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Short Communication

Germination of conidia and blastospores of *Paecilomyces fumosoroseus* on the cuticle of the silverleaf whitefly, *Bemisia argentifolii*

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The mitosporic fungus *Paecilomyces fumosoroseus* (Wize) Brown & Smith infects more than 40 insect species [1] and produces blastospores in liquid medium [2] and conidia on solid media [3]. Because two types of propagules can be used for formulating *P. fumosoroseus* as well as many other fungal entomopathogens, it is important to determine which form might be best suited for use in commercial formulations. For example, a study comparing the bioactivity of *P. fumosoroseus* blastospores and *Beauveria bassiana* (Balsamo) Vuillemin conidia has shown that blastospores were four times more effective in infecting and killing the silverleaf whitefly (*Bemisia argentifolii* Bellows & Perring) [2]. In contrast, Vandenberg et al. [4] showed that the relative efficacy of blastospores or conidia of *P. fumosoroseus* against Russian wheat aphids (*Diuraphis noxia* (Mordvilko)) was similar. Hall [5] reported that blastospores of *Verticillium lecanii* (Zimmermann) Viégas were twice as pathogenic to an aphid as conidia when based on a numerical basis, but only 0.6 times as pathogenic when based on a volume basis, due to their larger size.

Germination rates of fungal propagules could play an important role in determining virulence. In fact, fast spore germination has been shown to increase insect pathogenicity for germinated conidia of *P. fumosoroseus* [6] and conidia of *Metarhizium anisopliae* (Metchnikoff) Sorokin used against three different insects [7–9]. Most studies on spore germination rates have examined conidial germination in vitro, with one study assessing time to conidial germination on the cuticle of an insect in an indirect manner, not using scanning electron microscopy [SEM, 9].

We used SEM to assess germination rate of conidia and blastospores of *P. fumosoroseus* (strain 612; Mycotech Corp., Butte, MT, USA) on the cuticle of *B. argentifolii*, a devastating insect pest throughout the world. *P. fumosoroseus* blastospores were obtained using the method of Jackson et al. (1997), yielding 1.2 $\times$ 10^9 blastospores per milliliter. For conidia, Petri plates dishes containing Sabouraud's dextrose maltose agar were inoculated with stock cultures of *P. fumosoroseus* and after 20 days incubation at 22°C, conidia were washed-off with 20 ml of sterile distilled water. Spores were filtered once through a sterile double layer of cheese cloth, and centrifuged to concentrate the spores, yielding 4.1 $\times$ 10^7 conidia per milliliter.

B. argentifolii were reared on cotton seedlings at 25°C, 60% RH, and a 12:12 (L:D) photoperiod. Leaves with 2nd-3rd instar nymphal stages were placed upside down inside open Petri plates under a Burkard Computer Controlled Spraying Apparatus (Burkard Scientific Ltd., Uxbridge, UK). Controls were sprayed with sterile distilled water. Immediately after spraying, each leaf was transferred to a Petri plate containing 2% Bacto-agar (Difco Laboratories, Michigan, USA) and sealed with parafilm. The plate was inverted so that the abaxial side of the leaf faced down, with the adaxial side touching the agar. Plates were stored in the dark at 24°C, and leaf sections containing nymphs were collected immediately after spraying, and 2, 4, 8, and 24 hours later, followed by lyophilization in a Dura-Top MP Freeze-Dryer (FTS Systems, New York, USA). Gold-palladium was used to coat the lyophilized samples, and a scanning electron microscope (JEOL, JSM-6400 V Scanning Microscope) was used to assess germination of fifty conidia or blastospores on each of three nymphs per sampling time. Germination was deemed positive when a germ tube was seen projecting from the spore. SAS GLM and LS means [10] were used

Table 1. Percent germination (mean ± s.e.) for conidia and blastospores of *P. fumosoroseus* on the cuticle of *B. argentifolii* at different sampling times (hours) after inoculation.

Hours	% Germination[1]	
	Conidia	Blastospores[2]
0	0	3 ± 1.9
2	0	$15 \pm 2.4^{**}$
4	0	$37 \pm 5.8^{**}$
8	31 ± 4.6	$43 \pm 5.7^{*}$
24	99 ± 0.7	96 ± 3.9

[1]Based on scanning electron microscopy observations using 3 nymphs per sampling time and 50 conidia or blastospores per nymph.
[2]Significantly different to conidia at $^{**}p < 0.01$ or $^{*}p < 0.05$.

to determine differences in germination at specific sampling times.

Immediately after spraying *B. argentifolii*, a small percentage (∼3%) of the blastospores were already germinating (Table 1), indicating that the conditions in the liquid medium were sufficient to induce germ tube formation. On the insect cuticle, the percent germination for blastospores was significantly higher than for conidia at 2, 4, and 8 hours after spraying, but not at 24 hours (Table 1). The faster germination rate of blastospores may provide them with an ecological advantage in inciting disease in *B. argentifolii* under less favorable environmental conditions; spores that germinate at a faster rate require a narrower window of free moisture for spore germination and penetration into the insect host. The longer the spore remains ungerminated, the more likely it could be affected by adverse environmental factors such as low relative humidity, UV radiation, and possibly, chemical components on the insect's cuticle [11]. In addition, spores that germinate rapidly are more likely to infect immature stages and thereby not be sloughed during ecdysis.

The faster germination rate of blastospores, which was also confirmed in an in vitro experiment (Jackson, unpubl. data), indicates that blastospores might be sequestering endogenous reserves while growing in a liquid medium that provides the spore with all necessary nutrients to trigger germination and that these propagules are not dependent on the external nutritional environment for rapid germination. On the other hand, the germination rate of conidia appears to be influenced by the external nutritional environment.

The rate of germination of the formulated spore could have important consequences on the success of fungal entomopathogens as biological control agents in tandem with other desirable attributes for mycoinsecticides such as rainfastness, high storage stability, and desiccation tolerance [12]. We have demonstrated that blastospores of *P. fumosoroseus* germinate at a significantly faster rate than conidia on the cuticle of *B. argentifolii*. The use of blastospores as the active ingredient in a formulation should prove to be advantageous over conidia if a faster germination rate on the insect's cuticle can be obtained after applying blastospore-based formulated material.

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References

1. Smith P. Control of *Bemisia tabaci* and the potential of *Paecilomyces fumosoroseus* as a biopesticide. Biocon News Info 1993; 14: 71N–78N.
2. Jackson MA, McGuire MR, Lacey LA, Wraight SP. Liquid culture production of desiccation tolerant blastospores of the bioinsecticidal fungus *Paecilomyces fumosoroseus.* Mycol Res 1997; 101: 35–41.
3. Inch JMM, Humphreys AM, Trinci APJ, Gillespie AT. Growth and blastospore formation by *Paecilomyces fumosoroseus*, a pathogen of brown planthopper (*Nilaparvata lugens*). Trans Br Mycol Soc 1986; 87: 215–222.
4. Vandenberg JD, Jackson MA, Lacey LA. Relative efficacy of blastospores and aerial conidia of *Paecilomyces fumosoroseus* against the Russian wheat aphid. J Invertebr Pathol 1998; 72: 181–183.
5. Hall RA. Pathogenicity of *Verticillium lecanii* conidia and blastospores against the aphid *Macrosiphoniella sanborni*. Entomophaga 1979; 12: 191–198.
6. Fargues J, Maniania N, Delmas JC Infectivity of propagules of *Paecilomyces fumosoroseus* during in vitro development to *Spodoptera frugiperda*. J Invertebr Pathol 1994; 64: 173–178.
7. Al-Aidroos K, Roberts DW. Mutants of *Metarhizium anisopliae* with increased virulence towards mosquito larvae. Can J Genet Cytol 1978; 20: 211–219.
8. Samuels KDZ, Heale JB, Llewellyn M. Characteristics relating to the pathogenicity of *Metarhizium anisopliae* toward *Nilaparvata lugens*. J Invertebr Pathol 1989; 53: 25–31.
9. Hassan AEM, Dillon RJ, Charnley AK. Influence of accelerated germination of conidia on the pathogenicity of *Metarhizium anisopliae* for *Manduca sexta*. J Invertebr Pathol 1989; 54: 277–279.
10. SAS Institute Inc. SAS/STAT User's Guide, Version 6, Fourth Edition, Vol. 2. The SAS Institute, Inc., Cary, NC. 1989; 846 pp.

11. Vega FE, Dowd PF, McGuire MR, Jackson MA, Nelsen TC. In vitro effects of secondary plant compounds on germination of blastospores of the entomopathogenic fungus *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes). J Invertebr Pathol 1997; 70: 209–213.
12. Wraight SP, and Carruthers RI. Production, delivery, and use of mycoinsecticides for control of insect pests on field crops. In: Hall FR and Menn JJ, eds. Biopesticides: Use and Delivery, New Jersey: Humana Press, 1999: 233–269.

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