while MS viability was lower in formulations with corn flour (76%), cornstarch (89%), or starch–flour (51%), these are still reasonably good survival rates for microbial propagules after 18 months storage.

Bioassays showed that MS–flour formulations significantly improved biocontrol efficacy by significantly increasing disease incidence and severity in hemp sesbania seedlings compared to the other formulations tested (Tables 2 and 3). How flour formulations improve MS biocontrol efficacy is unclear. Since MS are soil incorporated, bioherbicide efficacy was dependent on contact with the emerging hemp sesbania seedlings. One major difference in the cornstarch and the corn flour formulations is that the pregelatinized flour contains ~10% protein. The pregelatinized cornstarch is essentially devoid of nitrogen. The nitrogen provided by flour-formulated MS may have aided the outgrowth of hyphae from germinating MS and thereby increased the chance of contact with an emerging hemp sesbania seedling. It is also possible that the rapid production of high spore concentrations by the germinating MS–flour formulation was responsible for the higher incidence of disease on emerging hemp sesbania seedlings. Previous studies with Pesta (wheat flour and kaolin) formulations of C. truncatum biomass showed that hyphal growth and sporulation occurred after the particles were rehydrated (Connick et al., 1991).

Sporogenic germination, a process wherein sclerotial propagules produce conidia directly, occurred when nonformulated dried microsclerotia were incubated at 28°C on Noble water agar (Fig. 2). Noble agar is a refined agar with very little nutritive value. The ability of nonformulated, dried microsclerotia to produce conidia on water agar suggests that MS are capable of using endogenous reserves for germination and sporulation. Previous studies have shown that sclerotia of Colletotrichum coccodes undergo sporogenic germination and that these sclerotial masses originated as incipient acervuli (Tu, 1980; Blakeman and Hornby, 1966; Coley-Smith and Cooke, 1971). If the MS of C. truncatum are structurally and physiologically equivalent to these “incipient acervuli,” sporogenic germination may represent an important mechanism in providing the conidial inoculum necessary to infect emerging hemp sesbania seedlings.

Conidia production on water agar was significantly higher in MS–flour preparations. This would be expected since flour provides an abundant supply of carbon and nitrogen for the germinating microsclerotium. If conidia, rather than germinating microsclerotia, are primarily responsible for infecting hemp sesbania seedlings, an association between increased sporulation by the MS–flour formulation and increased infection of hemp sesbania seedlings would be expected. This association is predicated on the assumption that the MS–flour formulation also produced higher conidial concentrations during germination in soil. Studies to quantify conidia production by microsclerotia of C. truncatum in soil are currently in progress.

After 6 days of incubation on water agar, the MS–starch:flour formulation produced significantly more conidia than nonformulated MS or starch-formulated MS in vitro, yet they did not exhibit increased biocon-
control efficacy in plant studies (Fig. 1, Tables 2 and 3). Although a lack of enhanced biocontrol efficacy by the MS–starch:flour formulation suggested that increased conidiation may not be a factor in inciting disease in emerging hemp sesbania seedlings, it should be noted that a significant increase in conidia production by flour-formulated MS was seen compared to all other MS formulations after 3 days of incubation on water agar plates (Fig. 1). Early conidiation may be required for increased level of disease in emerging hemp sesbania seedlings. Increased conidiation by MS after hemp sesbania seedlings have emerged from the soil may be ineffective in enhancing disease. More information on the timing and mode of C. truncatum infection of hemp sesbania seedlings is needed. Studies are currently underway to resolve these questions.

The formation of so-called secondary sclerotia by germinating sclerotia is considered a survival mechanism whereby the fungus is able to persist in soil in the absence of a suitable host for colonization (Coley-Smith and Cooke, 1971). While starch-formulated microsclerotia did not produce more conidia, they did form large numbers of secondary microsclerotial structures in water agar (Fig. 2). The production of additional microsclerotia by the germinating MS–starch formulation follows a nutritional pattern similar to that seen in liquid cultures where a high concentration of carbohydrate (8% glucose) was required for microsclerotia formation (Jackson and Bothast, 1990). Although starch-formulated MS did not improve biocontrol efficacy under the conditions of our study, field trials with starch formulations are warranted to examine the long-term effect of increasing the concentration of microsclerotial propagules in soil.

In conclusion, formulating C. truncatum microsclerotia with pregelatinized corn flour appears to provide a method for improving the biocontrol efficacy of these propagules. Field trials with flour and starch formulations are needed to evaluate their practical value as bioherbicides. This study has demonstrated that the exogenous nutritional environment can significantly impact the mode of growth (sporulation or secondary microsclerotia development) and the biocontrol efficacy of C. truncatum microsclerotia. An understanding of how soil-incorporated C. truncatum conidia and microsclerotia infect emerging hemp sesbania seedlings should help us develop formulations which improve biocontrol efficacy.

ACKNOWLEDGMENTS

The authors thank Angela R. Payne and Patricia M. Soncasie for their excellent technical assistance during this study.

REFERENCES


