MYCOSCIENCE 55 (2014) 21–26

Available online at www.sciencedirect.com

MYCOSCIENCE

ISSN 1340-3540 (print), 1618-2545 (online)

journal homepage: www.elsevier.com/locate/myc

Full paper

# Modeling analysis on sporulation capacity, storage and infectivity of the aphid-specific pathogen *Conidiobolus obscurus* (Entomophthoromycota: Entomophthorales)

*Xiang Zhou, Kai Guo, Sheng-Feng Mao*, Wei He, Yun-Yun Zhu*

*Provincial Engineering Laboratory of Biopesticide Preparation, School of Forestry and Biotechnology, Zhejiang Agricultural and Forestry University, Lin'an 311300, People's Republic of China*

ARTICLE INFO

*Article history:*
Received 28 June 2012
Received in revised form
29 August 2012
Accepted 15 April 2013
Available online 20 May 2013

*Keywords:*
Alginate formulation
Aphid control
Bioassay
*Myzus persicae*

ABSTRACT

Entomophthorales are important natural enemies against agroforestry pests. *Conidiobolus obscurus* in this order, a common obligate aphid pathogen, possesses features of rapid growth *in vitro* and ease to mass production. This study sought to evaluate the potential of *C. obscurus* in aphid biocontrol, by modeling analyzing on the sporulation capacity and storage of its alginate formulation and infectivity to *Myzus persicae*. The *C. obscurus* mycelia-entrapping alginate pellets discharges 0.12–18.26 × $10^4$ conidia per pellet at 4–32 °C. The optimal temperature for the fungal sporulation was computed as 23.3 °C. Each pellet could sporulated for 7 d, releasing 22.3-fold more conidia than a cadaver at 24 °C. Moreover, it had longevity of 8 mo at 4 °C, with half decline time of 2.3 mo. The infectivity of *C. obscurus* was assessed by multi-concentration bioassays at 10–28 °C and 8–16 h light per d. The median lethal concentration ($LC_{50}$) at each temperature-photoperiod regime was computed based on the morality-concentration trend. The $LC_{50}$ values reached the lowest one of 15 conidia per $mm^2$ at 28 °C and 16:8 L:D cycles. The total results suggest that *C. obscurus* mycelia-inclusive alginate pellets meet the requirement of aphid biocontrol in the high-temperature surroundings of 24–28 °C.



## 1. Introduction

Many fungi in the order of Entomophthorales succeed in regulating arthropod host populations in worldwide agroforestry ecosystems (Pell et al. 2001; Barta and Cagáň 2006). Unfortunately, few applicable mycoinsecticides made of them is manufactured for pest control (Faria and Wraight 2007). The difficulties in mass production, storage, formulation, field release and the fungi sensitive to environmental conditions contribute to the impediments (Wilding and Latteur 1987; Milner 1997; Jackson et al. 2010).

Only the conidia can infect hosts for mycosis transmission within host populations, so the capacity of an entomophthoralean formulation containing or producing conidia, and the infectivity indicate its biocontrol potential. Their mucilaginous conidia are difficult to be harvested and formulated for field applications (Wilding and Latteur 1987; Pell et al. 2001). Batta et al. (2011) reported a water-in-oil formulation

* *Corresponding author*. Tel.: +86 571 61069780; fax: +86 571 63740809.
E-mail addresses: xzhou.zafu@gmail.com (X. Zhou), maosf@126.com (S.-F. Mao).
1340-3540/$ – see front matter 
http://dx.doi.org/10.1016/j.myc.2013.03.005

of secondary conidia (capilliconidia) of *Zoophthora radicans* (Bref.) A. Batko against diamondback moth. But few details about mass production and harvest of those conidia were revealed. Resting spores of great anti-adversity can be mass-produced in certain species and easily formulated. It failed to apply in open fields because of its inoperable synchronous germination (Latgé et al. 1983). Thus, most entomophthoralean formulations focused on mycelia due to the capability of producing conidia.

Mycelia-inclusive cadavers and cadaver powder were the first attempt and restricted to small-scale studies for the difficulty in mass production (Milner et al. 1982; Wilding et al. 1986, 1990). Mass production of mycelia *in vitro* then succeeded in submerged fermentation (Latteur and Godefroid 1983). Based on this, McCabe and Soper (1985) developed a marcescence process to formulate mycelia, but it had a constraint of narrow suitable range of environmental conditions, especially of air humidity and temperature (Wilding 1969; Wilding and Latteur 1987; Wraight et al. 2003). Recently, a humectant formulation was developed, which could sporulate even at unsaturated regimes of 86–97% relative humidity (Zhou and Feng 2009). Still, the problem is that mycelia outgrowing on the granular formulation affect the procedure of release in fields. Efforts were also made to immobilize mycelia in an alginate matrix (Shah et al. 1998, 1999; Zhou and Feng 2010b). Mycelia-entrapping alginate pellets were easy to formulate and field release, but they like other entomophthoralean formulations could never be stored over half a year (Shah et al. 2000; Feng and Hua 2005; Zhou and Feng 2010b).

Besides, another important factor in determining the effectiveness of the formulations is the response to temperature compared with that of the goal pests (Guzmán-Franco et al. 2008). For example, *Pandora neoaphidis* (Remaud. & Hennebert) Humber and *P. nouryi* (Remaud. & Hennebert) Humber have thermal preference for 10–20 °C, so their formulations probably performed in aphid biological control better in cool climatic conditions (Shah et al. 2002; Zhou and Feng 2010b). Considering their host aphid pests infest over 20 °C such as *Myzus persicae* has the optimal temperature of 26.7 °C (Davis et al. 2006), their practical potential of the formulation is reasonably limited. It necessitates evaluating the optimal temperature range of a biocontrol agent before practical application.

*Conidiobolus obscurus* (I.M. Hall & P.H. Dunn) Remaud. & S. Keller is a pathogen specific only to aphids, widespread by infected host alatae disseminating and found in America, Europe, Asia and Africa (Barta and Cagáň 2006; Zhang et al. 2007). *Conidiobolus obscurus* is easy for mycelial mass production and formulation, and has a comparatively high optimal temperature for growth. The present study sought to manufacture an alginate formulation of *C. obscurus* for aphid control and perform modeling analyses for the fungal biocontrol potential.

## 2. Materials and methods

### 2.1. Fungal isolate and culture

An isolate of *C. obscurus* used in this study was acquired from the USDA-ARS Collection of Entomopathogenic Fungal Cultures (RW Holley Center for Agriculture and Health, Ithaca, NY; ARSEF accession number: 7217). The isolate was maintained on the slants of modified Sabouraud dextrose agar at 4 °C and recovered twice a year at 24 °C. The enhancement of virulence of the isolate was performed with host passage of the green peach aphid *Myzus persicae* (Sulzer) before the following experiments (Hayden et al. 1992).

The rejuvenated isolate was routinely cultured on the plates of Sabouraud dextrose agar (v/v: 90%) plus egg yolk (v/v: 11.5%) and milk (v/v: 8.5%) at 24 °C and 12:12 L:D cycles. Homogenized mycelial liquid was obtained by two steps: firstly, inoculating mashed culture pieces in 20 ml Sabouraud dextrose broth (SDB) plus 0.1% (v/v) emulsified sesame oil in a 100-ml flask and shaking at 150 rpm for 3 d at 24 °C; secondly, transferring 2 ml of rough mycelial liquid into 50 ml SDB in a 150-ml flask for additional 3-d shaking at the same regime. The mycelial liquid was then used to manufacture mycelia-entrapping alginate pellets and sporulating plates. After dehydrated into mycelial mats, they were laid uniformly onto 90-mm-diameter dishes to prepare sporulating plates for inoculating aphids.

### 2.2. Manufacture and storage of alginate pellets

Alginate pellets entrapping *C. obscurus* mycelia were manufactured using a previous method (Shah et al. 1998; Zhou and Feng 2010b) with modifications. One gram sodium alginate, 1 g millet powder and 0.06 g dihydrostreptomycin were added to 50 ml of mycelial liquid (ca. 25 mg dry biomass per milliliter). Millet powder was prepared by mechanically grinding shelled millet grains. After shaking at 150 rpm for 3 h at 24 °C, the homogenized mixture was instilled into 1% (w/v) autoclaved calcium chloride solution with 2-mm-diameter opening pipettes, forming 5-mm-diameter pellets (–20 pellets per ml mixture). The gelatinized pellets were rinsed with sterile water and harvested by filtering through a funnel.

Fresh pellets were parafilm-sealed in Petri dishes and stored at 4 °C. Seven pellets were arbitrarily taken at 30-day intervals during the storage period for estimating the decline in their sporulation capacities, the method as described below, until sporulation was not detected from sampled pellets.

### 2.3. Assessment of sporulation capacity

Alginate pellets were evaluated individually for their sporulation capacities using spore collectors (22 mm high × 13 mm diameter) described elsewhere (Hua and Feng 2003). Each fresh pellet taken arbitrarily was maintained for 7 d in the lid of a spore collector, discharging all primary conidia into 200 μl of 0.5% dodecyl sodium sulfate in the bottom of the collector at 4, 12, 20, 24, 28 and 32 °C with the same photoperiod (12:12 L:D). The conidia were dispersed as morphologically-unchanged single cell in the surfactant solution for counting. Sporulation capacity of each pellet was assessed by microscopically counting from three samples of the conidial suspension. Each temperature treatment included seven pellets arbitrarily taken as replicates.

Temporal sporulation pattern of the pellets were daily monitored for 7 d at 24 °C using the same method and spore collectors. The sporulation capacity of pellets with different storage time and fresh adult cadavers of *M. persicae* killed by *C. obscurus* in laboratory were also estimated at 24 °C.