Submerged Fermentation of *Colletotrichum truncatum* for Biological Control of Scentless Chamomile

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Abstract

Colletotrichum truncatum is being developed for biocontrol of scentless chamomile (*Matricaria perforata*). For potential mass production, a study was conducted to determine the possibility of submerged culturing. Fungal cultures were grown in V8-juice broth or a basal-salts liquid supplemented with carbon (C) and nitrogen (N) sources (C:N ratio at 10:1) in 500-ml flasks at 16 °C, 22 °C, or 28 °C in darkness to determine the impact of temperature. Sporulation was highest in the basal-salts medium at 16 °C, yielding about 1 x 10^6 sp/ml after two weeks. Further improvements were explored by examining the effects of various C and N sources, C concentrations, and C:N ratios. After comparison of nine C sources and eight organic and inorganic N sources, glucose and casamino acids were considered to be effective supplements to the basal-salts medium. Increasing glucose concentration from 5 to 40 g/L enhanced spore yields, but decreased biocontrol efficacy when glucose was 20 g/L or higher. In comparison to non-treated controls, spores produced at 5 g/L glucose reduced fresh weight of scentless chamomile by approximately 75% as opposed to only 39% by spores produced at 40 g/L glucose. Media amended with 10 g/L glucose supported high levels of sporulation without compromising the efficacy of spores. No significant difference in sporulation or efficacy was observed among C:N ratios ranging from 5:1 to 40:1.

Introduction

Matricaria perforata Merat. is commonly known as scentless chamomile, mayweed, false chamomile, or scentless mayweed. The plant can be an annual, biennial, or short-lived perennial on Canadian prairies, and has been a very adaptable species since introduced to Canada from northern Europe and western Asia (Royer and Dickinson, 1999). Scentless chamomile is now considered a noxious weed in Western Canada. It is a difficult weed to control because it is highly competitive and naturally tolerant to most post-emergent herbicides, which are ineffective at crop-tolerant rates after the 4-leaf stage (Peng *et al.,* 2000). Most effective strategies for controlling this weed are necessary to curb problems caused by heavy infestation in crops, chemical-fallow rotations, pastures, sloughs, and along municipal roads, rails, and highways. Researchers at Agriculture and Agri-Food Canada in Saskatoon have identified a group of fungal pathogens (*Colletotrichum* spp.) that selectively attack scentless chamomile, showing promise

for biological control. One of these isolates (00-3B1) has been tentatively identified as *Colletotrichum truncatum*. In order to research and develop this biological control strategy effectively, it is imperative to produce efficacious fungal spores efficiently on a large scale. Initial production methods using solid agar media gave inconsistent results, and the process would not be economical for large-scale productions. Other *Colletotrichum* species have been successfully produced using submerged fermentation, and strategies to manipulate the physical, environmental, and nutritional conditions of liquid media can be used to improve fitness of *Colletotrichum* for potential biological control (Jackson and Bothast, 1990; Jackson *et al.,* 1996; Schisler *et al.,* 1995).

The objectives of this study were to: 1) determine if it was possible for the fungus to sporulate in a defined liquid medium; 2) understand more conducive lighting and temperature conditions for maximum spore yield, 3) and explore improvement of sporulation by manipulating carbon and nitrogen composition in the medium.

Results and Discussions

Effect of light: Light was not a significant factor for sporulation of *Colletotrichum* sp. 00-3B1. After two weeks, spore concentration in treatments of continuous darkness, 14 h light/10 h dark, and continuous light were not significantly different (Figure 1).

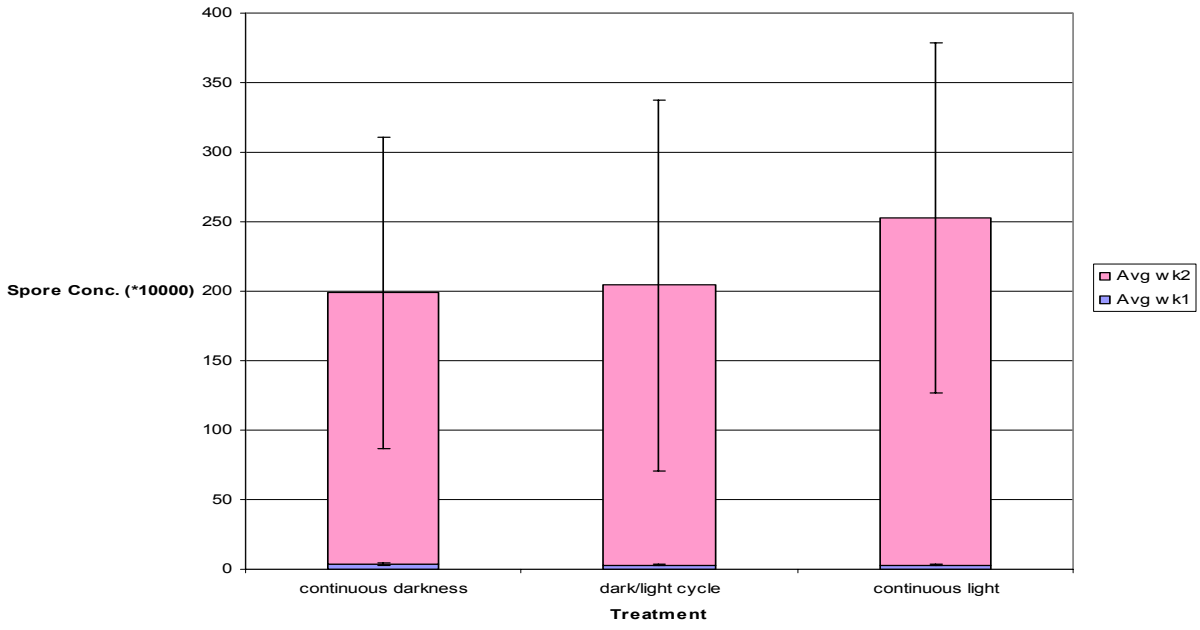

Figure 1. Effect of light on sporulation of 00-3B1 in submerged culture (data pooled from 2 trials).

Effect of seed inoculum concentration: Increasing initial spore numbers added to the medium generally produced higher spore concentration in submerged cultures (Figure 2). But it is not practical to add extremely large numbers of spores as seed inoculum.

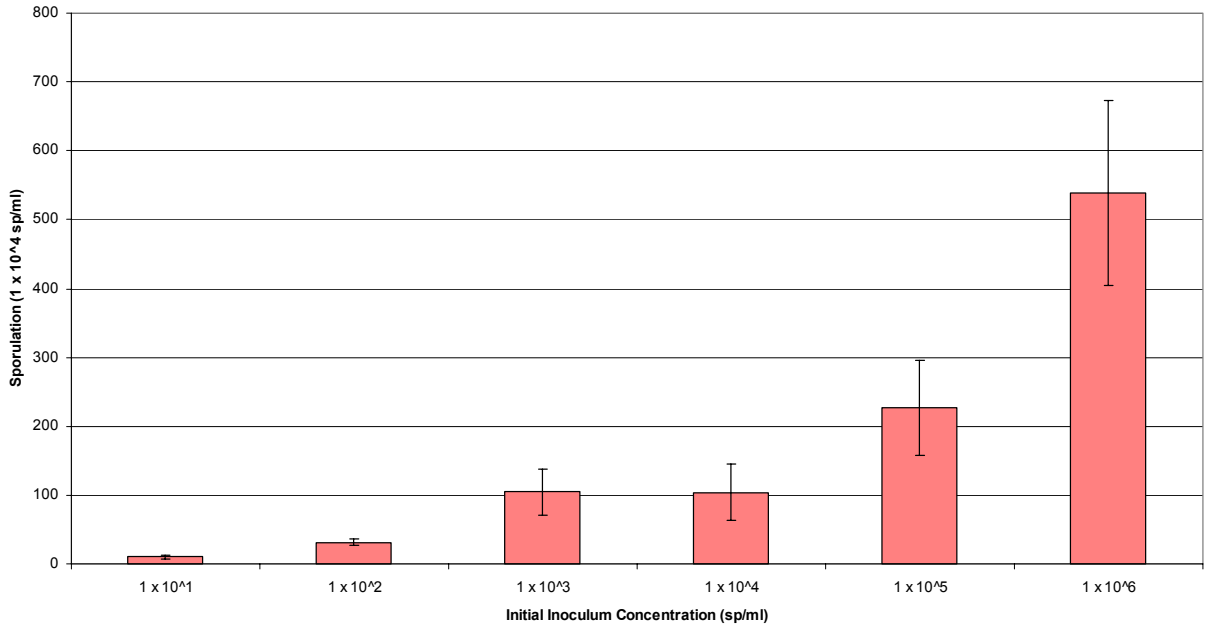

Figure 2. Effect of seed inoculum concentration on sporulation of 00-3B1 in submerged culture.

Effect of carbon sources: When comparing different carbon sources in liquid media, the highest spore yield was with glucose, with an average sporulation of 1.98 x 10^6 sp/ml after 2 weeks (Table 1). Spores produced using different carbon sources showed slight variation in efficacy, but all significantly reduced the fresh weight of scentless chamomile as compared to the control, when applied with an airbrush sprayer at 1 x 10^5 or 1 x 10^6 sp/ml (Figure 3).

Table 1. Effect of Carbon Sources on Sporulation of 00-3B1 in Submerged Culture.

	Spore Yield (10^4 sp/ml)	
Treatment	**7 days**	**14 days**
glucose	2 bc	198 a
trehalose	1 c	55 ab
sucrose	1 c	38 b
maltose	4 b	25 b
lactose	1 c	12 b
cellulose	11 a	7 b
glycerol	1 c	7 b
fructose	1 c	3 b
mollasses	0 c	0 b

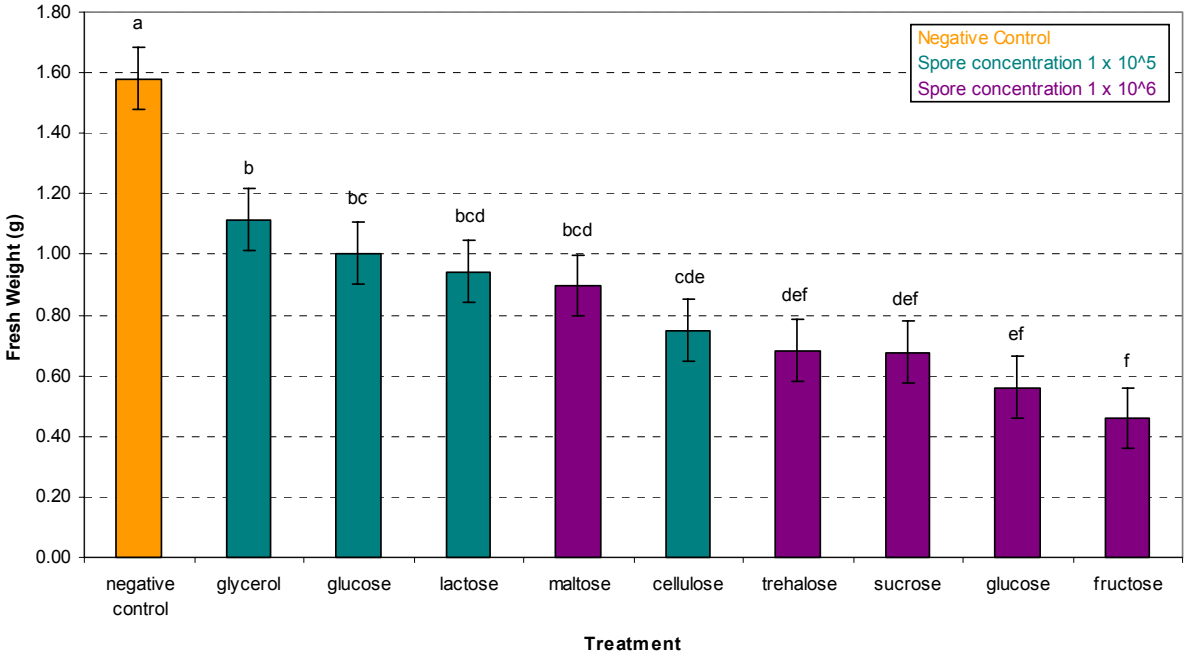

Figure 3. Weed-control efficacy of 00-3B1 spores produced in different carbon sources.

Effect of nitrogen sources: When comparing different nitrogen sources in liquid media, the highest spore yield was with casein hydrolysate or casamino acids, with an average sporulation of 1.84 x 10^6 sp/ml and 4.2 x 10^5 sp/ml respectively, after 2 weeks (Table 2). Spores produced with these two nitrogen sources caused a similar level of fresh weight reduction on scentless chamomile when applied at 9.0 x 10^5 – 1.0 x 10^6 sp/ml with an airbrush sprayer.

Table 2. Effect of Nitrogen Sources on Sporulation of 00-3B1 in submerged culture.

	Spore Yield (10^4 sp/ml)	
Treatment	**7 days**	**14 days**
Casein hydrolysate	0 b	184 a
Casamino acids	14 a	42 a
Tryptone	1 b	30 b
Ammonium nitrate	1 b	24 b
Potassium nitrate	1 b	13 b
Leucine	4 ab	9 b
Cottonseed hydrolysate	1 b	8 b
Glutamic acid	1 b	4 b

Effect of carbon concentration: Glucose concentrations ranging from 5 g/L – 40 g/L supported growth and sporulation of *Colletotrichum* sp. 00-3B1 (Figure 4). Although higher glucose concentrations often resulted in increased spore yield, the spores produced at these concentrations were less efficacious against scentless chamomile (Figure 4 and 5). A concentration of 10 g/L resulted in high spore yields while maintaining spore efficacy in control of scentless chamomile.

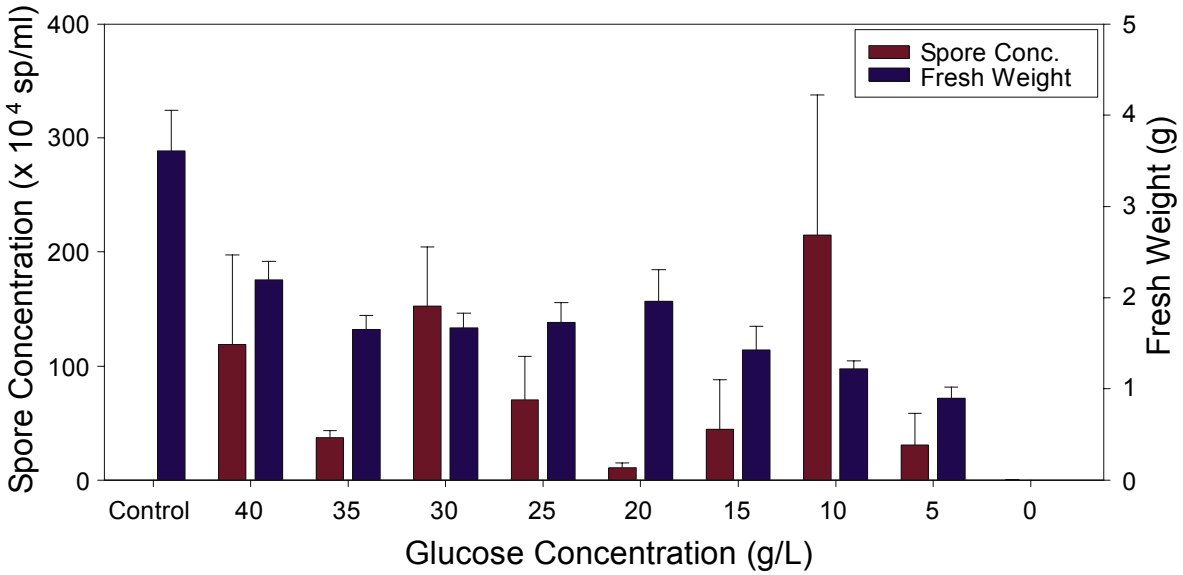

Figure 4. Effect of carbon concentration on 00-3B1 spore yield and efficacy

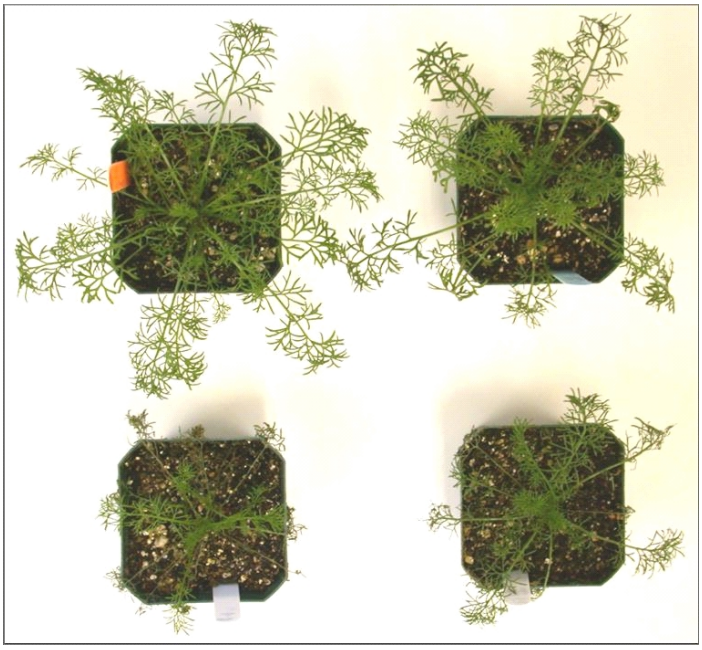

Figure 5. Suppression of scentless chamomile by *Colletotrichum* sp. 00-3B1 produced at different glucose concentrations (from top left, then clockwise: control, 40g/L, 5 g/L, and 10g/L).

Effect of C:N ratio: In preliminary studies, there was no significant difference in spore yield or efficacy of spores produced in various C:N ratios using glucose and casamino acids (Figure 6), although this factor was found to be important in liquid fermentation of a similar Colletotrichum fungus (Jackson and Bothast, 1990).

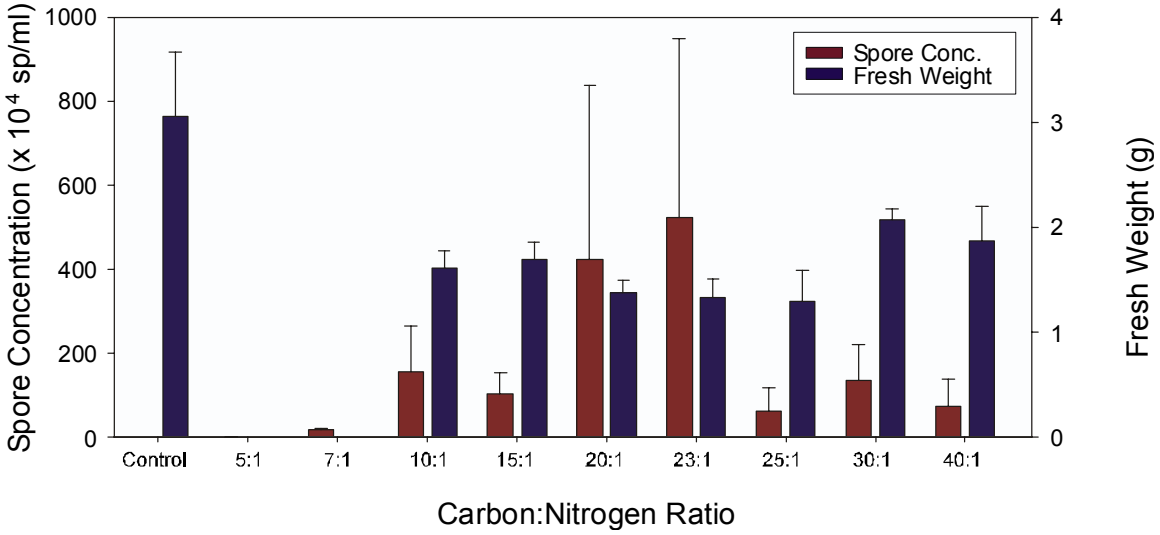

Figure 6. Effect of carbon-to-nitrogen ratio on 00-3B1 spore yield and efficacy.

Conclusions

Preliminary research has shown that submerged culturing can be used to produce efficacious Colletotrichum spores for biocontrol of scentless chamomile. Physical, environmental, and nutritional factors can be manipulated to further enhance spore production. There is a potential to enhance efficacy of biological control through manipulation of nutritional composition in liquid media.

References

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