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Variability in thermal responses among *Furia gastropachae* isolates from different geographic origins

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Abstract

The effect of temperature ranging from 5–30 °C on *in vitro* vegetative growth and conidial germination of isolates of the entomophthoralean fungus *Furia gastropachae* was investigated. Eleven isolates were used for growth studies; two from Maryland, six from New York, and three from Ontario. A subset of four isolates, one each from Maryland and New York and two from Ontario, were used in conidial germination experiments. Growth and germination were significantly associated with temperature for all isolates, occurring throughout the range 5–30 °C, though both processes were inhibited to varying degrees at upper and lower extremes. Temperature optima for growth ranged from 20 to 27 °C, and for germination from 20 to 25 °C. Although significant variability was observed among isolates in growth at temperatures above 13 °C, temperature optima were not significantly different among isolates, and variability did not appear to relate to the geoclimatic origins of the isolates. In contrast, germination responses to temperature did appear to be related to geographic origin. *Furia gastropachae* isolates from New York and Maryland germinated more slowly at 10 °C than did Ontario isolates, although the percentage of conidia ultimately germinating at each temperature was the same for all isolates. The New York and Maryland isolates performed much better at 30 °C, with significantly greater overall germination and secondary conidial discharge, than the Ontario isolates. Compared with other isolates at 30 °C, Ontario isolates were the least active, often failing to successfully discharge any secondary conidia.

Keywords: Entomopathogenic fungi; Temperature; Biological control; Forest tent caterpillar; *Malacosoma disstria*

1. Introduction

The development of fungal epizootics in insect populations is highly dependent on the abiotic environment (Carruthers and Soper, 1987; Hajek, 1997). Moisture is critical, with most entomopathogenic fungi requiring conditions approaching saturation to successfully invade hosts. Temperature is also important, although its effect on fungal activity varies greatly among fungal species and even isolates within a species (e.g., Fargues et al., 1997; Ouedraogo et al., 1997). Temperature optima are often, though by no means always, related to ambient conditions in the area of origin of a fungal isolate (e.g., Fargues et al., 1992; Papierok et al., 1993; Vidal et al., 1997). Thus, for example, while most of the predominantly temperate entomophthoralean fungi perform optimally at 20 °C or below (Hall and Papierok, 1982), an Israeli isolate of *Zoophthora radicans* showed maximal infection of aphid hosts at 25 °C (Milner and Lutton, 1983).

Optimal temperatures for epizootic development are the result of the combined effect of temperature on several stages of the fungal life cycle, including conidial germination and hyphal growth. Temperature has been shown to influence the rate and level of germination of conidia for many hypocrealean and entomophthoralean fungi (e.g., Luz and Fargues, 1997). For entomophthoralean species, temperature can further influence the mode of germina-

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tion (e.g., Morgan et al., 1995). Temperature is the principal abiotic factor limiting vegetative growth of fungi within the host hemocoel (Carruthers et al., 1985) and its importance is amplified by the exhibition of "behavioral fever" by several host species, which allows infected insects to cure themselves of mycoses by elevating their internal body temperature (Roy et al., 2006). A thorough understanding of the relationship between temperature and fungal activity is critical both to the prediction of epizootics and the selection of isolates for biological control.

Furia gastropachae is an entomophthoralean pathogen of the forest tent caterpillar, *Malacosoma disstria* (Lepidoptera: Lasiocampidae), a common defoliator of North American hardwoods. *Furia gastropachae* is widely distributed, causing epizootics as far north as Ontario and North Dakota and as far south as Alabama and Florida (Filotas et al., 2003). Previously reported laboratory studies indicated that virulence of *F. gastropachae* is affected by temperature, with infection optimized at 15–20 °C (Filotas and Hajek, 2004; Filotas et al., 2006). During virulence studies, percent infection was maximized at cooler temperatures for an isolate originating from a more northern location (Filotas et al., 2006), suggesting that thermal relationships may be related to the isolates' geographic origins.

The objective of the present study was to further investigate whether isolates of this widely distributed entomopathogen varied in relation to activity by temperature according to geoclimatic origin of isolates. This relationship was evaluated by quantifying *in vitro* growth and germination of *F. gastropachae* isolates from varying geographic origins in North America across a range of temperatures. To further evaluate upper thermal activity limits, discharge of secondary conidia at 30 °C was compared among isolates.

2. Materials and methods

2.1. Fungal isolates

Eleven isolates of *F. gastropachae* were obtained from six locations at varying latitudes (Table 1). All isolates were obtained either from field-collected cadavers or from laboratory-reared larvae infected by exposure to *F. gastropachae* resting spores in field-collected soil (Filotas, 2002). We know of no isolates of this fungus from locations further south than the isolates included in this study and we were unable to isolate *F. gastropachae* from more southern locations. All isolates are currently maintained under liquid nitrogen in the United States Department of Agriculture, Agricultural Research Service Collection of Entomopathogenic Fungal Cultures (ARSEF), Ithaca, New York. Isolates were grown as hyphae in 95% Grace's insect tissue culture medium + 5% fetal bovine serum (Gibco/BRL, Gaithersburg, MD) at 20 °C in the dark and were subcultured up to six times every 6–8 days. To obtain mycelia for use in assays, 0.3 ml of liquid culture was inoculated onto egg yolk/Sabouraud maltose agar (EYSMA: Papierok and Hajek, 1997) and incubated in constant darkness at 20 °C for 6–10 days.

2.2. In vitro growth studies

A cork-borer was used to cut four-mm diameter disks of unsporulated mycelium from six to eight day old cultures. These were then placed upside down in the center of sterile EYSMA in 90-mm Petri dishes and incubated in constant darkness at 5, 10, 13, 15, 17, 20, 23, 25, 27, or 30 °C for 16 days. Surface radial growth was measured in mm every other day using two diameters, at right angles to each other, drawn on the bottom of each dish. Any plates

Table 1
Mode of isolation, geographic origin and collection dates for isolates of *Furia gastropachae* used in bioassays

Latitude (°N)	Isolate[a]	Isolation method[b]	Year	Location	Geoclimatic grouping[c]
38.2	ARSEF 5545	Field	1997	Snow Hill, Maryland, USA	3
38.2	ARSEF 5546	Field	1997	Snow Hill, Maryland, USA	3
42.5	ARSEF 5351	Field	1996	Dryden, New York, USA	2
42.6	ARSEF 5547	Field	1997	Cortland, New York, USA	2
43.5	ARSEF 5541	Lab	1997	Richland, New York, USA	2
43.5	ARSEF 5542	Lab	1997	Richland, New York, USA	2
43.5	ARSEF 5543	Lab	1997	Richland, New York, USA	2
43.5	ARSEF 5544	Lab	1997	Richland, New York, USA	2
46.3	ARSEF 5869/FPMI 713	Field	1982	Webbwood, Ontario, CAN	1
47.5	ARSEF 5871/FPMI 992–2a	Field	1986	New Liskeard, Ontario, CAN	1
47.5	ARSEF 5872/FPMI 922–3a	Field	1986	New Liskeard, Ontario, CAN	1

All isolates obtained from infected fourth or fifth instar *M. disstria* cadavers.

[a] ARSEF, Agriculture Research Service Collection of Entomopathogenic Fungal Cultures, USDA, Ithaca, NY, USA; FPMI, Forest Pest Management Institute Collection of Entomopathogenic Fungal Cultures, Sault Ste. Marie, Ontario, Canada (FPMI isolates originated in Sault Ste. Marie, now maintained in ARSEF collection).

[b] Field, isolation from naturally infected field-collected cadavers; Lab, isolation via infection of laboratory-reared *M. disstria* larvae with field-collected resting spores.

[c] Geoclimatic grouping for tests of the effect of origin: 1, Ontario isolates; 2, New York isolates; 3, Maryland isolates.