

Trichothecene production and sporulation by *Myrothecium verrucaria* in response to substrate composition

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Introduction and Objectives

Myrothecium verrucaria is an aggressive pathogen of several weeds and is being evaluated as a bioherbicide, especially for the control of kudzu

Safe production and application of the fungus is undermined by the production by the fungus of macrocyclic trichothecene mycotoxins

This study measured the level of trichothecenes and conidia production on agar-based media with varying carbon and nitrogen sources to determine if toxin production and sporulation could be uncoupled and to find substrates that yielded spores with reduced toxin levels

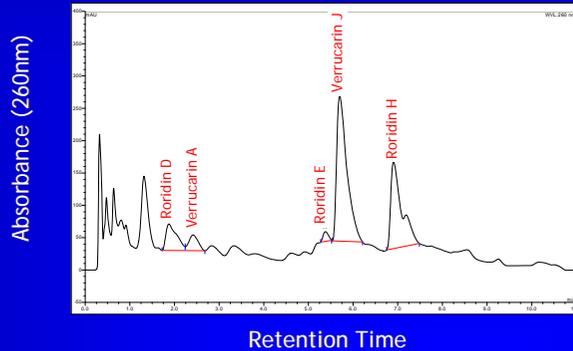
Methods

Agar plates were prepared by addition of spores of *M. verrucaria* to molten agar with complex or defined nutrient sources

After 3, 5, 9 or 15 days of incubation the plates were extracted with ethanol for trichothecene quantitation or scraped with water to measure the sporulation level

HPLC Measurement of Trichothecenes

Potato Dextrose Agar



Ammonium nitrate + sucrose defined medium

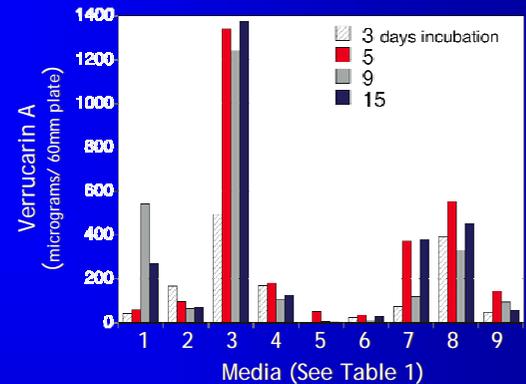
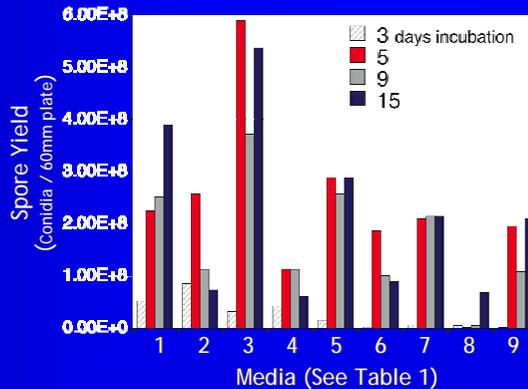
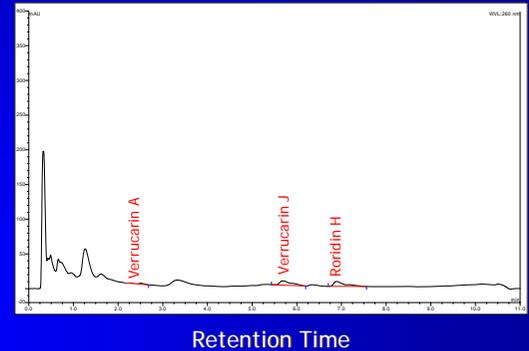


Table 1. Media tested

Media	Description	Nitrogen ^a (g l ⁻¹)	Carbon ^b (g l ⁻¹)	C : N ratio
1	Potato Dextrose Agar	0.92	9.97	10.9
2	Vogel's Ammonium nitrate 2 g L ⁻¹ Sucrose 15 g L ⁻¹ Yeast Extract 2 g L ⁻¹	0.91	7.82	8.6
3	Vogel's Ammonium nitrate 2 g L ⁻¹ Molasses 30 g L ⁻¹	0.82	12.87	15.7
4	Vogel's Ammonium nitrate 2 g L ⁻¹ Molasses 15 g L ⁻¹	0.72	6.63	9.2
5	Vogel's Ammonium nitrate 2 g L ⁻¹ Glucose 15 g L ⁻¹	0.7	6.61	9.4
6	Vogel's medium ^a Ammonium nitrate 2 g L ⁻¹ Sucrose 15 g L ⁻¹	0.7	6.92	9.9
7	Vogel's Ammonium nitrate 2 g L ⁻¹ Sucrose 30 g L ⁻¹	0.7	13.23	18.9
8	Vogel's Ammonium sulfate 2.4 g L ⁻¹ Sucrose 15 g L ⁻¹	0.51	6.92	13.6
9	Vogel's Potassium nitrate 2.4 g L ⁻¹ Sucrose 15 g L ⁻¹	0.33	6.92	21.0

Conclusions

- The level of sporulation and trichothecene production is readily manipulated by alteration of the media composition
- A defined media has been identified that produces nearly undetectable levels of trichothecenes and sporulation levels similar to those produced on Potato Dextrose Agar

Published results with more extensive discussion:

Weaver, Mark A., Robert E. Hoagland, C. Douglas Boyette, and Robert M. Zablotowicz. 2009. Macrocyclic trichothecene production and sporulation by a biological control strain of *Myrothecium verrucaria* is regulated by cultural conditions. *World Mycotoxin Journal* 2(1): 35-43.

Thanks to:

Bruce Jarvis for providing trichothecene standards
Laura Bennett and Carol Benson for technical assistance

This and additional publications are available online at:
<http://www.ars.usda.gov/pandp/docs.htm?docid=12420>