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Attraction of *Myzus persicae* (Hemiptera: Aphididae) to the Entomopathogenic Fungus *Beauveria bassiana*

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Abstract

Beauveria bassiana (Balsamo) Vuillemin infects a wide variety of insects, including the green peach aphid, *Myzus persicae* (Sulzer). Volatiles emitted from *B. bassiana* can act as attractive or repellent semiochemicals, with most responses reported to date resulting in insects avoiding *B. bassiana*. Since insects can detect 'enemy-specific volatile compounds', we hypothesized the preference behavior of *M. persicae* would be influenced by volatile emissions from *B. bassiana*. We conducted Petri dish and Y-tube olfactometer bioassays to characterize the preference of *M. persicae* to *B. bassiana* strain GHA. During Petri dish bioassays, more apterous and alate *M. persicae* were recorded in the vicinity of agar colonized by *B. bassiana* compared to agar, or *Fusarium proliferatum* (Matsushima) Nirenberg and *Ambrosiella grosmanniae* Mayers, McNew, & Harrington as representatives of non-entomopathogenic fungi. Petri dish bioassays also determined that apterous and alate *M. persicae* preferred filter paper saturated with 1×10^7, 1×10^6, and 1×10^5 *B. bassiana* conidia/ml compared to Tween 80. Y-tube bioassays documented that more apterous and alate *M. persicae* oriented upwind to volatiles from *B. bassiana* mycelia compared to agar. Apterous and alate *Myzus persicae* were also preferentially attracted to 1×10^7 and 1×10^6 *B. bassiana* conidia/ml compared to Tween-80 during Y-tube bioassays. These results complement a previous finding that the mosquito *Anopheles stephensi* Liston is attracted to volatiles from *B. bassiana*. Future studies aimed at characterizing the olfactory mechanism leading to attraction of *M. persicae* to *B. bassiana* could aid in optimizing lure-and-kill strategies.

Keywords: *Beauveria bassiana*, *Myzus persicae*, microbial volatile organic compounds, entomopathogenic fungi

The green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), is a polyphagous insect pest on a worldwide scale of crops grown under field and controlled-environment settings (Dedryver et al. 2010). Conventional insecticides are primarily used for managing *M. persicae* (Dewar 2007), but entomopathogenic fungi show promise as a microbial control tactic. *Myzus persicae* and other aphids have been the subject of studies evaluating entomopathogenic fungi because these insects are susceptible to natural fungal epizootics (Milner 1997).

Beauveria bassiana (Balsamo) Vuillemin (Ascomycota: Hypocreales) is a ubiquitous entomopathogenic fungus documented to infect a variety of insect hosts throughout temperate and tropical areas of the world (Zimmermann 2007). Pathogenicity of *B. bassiana* is a function of spores attaching to the insect cuticle followed by germination and penetration by the hyphae into the hemocoel in conjunction with the release of insecticidal metabolites, proteins, and enzymes (Zimmermann 2007, Ortiz-Urquiza et al. 2010, Cheong et al. 2020).

Beauveria bassiana has commonly been isolated from infected insects, but it has also been isolated as a naturally occurring and artificially introduced endophyte from a variety of plants (Ownley et al. 2008; Vega 2008; Allegrucci et al. 2020). Furthermore, *B. bassiana* has been recovered from diverse soil types ranging from peat bogs, alpine soil, and desert soil (Zimmermann 2007). While more virulent entomopathogens exist, Jandricic et al. (2014) noted that *B. bassiana* has been the focus of formulation development due in part to its adaptability and comparatively stable production on a commercial scale. In 2014, 45 *B. bassiana* formulations were commercially available on an international scale representing 37.2% of the mycoinsecticide market (Faria and Wraight 2007, Jaronski 2014).

A growing body of evidence indicates that volatiles emitted from *B. bassiana* can function as semiochemicals to insects (Davis et al. 2013). For instance, the ectoparasitoid *Cephalonomia tarsalis* (Ash.) (Lord 2001), the generalist predators *Anthocoris nemorum* L. (Meyling and Pell 2006) and *Coccinella septempunctata* L. (Ormond et al. 2011), and the termites *Macrotermes michaelseni* (Sjöstedt) (Mburu et al. 2009) and *Coptotermes formosanus* Shiraki (Hussain et al. 2010) avoid *B. bassiana* mycelia and/or conidia following the detection of fungal volatiles and/or direct contact. In contrast, George et al. (2013) reported that the mosquito *Anopheles stephensi* Liston was highly attracted to volatile emissions from *B. bassiana* conidia. Volatiles from *B. bassiana* conidia were also more attractive than emissions from the entomopathogenic fungus *Metarhizium anisopliae* and a *Penicillium* sp. Assessing the behavioral response of *M. persicae* to *B. bassiana* could also help to optimize this entomopathogenic fungus as a pest control tactic. For instance, avoidance of *B. bassiana* due to the detection of enemy-specific volatile compounds (Dicke and Grostal 2001) could potentially reduce the effectiveness of formulations.

Based on these aforementioned studies, we hypothesized that the preference behavior of *M. persicae* would be influenced by volatile emissions from *B. bassiana*. ~~To test this hypothesis, we conducted a series of Petri dish and Y-tube olfactometer bioassays to characterize the preference of *M. persicae* to mycelia and conidia of *B. bassiana*.~~

Materials and Methods

Insects

A colony of *M. persicae* was maintained on *Zinnia elegans* cv. Purity White (BFG Supply Co., Burton, OH) in nylon mesh cages (45 cm × 48 cm × 77 cm; l × w × h) using a 16:8 hrs L:D

photoperiod, 23 °C, and 70 ± 5% R.H. Plants were fertilized with all-purpose micronutrient 20-20-20 (N:P:K) (Jack's Classic, J. R. Peters, Inc., OH) through a drip irrigation system. The colony was supplemented with fresh *Z. elegans* plant material every 30 d. Apterous and alate *M. persicae* were collected from the colony on the day of each bioassay and transferred using a paint brush to a petri dish containing moistened filter paper. Specimens were held for 30 min in the Petri dishes at room temperature until used in bioassays.

Fungi

Agar cubes. Pure mycelial cultures of *B. bassiana* strain GHA growing on 2% malt extract agar (MEA, Sigma-Aldrich, St. Louis, MO) were obtained from the USDA-ARS Collection of Entomopathogenic Fungal Cultures (ARSEF; Ithaca, NY). Mycelial cultures of the plant pathogen *Fusarium proliferatum* (Matsushima) Nirenberg and the nutritional fungal symbiont of ambrosia beetles *Ambrosiella grosmanniae* Mayers, McNew and Harrington strain XgOH11 growing on 2% MEA were obtained from the USDA-ARS Robert W. Holley Center for Agriculture & Health in Ithaca, NY as representatives of non-entomopathogenic fungi for use as negative controls (Castrillo et al. 2016; Mayers et al. 2015). All cultures were maintained on 2% MEA and stored at 25 ± 5°C (0:24 hrs L:D) in the USDA-ARS Horticultural Insects Research Lab. Mycelial cultures were allowed to grow on the MEA plates for about 14 d before use in Petri dish and Y-tube olfactometer bioassays. Cubes of colonized agar were collected after 21 d from the interior of the colonized plates using a sterilized spatula for ~~use~~ in bioassays as described below.

Conidial suspension. Dried conidia of *B. bassiana* were prepared according to Castrillo et al. (2008) and obtained from ARSEF for use in Petri dish and Y-tube bioassays. A stock

119 solution was first prepared by suspending 50 mg of dried conidia in 1 mL of aqueous 0.01%
120 Tween-80 (Sigma-Aldrich, St. Louis, MO). After vortexing the solution for 15 min, the
121 conidia/mL concentration of the stock was measured using a Neubauer hemocytometer
122 (Spencer Bright Line, Buffalo, NY, USA) following Castrillo et al. (2011). Serial dilutions
123 were then prepared in 0.01% Tween 80 (aq) to obtain 1×10^7, 1×10^6, 1×10^5, and 1×10^4
124 conidia/mL for use in bioassays ~~as described below~~.
125
126 Petri Dish Bioassays
127 *Agar cubes*. Two-choice bioassays were conducted using borosilicate glass Petri dishes (100
128 mm in diam., 15 mm height) as an arena to characterize the preference behavior of apterous
129 and alate *M. persicae*. For bioassay purposes, two circles (2.5 cm diam., 4.9 cm^2 area)
130 positioned 3.5 cm apart on opposite sides of the Petri dishes were drawn using a marker on the
131 external surface of the bottom dish. To test mycelial cultures, two cubes of agar (1 cm × 0.7
132 cm; L × W) were placed randomly within each of the two circles in the Petri dishes to permit
133 the following comparisons: (1) *B. bassiana* mycelial cultures vs. agar alone, (2) *B. bassiana*
134 mycelia vs. *F. proliferatum* mycelia, and (3) *B. bassiana* mycelia vs. *A. grosmanniae* mycelia.
135 *Conidial suspension*. To test conidial suspensions, a 100 µl aliquot of 1×10^7 to 1×10^4 *B.*
136 *bassiana* conidia/mL or Tween-80, equidistant to each other at random positions, was applied
137 to saturate a section of filter paper (2 cm × 1.5 cm; L×W; Whatman No.1, Thermo Fisher
138 Scientific).
139 After the treatments were arranged, adult *M. persicae* (n = 10 apterous or n = 5 alates per
140 Petri dish) were transferred using a paintbrush to the center of each Petri dish. The number of
141 *M. persicae* within each of the two circles (7.1 cm^2) containing a treatment (*B. bassiana*

mycelial cultures or conidial suspensions) and control (agar alone or Tween-80) were then counted at 5 min intervals for a total duration of 20 min. Each bioassay was used to compare apterous and alate responses to *B. bassiana* mycelial cultures (cubes) and conidial suspensions, and was repeated 25 times (n = 25). Bioassays to compare apterous *M. persicae* responses to *B. bassiana* mycelial cultures vs. *F. proliferatum*, and *A. grosmanniae* were repeated 20 times (n = 20). Petri dishes were rinsed with 70% ethanol, autoclaved for 30 min, and allowed to cool before use in all bioassays. Petri dish bioassays were carried out under ambient laboratory conditions (25 ± 1°C; 60 ± 10% RH) between 09:00 h and 16:00 h for both forms of aphids.

Y-Tube Bioassays

The response of *M. persicae* to volatile emissions from mycelial cultures and conidia of *B. bassiana* was further assessed using a glass Y-tube olfactometer consisting of a 200 mm stem length, 230 mm arm length with a 60° angle, and internal diameter of 25 mm (Analytical Research Systems, Gainesville, Florida, USA). Air generated from an oil free pump was purified through a three-stage activated charcoal filtration system, regulated using flowmeters, and humidified with distilled and deionized water before passing through custom-made glass stimulus tubes secured via ground glass joints into each arm of the Y-tube (Analytical Research Systems). The Y-tube was placed horizontally on a table and a constant airflow of 100 ml/min was maintained through each arm for a total flow of 200 ml/min.

To test the response of *M. persicae* to mycelial cultures, two cubes of agar (1 cm × 0.7 cm; L× W) colonized by *B. bassiana* mycelia or agar alone were placed within the glass stimulus tubes. A mesh screen was incorporated into the end (e.g. outlet) of the glass stimulus tubes. To test conidial suspensions, a 100 µl aliquot of 1×10^7 to 1×10^4 *B. bassiana* conidia/mL or

Tween-80 was applied to a piece of filter paper positioned within the stimulus tubes (2 cm × 1.5 cm; L×W; Whatman No.1, Thermo Fisher Scientific). Apterous (n = 10) and alate (n = 5) *M. persicae* were separately released at the base of the Y-tube and the number of aphids present in both arms of the Y-tube were monitored at 5 min intervals over a total duration of 20 min. Specimens that remained in the stem of the Y-tube were considered non-responsive and were not counted. Y-tube olfactometer bioassays were carried out under ambient laboratory conditions (25 ± 1°C; 60 ± 10% RH) between 09:00 h and 16:00 h. A total of n = 20 replicates were conducted with apterous and alate aphids to compare the response to *B. bassiana* mycelia vs. agar. A total of n = 10 replicates were conducted with apterous and alate aphids to compare the response to *B. bassiana* conidial suspensions vs. Tween. To eliminate a directional bias, odor source positions were alternated after each replicate within a bioassay.

Statistical Analyses

Time-course count data of *M. persicae* responding to selected stimuli in Petri dish and Y-tube olfactometer bioassays were first analyzed using a repeated measures ANOVA ($\alpha = 0.05$; Proc GLM, SAS Institute Inc., Carry, NC, USA) to test for a significant between-subject effect. Specifically, between-subject effects were tested in the number of *M. persicae* responding to agar colonized by *B. bassiana* mycelia vs. agar alone, *B. bassiana* mycelia vs. *F. proliferatum* and *A. grosmanniae*, and *B. bassiana* conidia vs. Tween-80. If a significant between-subject effect was detected, the ANOVA was done at each date ($\alpha = 0.05$; SAS Institute). Analyses were conducted using count data, but proportions are presented in the figures. Data were evaluated for normality and for homogeneity of variances and no transformations were needed.

Results

Petri Dish Bioassays

During time-course Petri dish bioassays, repeated measures ANOVA detected a significant between-subject effect in the counts of apterous (Fig. 1A: $F_{1,48} = 80.74$; $P < 0.0001$) and alate (Fig. 1B: $F_{1,48} = 29.19$; $P < 0.0001$) *M. persicae* that were recorded within a 7.1 cm^2 area around an agar cube colonized by the entomopathogenic fungus *B. bassiana* vs. agar alone. Significantly more apterous and alate *M. persicae* were observed in the vicinity of agar cubes colonized by *B. bassiana* compared to agar alone at each of the 5 min time points over the 20 min bioassay (Figs. 1A-B, Table 1).

A significant between-subject effect was also detected in the counts of apterous *M. persicae* that were in the vicinity of an agar cube colonized by *B. bassiana* compared to the plant pathogen *F. proliferatum* (Fig. 1C: $F_{1,38} = 378.10$; $P < 0.0001$) and the ambrosia beetle fungal symbiont *A. grosmanniae* (Fig. 1D: $F_{1,38} = 182.14$; $P < 0.0001$). Significantly more apterous *M. persicae* were observed in the vicinity of agar cubes colonized by *B. bassiana* compared to *F. proliferatum* (Fig. 1C) and *A. grosmanniae* at each of the 5 min time points over the 20 min bioassay (Figs. C-D, Table 1).

Subsequent Petri dish bioassays detected a significant between-subject effect in the counts of apterous *M. persicae* that were within a 7.1 cm^2 area around a piece of filter paper saturated with 1×10^7 (Fig. 2A: $F_{1,48} = 71.02$; $P < 0.0001$), 1×10^6 (Fig. 2C: $F_{1,48} = 74.14$; $P < 0.0001$), and 1×10^5 (Fig. 2E: $F_{1,48} = 42.99$; $P < 0.0001$) *B. bassiana* conidia/ml compared to a Tween 80 control. Specifically, more apterous *M. persicae* were observed in the vicinity of filter paper treated with 1×10^7, 1×10^6, and 1×10^5 *B. bassiana* conidia/ml at each of the 5 min time points over the 20 min bioassay (Figs. 2A,C,E; Table 1). In contrast, no difference was

211 detected in the counts of apterous and alate *M. persicae* that selected filter paper saturated with
212 1×10^4 *B. bassiana* conidia/ml compared to a Tween 80 control. (Fig. 2G: $F_{1,48} = 0.31$; $P =$
213 0.58; Fig. 2H: $F_{1,48} = 0.00$; $P = 1.0$).

214 A significant between-subject effect was also detected in the counts of alate *M. persicae*
215 that were recorded in the vicinity of filter paper treated with 1×10^7 (Fig. 2B: $F_{1,48} = 7.02$; $P =$
216 0.011), 1×10^6 (Fig. 2D: $F_{1,48} = 31.62$; $P < 0.0001$), and 1×10^5 (Fig. 2F: $F_{1,48} = 14.84$; $P =$
217 0.0003) conidia/ml compared to the Tween 80 control. Significantly more alate *M. persicae*
218 were observed in the vicinity of filter paper saturated with 1×10^7 conidia/ml compared to the
219 Tween 80 control at the 20 min time point (Fig. 2B; Table 1), while more alate *M. persicae*
220 were recorded in the vicinity of filter paper treated with 1×10^6 (Fig. 2D) and 1×10^5 (Fig. 2F)
221 at each of the 5 min time points over the 20 min bioassay.
222
223 Y-Tube Olfactometer Bioassays
224 Repeated measures ANOVA detected a significant between-subject effect in the counts of
225 apterous (Fig. 3A: $F_{1,38} = 23.58$; $P < 0.0001$) and alate (Fig. 3B: $F_{1,38} = 19.50$; $P < 0.0001$) *M.*
226 *persicae* that oriented towards an agar cube colonized by *B. bassiana* vs. an agar control during
227 time-course Y-tube bioassays. Significantly more apterous and alate *M. persicae* oriented
228 upwind in the Y-tube in response to volatile stimuli from agar cube colonized by *B. bassiana*
229 vs. the agar control at each of the 5 min time points over the 20 min bioassay (Fig. 3A-B, Table
230 1).

231 A significant between-subject effect was detected in the counts of apterous *M. persicae* that
232 oriented upwind during Y-tube bioassays to filter paper saturated with 1×10^7 (Fig. 4A: $F_{1,18} =$
233 12.73; $P = 0.002$) and 1×10^6 (Fig. 4C: $F_{1,18} = 37.41$; $P < 0.0001$) *B. bassiana* conidia/ml as

compared to a Tween-80 control. Significantly more apterous *M. persicae* selected volatile stimuli from the 1×10^7 and 1×10^6 conidia/ml suspension at each of the 5 min time points over the 20 min bioassay (Fig. 4A, C; Table 1). A significant between-subject effect was also detected in the counts of alate *M. persicae* that oriented upwind during Y-tube bioassays to 1×10^7 (Fig. 4B: $F_{1,18} = 11.30$; $P = 0.004$) and 1×10^6 (Fig. 4D: $F_{1,18} = 36.49$; $P < 0.0001$) conidia/ml compared to the control. Significantly more alate *M. persicae* selected the 1×10^7 and 1×10^6 conidial suspension at each of the 5 min time points over the 20 min bioassay (Fig. 4B, D; Table 1). In contrast, there was not a significant between-subject effect in the attraction of apterous (Fig. 4E: $F_{1,18} = 0.95$; $P = 0.34$) and alate (Fig. 4F: $F_{1,18} = 0.20$; $P = 0.7$) *M. persicae* to volatile stimuli from 1×10^5 conidia/ml vs. the Tween-80 control.

Discussion

Volatiles from entomopathogenic and antagonistic fungi are increasingly being recognized for their capability to influence insect behavior (Holighaus and Rohlfs 2016; Cale et al. 2016). During still-air Petri dish bioassays, our current study demonstrated a preference of *M. persicae* for *B. bassiana* mycelia and conidia, but not the fungal plant pathogen *F. proliferatum* or the ambrosia beetle nutritional fungal symbiont *A. grosmanniae*. Notably, the ambrosia beetle *Xylosandrus germanus* (Blandford) exhibited an arrestment response to volatile emissions of its nutritional fungal symbiont, *A. grosmanniae*, but not *B. bassiana* or *F. proliferatum* (Ranger et al. 2021). Our current study also demonstrated *M. persicae* were attracted to volatiles emitted from *B. bassiana* mycelia and conidia Y-tube olfactometer bioassays. The attraction of *M. persicae* to *B. bassiana* mycelia and conidia complements George et al. (2013) that demonsrated attraction of *A. stephensi* to volatiles from *B. bassiana*.

While certain insects can detect and avoid *B. bassiana* (Lord 2001, Meyling and Pell 2006, Mburu et al. 2009, Hussain et al. 2010, Ormond et al. 2011), it is conceivable that as a parasitic, entomopathogenic fungus, *B. bassiana* would benefit from evolving to attract rather than repel insect hosts.

Understanding the behavioral response of insects to *B. bassiana* and other entomopathogenic fungi could aid in implementing the use of formulations. For instance, George et al. (2013) demonstrated that mosquitoes are attracted to conidia of *B. bassiana* and *Heliothis subflexa* caterpillars infected with *B. bassiana*. Subsequent experiments demonstrated that cloth treated with oil-formulated *B. bassiana* conidia also attracted female mosquitoes, which resulted in 95% of the attracted females becoming infected with *B. bassiana* after one minute of contact. In contrast, certain insects avoid substrates treated with *B. bassiana*. The generalist predator *A. nemorum* avoids leaf surfaces treated with conidia of *B. bassiana* (Meyling and Pell 2006), and the seven-spot ladybird beetle *C. septempunctata* can detect and avoid lethal densities of *B. bassiana* conidia on leaves and soil (Ormond et al. 2011). Future studies are warranted to characterize the response of *M. persicae* to plants or other substrates treated with *B. bassiana* formulations.

The mechanism by which *B. bassiana* volatile emissions function as semiochemicals in attracting or repelling insects is not well understood, but insight is being gained into the array of ubiquitous and less common volatile compounds emitted from *B. bassiana*. Using solid phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS), Crespo et al. (2008) detected six compounds emitted from mycelia of culture-grown *B. bassiana* including two unidentified diisopropylnaphthalene compounds and two unidentified sesquiterpenes. Hussain et al. (2010) tentatively identified 11 compounds by SPME-GC-MS emitted from

culture-grown *B. bassiana*, including the monoterpenes 2-thujene and 2-isopropyl-5-methyl-3-cyclohexen-1-one and an isomer of naphthalene identified as azulene. Similarly, Bojke et al. (2018) detected two sesquiterpenes (γ-gurjunen and squalene), fatty acids, and 3-methylbutanal in volatile emissions from mycelia of *B. bassiana* by SPME-GC-MS. Most recently, Ranger et al. (2021) reported a tentatively identified sesquiterpene (i.e., β-elemene) along with a variety of ubiquitous compounds consisting of three alcohols (i.e., 2-methyl-1-butanol, 3-methyl-1-butanol, 1-octen-3-ol), one aldehyde (i.e., 2-octenal), and two esters (i.e., methyl benzoate, methyl cinnamate).

Blends of compounds emitted from *B. bassiana* and other fungi likely play more of a role in influencing insect behavior than individual compounds (Davis et al. 2013). Subsequent electrophysiological and behavioral experiments are warranted to identify volatile blends and/or individual compounds from *B. bassiana* that attract *M. persicae*. 'Lure and kill' strategies might be facilitated by identifying and selecting attractive strains of *B. bassiana* and/or by incorporating semiochemicals into *B. bassiana* formulations. For example, combining a pheromone and auto-infection system disseminating *B. bassiana* resulted in high mortality of the sweet potato weevil, *Cylas formicarius* (Fabricius) (Yasuda 1999). Attracting the spruce bark beetle *Ips typographus* to pheromone-baited traps treated with *B. bassiana* led to reduced burrowing by the foundress beetles and a failure to produce offspring within the host tree galleries (Kreutz et al. 2004). Deploying pheromone lures in plots treated with *B. bassiana* resulted in higher mortality of the banana weevil *Cosmopolites sordidus* (Germar) compared to plots treated with *B. bassiana* but absent of the pheromone lures (Tinzaara et al. 2007).

Since *B. bassiana* conidia can be transported through the air, it is possible that aphids encountered conidia while moving upwind during our Y-tube olfactometer bioassays. Other studies that have assessed the orientation of insects to *B. bassiana* mycelia and conidia could also have been limited by this airborne dispersal (Lord 2001, Meyling and Pell 2006, Mburu et al. 2009, Hussain et al. 2010, Ormond et al. 2011, George et al. 2013). Testing conidial suspensions in Tween-80 applied to filter paper may have reduced the movement of conidia during our current study and the aforementioned studies. Furthermore, a mesh screen was downwind of the *B. bassiana* mycelia and conidia in the Y-tube olfactometer used during our current study, which could have impeded further downwind movement of the conidia.

Overall, our current study found that apterous and alate *M. persicae* were attracted to mycelia and conidia of *B. bassiana*. While the majority of studies conducted to date have demonstrated avoidance of *B. bassiana* by insects following the detection of 'enemy-specific volatile compounds', our results complement the finding that mosquitoes are attracted to volatiles emitted from *B. bassiana* (George et al. (2013). Future studies aimed at characterizing the olfactory basis for attraction of *M. persicae* to *B. bassiana*, along with behavioral and olfactory responses of other insects, would provide important insights into the capability of volatiles from entomopathogenic and antagonistic fungi to influence insect behavior. Enhancing the attraction of *M. persicae* to *B. bassiana* could also be useful for lure-and-kill strategies.

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References Cited

Allegrucci, N., M. S. Velazquez, M. L. Russo, M. F. Vianna, C. Abarca, and A. C. Scorsetti. 2020. Establishment of the entomopathogenic fungus *Beauveria bassiana* as an endophyte in *Capsicum annuum* and its effects on the aphid pest *Myzus persicae* (Homoptera: Aphididae). Rev. Biol. Trop. 68: 1084–1094.

Biryol, S., Z. Demirbağ, P. Erdoğan, and I. Demir. 2022. Development of *Beauveria bassiana* (Ascomycota: Hypocreales) as a mycoinsecticide to control green peach aphid, *Myzus persicae* (Homoptera: Aphididae) and investigation of its biocontrol potential. J. Asia-Pac. Entomol. 25: 101878.

Bojke, A., C. Tkaczuk, P. Stepnowski, and M. Gołębiowski. 2018. Comparison of volatile compounds released by entomopathogenic fungi. Microbiol. Res. 214: 129–136.

Cale, J. A., R. M. Collignon, J. G. Klutsch, S. S. Kanekar, A. Hussain, and N. Erbilgin. 2016. Fungal volatiles can act as carbon sources and semiochemicals to mediate interspecific interactions among bark beetle-associated fungal symbionts. PLoS One. 11: e0162197.

Castrillo, L. A., T. A. Ugine, M. J. Filotas, J. P. Sanderson, J. D. Vandenberg, and S. P. Wraight. 2008. Molecular characterization and comparative virulence of *Beauveria bassiana* isolates (Ascomycota: Hypocreales) associated with the greenhouse shorefly, *Scatella tenuicosta* (Diptera: Ephydrididae). Biol. Control. 45: 154–162.

Castrillo, L. A., M. H. Griggs, C. M. Ranger, M. E. Reding, and J. D. Vandenberg. 2011. Virulence of commercial strains of *Beauveria bassiana* and *Metarhizium brunneum* (Ascomycota: Hypocreales) against adult *Xylosandrus germanus* (Coleoptera: Curculionidae) and impact on brood. Biol. Control. 58: 121–126.

Castrillo, L. A., M. H. Griggs, and J. D. Vandenberg. 2016. Competition between biological control fungi and fungal symbionts of ambrosia beetles *Xylosandrus crassiusculus* and *X. germanus* (Coleoptera: Curculionidae): mycelial interactions and impacts on beetle brood production. Biol. Control. 103: 138–146.

Crespo, R., N. Pedrini, M. P. Juárez, and G. M. Dal Bello. 2008. Volatile organic compounds released by the entomopathogenic fungus *Beauveria bassiana*. Microbiol. Res. 163: 148–151.

Cheong, P. C., T. R. Glare, M. Rostás, S. Haines, J. J. Brookes, and S. Ford. 2020. Lack of involvement of chitinase in direct toxicity of *Beauveria bassiana* cultures to the aphid *Myzus persicae*. J. Invertebr. Pathol. 169: 107276.

Davis, T. S., T. L. Crippen, R. W. Hofstetter, and J. K. Tomberlin. 2013. Microbial volatile emissions as insect semiochemicals. J. Chem. Ecol. 39: 840–859.

Dedryver, C. A., A. Le Ralec, and F. Fabre. 2010. The conflicting relationships between aphids and men: a review of aphid damage and control strategies. C. R. Biol. 333: 539–553.

Dewar, A. M. 2007. Chemical control, pp. 391–422. *In* H. F. Van Emden, and R. Harrington (eds.), Aphids as crop pests. CAB International, Wallingford, UK.

Dicke, M., and P. Grostal. 2001. Chemical detection of natural enemies by arthropods: an ecological perspective. Annu. Rev. Ecol. Evol. Syst. 32: 1–23.

de Faria, M. R., and S. P. Wraight. 2007. Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. Biol. Control. 43: 237–256.

George, J., N. E. Jenkins, S. Blanford, M. B. Thomas, and T. C. Baker. 2013. Malaria Mosquitoes Attracted by Fatal Fungus. PLoS ONE. 8: e62632.

Holighaus, G., and M. Rohlfs. 2016. Fungal allelochemicals in insect pest management. Appl. Microbiol. Biotechnol. 100: 5681–5689.

Hussain, A., M-Y. Tian, Y-R. He, J. M. Bland, and W-X. Gu. 2010. Behavioral and electrophysiological responses of *Coptotermes formosanus* Shiraki towards entomopathogenic fungi volatiles. Biol. Control. 55: 166–173.

Jandricic, S. E., M. Filotas, J. P. Sanderson, and S. P. Wraight. 2014. Pathogenicity of conidia -based preparations of entomopathogenic fungi against the greenhouse pest aphids *Myzus persicae*, *Aphis gossypii*, and *Aulacorthum solani* (Hemiptera: Aphididae). J. Invertebr. Pathol. 118: 34–46.

Jaronski, S.T. 2014. Mass production of beneficial organisms, pp. 357–413. *In* (Chapter 11) Mass production of entomopathogenic fungi: state of the art. Elsevier Inc. Sidney, MT, USA.

Kreutz, J., G. Zimmermann, and O. Vaupel. 2004. Horizontal transmission of the entomopathogenic fungus *Beauveria bassiana* among the spruce bark beetle, *Ips typographus* (Col., Scolytidae) in the laboratory and under field conditions. Biocontrol Sci. Technol. 14: 837–848.

Lord, J. C. 2001. Response of the wasp *Cephalonomia tarsalis* (Hymenoptera: Bethylidae) to *Beauveria bassiana* (Hyphomycetes: Moniliales) as free conidia or infection in its host, the sawtoothed grain beetle, *Oryzaephilus surinamensis* (Coleoptera: Silvanidae). Biol. Control. 21: 300–304.

Mantzoukas, S., and I. Lagogiannis. 2019. Endophytic colonization of pepper (*Capsicum annum*) controls aphids (*Myzus persicae* Sulzer). Appl. Sci. 9: 2239.

Mayers, C. G., D. L. McNew, T. C. Harrington, R. A. Roeper, S. W. Fraedrich, P. H. Biedermann, L. A. Castrillo, and S. E. Reed. 2015. Three genera in the Ceratocystidaceae are the respective symbionts of three independent lineages of ambrosia beetles with large, complex mycangia. Fungal Biol. 119: 1075–1092.

Mburu, D. M., L. Ochola, N. K. Maniania, P. G. N. Njagi, L. M. Gitonga, M. W. Ndung'u, A. K. Wanjoya, and A. Hassanali. 2009. Relationship between virulence and repellency of entomopathogenic isolates of *Metarhizium anisopliae* and *Beauveria bassiana* to the termite *Macrotermes michaelseni*. J. Insect Physiol. 55: 774–780.

Meyling, N. V., and J. K. Pell. 2006. Detection and avoidance of entomopathogenic fungi by a generalist insect predator. Ecol. Entomol. 31: 162–171.

Milner, R. J. 1997. Prospects for biopesticides for aphid control. Entomophaga. 42: 227–239.

Ormond, E. L., A. P. M. Thomas, J. K. Pell, S. N. Freeman, and H. E. Roy. 2011. Avoidance of a generalist entomopathogenic fungus by the ladybird, *Coccinella septempunctata. FEMS* Microbiol. Ecol. 77: 229–237.

Ortiz-Urquiza, A., L. Riveiro-Miranda, C. Santiago-Álvarez, and E. Quesada-Moraga. 2010. Insect-toxic secreted proteins and virulence of the entomopathogenic fungus *Beauveria bassiana*. J. Invertebr. Pathol. 105: 270–278.

Ownley, B. H., M. R. Griffin, W. E. Klingeman, K. D. Gwinn, J. K. Moulton, and R. M. Pereira. 2008. *Beauveria bassiana*: endophytic colonization and plant disease control. J. Invertebr. Pathol. 98: 267–270.

Prince, G., and D. Chandler. 2020. Susceptibility of *Myzus persicae*, *Brevicoryne brassicae* and *Nasonovia ribisnigri* to fungal biopesticides in Laboratory and Field experiments. Insects. 11: 55.

Ranger, C. M., M. Dzurenko, J. Barnett, R. Geedi, L. Castrillo, M. Ethington, M. Ginzel, K. Addesso, and M. E. Reding. 2021. Electrophysiological and Behavioral Responses of an Ambrosio Beetle to Volatiles of its Nutritional Fungal Symbiont. J. Chem. Ecol. 47: 463–475.

Tinzaara, W., C. S. Gold, M. Dicke, A. Van Huis, C. M. Nankinga, G. H. Kagezi, and P. E. Ragama. 2007. The use of aggregation pheromone to enhance dissemination of *Beauveria bassiana* for the control of the banana weevil in Uganda. Biocontrol Sci. Technol. 17: 111–124.

Vega, F. E. 2008. Insect pathology and fungal endophytes. J. Invertebr. Pathol. 98: 277–279.

Yasuda, K. 1999. Auto-infection system for the sweet potato weevil, *Cylas formicarius* (Fabricius) (Coleoptera: Curculionidae) with entomopathogenic fungi, *Beauveria bassiana* using a modified sex pheromone trap in the field. Appl. Entomol. Zool. 34: 501–505.

Ye, C., Z. W. Wang, Y. L. Sheng, Z. G. Wang, G. Smagghe, O. Christiaens, J. Niu, and J. J. Wang. 2022. GNBP1 as a potential RNAi target to enhance the virulence of *Beauveria bassiana* for aphid control. J. Pest Sci. 95: 87–100.

Zimmermann, G. 2007. Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. Biocontrol Sci. Technol. 17: 553–596.

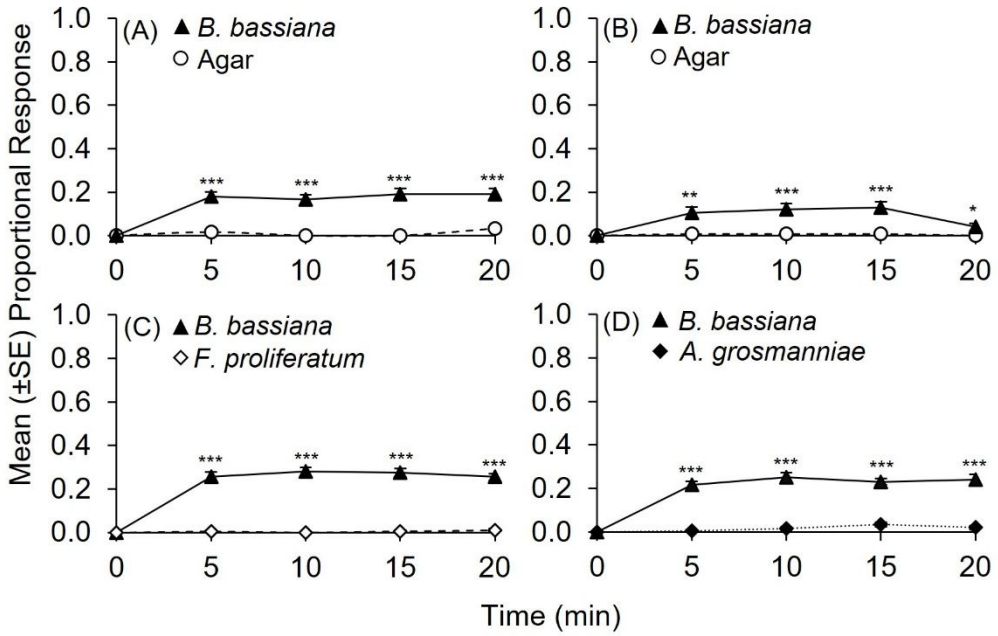

Figure 1. Proportion of apterous (A) and alate (B) forms of *Myzus persicae* within a 7.1 cm^2 area around an agar cube colonized by *Beauveria bassiana* vs. agar alone during Petri dish bioassays. The proportion of apterous *M. persicae* responding to agar colonized by *B. bassiana* vs. the plant pathogen *Fusarium proliferatum* (C), and *B. bassiana* vs. the ambrosia beetle fungal symbiont *Ambrosiella grosmanniae* (D). If a significant between-subject effect was detected by repeated measures ANVOA, then a paired student's t-test was used to test for differences between treatments at individual time points (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; See Table 1 for values). Count data were used for statistical analyses, but proportions are presented.

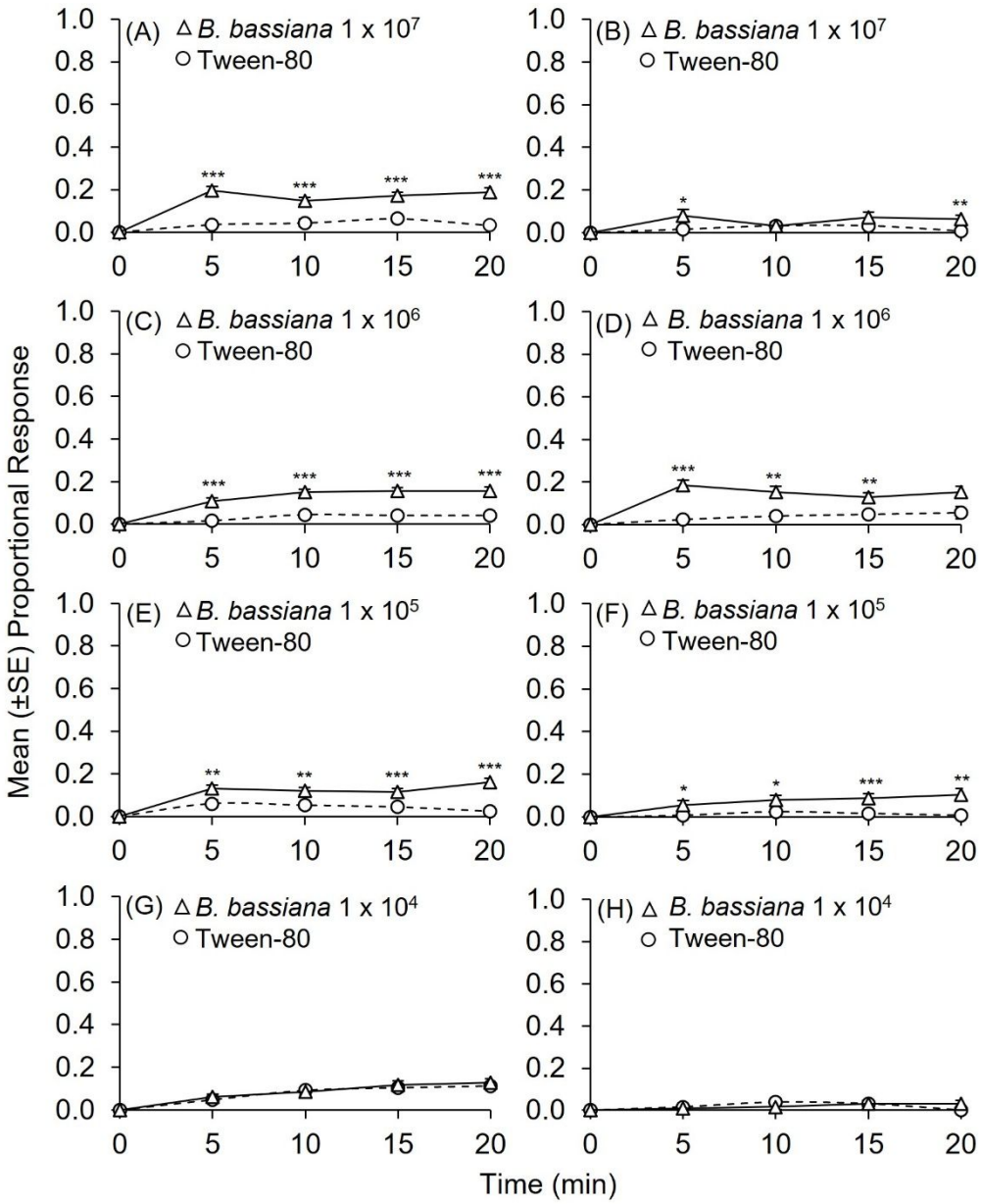

Figure 2. Proportion of apterous (A, C, E, G) and alate (B, D, F, H) forms of *Myzus persicae* within a 7.1 cm^2 area around filter paper containing a conidial suspension of *Beauveria bassiana* at 1×10^7 conidia/mL (A, B), 1×10^6 conidia/mL (C, D), 1×10^5 conidia/mL (E, F), and 1×10^4 conidia/mL (G, H) vs. Tween-80 and during Petri dish bioassays. If a significant between-subject effect was detected by repeated measures ANVOA, then a paired student's t-test was used to test for differences between treatments at individual time point (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; See Table 1 for values). Count data were used for statistical analyses, but proportions are presented.

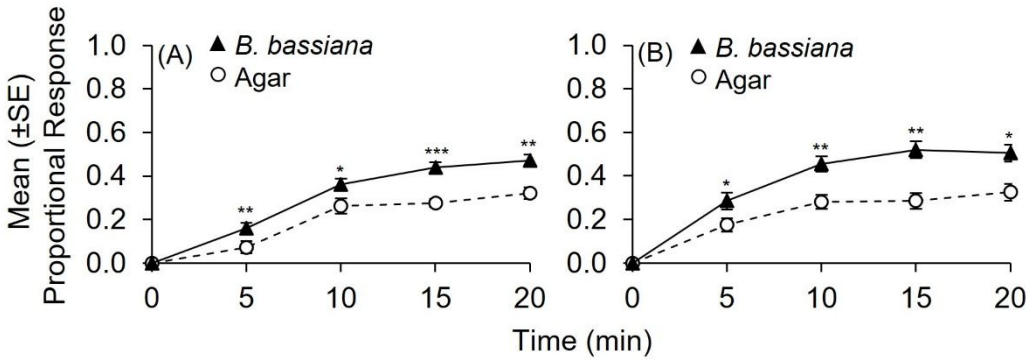

Figure 3. Proportion of apterous (A) and alate (B) forms of *Myzus persicae* responding to an agar cube colonized by *Beauveria bassiana* vs. agar during Y-tube olfactometer bioassays. If a significant between-subject effect was detected, then a paired student's t-test was used to test for differences between treatments at individual time point (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; See Table 1 for values). Count data were used for statistical analyses, but proportions are presented.

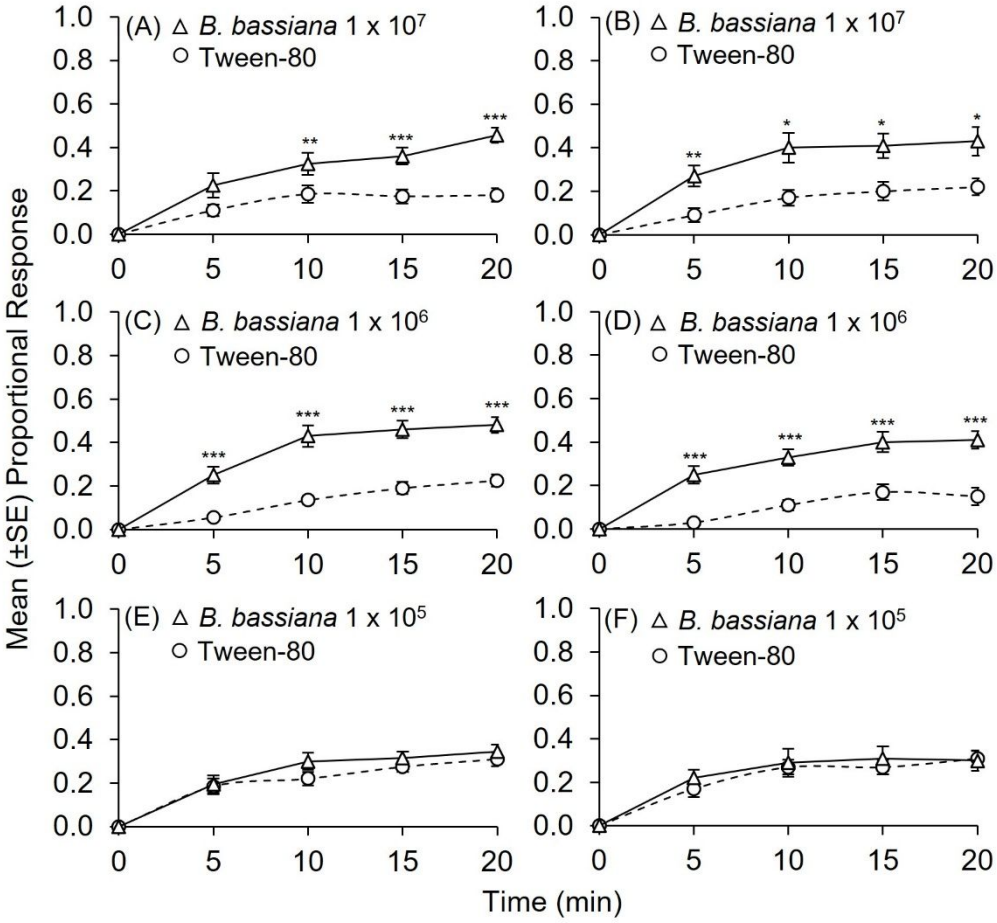

Figure 4. Proportion of apterous (A, C, E) and alate (B, D, F) forms of *Myzus persicae* that oriented towards a conidial suspension of *Beauveria bassiana* at 1×10^7 (A, B), 1×10^6 (C, D), and (E, F) 1×10^5 conidia/ml vs. Tween-80 during Y-tube bioassays. If a significant between-subject effect was detected, then a paired student's t-test was used to test for differences between treatments at individual time point (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; See Table 1 for values). Count data were used for statistical analyses, but proportions are presented.

Table 1. Statistical output from Petri dish and Y-tube olfactometer bioassays assessing the behavioral response of apterous (Ap) and alate (Al) *M. persicae* to the entomopathogenic fungus *B. bassiana*. Count data were analyzed but proportions are presented in the figures.

				Time (min)			
				5	10	15	20
Bioassay	Reps	Comparison	Form	*t*, df, *P* values			
Petri Dish	25	*Bb* vs. Agar	Ap	6.22, 24, <0.0001	8.50, 24, <0.0001	7.65, 24, <0.0001	5.78, 24, <0.0001
	25	*Bb* vs. Agar	Al	3.12, 24, 0.005	3.65, 24, 0.0013	4.24, 24, 0.0003	2.45, 48, <0.022
	20	*Bb* vs. *Fp*	Ap	11.18, 19, <0.0001	14.00, 19, <0.0001	13.08, 19, <0.0001	15.96, 19, <0.0001
	20	*Bb* vs. Ag	Ap	13.08, 19, <0.0001	10.64, 19, <0.0001	11.49, 19, <0.0001	8.90, 19, <0.0001
	25	10^7 Conidia vs. Tween	Ap	6.36, 24, <0.0001	5.10, 24, 0.0001	5.01, 24, <0.0001	6.74, 24, <0.0001
	25	10^6 Conidia vs. Tween	Ap	7.18, 24, <0.0001	7.69, 24, <0.0001	6.15, 24, <0.0001	4.92, 24, <0.0001
	25	10^5 Conidia vs. Tween	Ap	3.39, 24, 0.003	4.24, 24, 0.002	4.27, 24, 0.0004	6.83, 24, <0.0001
	25	10^4 Conidia vs. Tween	Ap	0.68, 24, 0.5	0.39, 24, 0.7	0.65, 24, 0.5	0.94, 24, 0.4
	25	10^7 Conidia vs. Tween	Al	2.14, 24, 0.043	0.00, 24, 1.00	1.55, 24, 0.13	3.06, 24, 0.01
	25	10^6 Conidia vs. Tween	Al	6.20, 24, <0.0001	3.65, 24, 0.0013	3.10, 24, 0.01	2.07, 24, 0.05
	25	10^5 Conidia vs. Tween	Al	2.30, 24, 0.031	2.59, 24, 0.02	3.67, 24, 0.001	3.36, 24, 0.003
	25	10^4 Conidia vs. Tween	Al	0.57, 24, 0.6	1.14, 24, 0.3	0.00, 24, 1.00	2.14, 24, 0.04
Y-Tube	20	*Bb* vs. Agar	Ap	3.17, 19, 0.01	2.30, 19, 0.033	5.05, 19, <0.0001	2.98, 19, 0.01
	20	*Bb* vs. Agar	Al	2.34, 19, 0.030	3.49, 19, 0.002	3.64, 19, 0.002	2.55, 19, 0.020
	10	10^7 Conidia vs. Tween	Ap	3.37, 9, 0.01	3.63, 9, 0.01	5.41, 9, 0.0004	6.21, 9, 0.0002
	10	10^6 Conidia vs. Tween	Ap	5.65, 9, 0.0003	5.36, 9, 0.001	5.01, 9, 0.001	5.45, 9, 0.0004
	10	10^5 Conidia vs. Tween	Ap	0.24, 9, 0.82	2.14, 9, 0.06	1.92, 9, 0.10	2.33, 9, 0.05
	10	10^7 Conidia vs. Tween	Al	5.51, 9, 0.0004	2.97, 9, 0.02	3.04, 9, 0.014	2.60, 9, 0.03
	10	10^6 Conidia vs. Tween	Al	4.97, 9, 0.001	5.66, 9, 0.0003	3.98, 9, 0.003	4.99, 9, 0.001
	10	10^5 Conidia vs. Tween	Al	1.86, 9, 0.096	0.31, 9, 0.76	0.65, 9, 0.53	0.23, 9, 0.82

A paired student's t-test was used to test for differences at individual time points (Figs. 1−4).