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Granulate ambrosia beetle, *Xylosandrus crassiusculus* (Coleoptera: Curculionidae), survival and brood production following exposure to entomopathogenic and mycoparasitic fungi

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HIGHLIGHTS

- *Xylosandrus crassiusculus* is susceptible to *Beauveria bassiana* and *Metarhizium brunneum*.
- Exposed females had lower survival rates and produced smaller brood.
- Exposure to mycoparasitic fungus affected symbiont growth in galleries.

GRAPHICAL ABSTRACT

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ABSTRACT

The granulate ambrosia beetle, *Xylosandrus crassiusculus*, is one of the most important exotic pests in orchards and nurseries in the US. The beetle has a wide host range, including some of the most popular and valuable trees in nurseries, and is difficult to control using chemical insecticides because of its cryptic habits. In this study we evaluated the susceptibility of *X. crassiusculus* to three commercial strains of entomopathogenic fungi and also examined gallery formation and brood production among females exposed to these biocontrol agents and a mycoparasitic fungus, *Trichoderma harzianum*. All three entomopathogens were virulent to *X. crassiusculus*: *Beauveria bassiana* strains GHA and Naturalis and *Metarhizium brunneum* F52 produced 76.7 ± 7%, 95.6 ± 1.1%, and 78.9 ± 7.8% mortality, respectively, among treated adult females at the highest dose (600 conidia/mm^2) 5 days after inoculation. Females exposed to beech stems treated with entomopathogenic fungi at the highest concentration had lower survival rates and produced fewer galleries. All concentrations tested, however, resulted in females with fewer offspring compared to control. Those exposed to *T. harzianum* strain KRL-AG2 produced galleries with sparse, patchy, or no symbiont growth, many of which had no or few brood present. Some of the females exposed to either *B. bassiana* or *M. brunneum* also had galleries with sparse mycelial growth. These results show the potential of entomopathogenic or mycoparasitic fungi in controlling *X. crassiusculus*, either directly by killing adult females and preventing or reducing brood production or indirectly by suppressing growth or establishment of their fungal symbiont in galleries.

1. Introduction

The granulate ambrosia beetle (formerly called Asian ambrosia beetle), *Xylosandrus crassiusculus* (Motschulsky) (Coleoptera: Curculionidae: Scolytinae), is native to Asia and an invasive pest in

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North America (Rabaglia et al., 2006). It was first discovered in South Carolina in 1974 (Anderson, 1974) and has since spread throughout the southeast, as far north as New York and west to Texas, with isolated pockets reported in Oregon and Washington (CAB International, 2013; Rabaglia et al., 2006; Mudge et al., 2001). It is considered one of the most important exotic pests of nursery grown trees in the US (Oliver and Mannion, 2001). *X. crassiusculus* attacks over 200 plant species, and host range includes woody ornamentals, fruit and nut trees (Wood and Bright, 1992; Schedl, 1963). Reported hosts include Bradford pear, crape myrtle, cherry, dogwood, magnolia, peach, and plum (Mizell et al., 1994). Beetles cause boring damage when they tunnel through sapwood to form galleries for brood production, and subsequent large cankers formed at site of attacks may cause death by girdling. Mizell and Riddle (2004) observed that 10 or more attacks per tree would kill all trees with a trunk caliper of less than 3 inches. Beetles may also carry secondary fungi (e.g., *Fusarium* spp.) or bacteria that could cause diseases (e.g., Beaver, 1989; Whitney, 1982), and abandoned galleries could be vulnerable to pathogenic or wood decay fungi (Hijii et al., 1991). Furthermore, beetles also attack sawn logs, reducing their value due to boring damage and discoloration from the growth of its fungal symbiont (Atkinson et al., 2011).

Like other ambrosia beetles, *X. crassiusculus* is associated with a symbiotic fungus. Females carry *Ambrosiella xylebori* Brader (Ascomycota: Microascales) in a specialized structure, a mycangium, located between the pro- and mesothoracic nota (Francke-Grossman, 1967). The fungus grows in galleries excavated by the adult females and serves as the food source of both adults and larvae (Beaver, 1989; Francke-Grossman, 1967). *X. crassiusculus* exhibits arrhenotokous parthenogenesis, with a female-biased sex ratio. Adult female progeny mate with their siblings before dispersal to found new galleries (Peer and Taborsky, 2004). Only adults, most often only females, spend a relatively short time outside host trees during dispersal and host selection. Males are smaller than females and have reduced, non-functional wings (Peer and Taborsky, 2004, 2007). Because of their cryptic habits, control strategies using conventional insecticides require repeated applications, sprayings timed to coincide closely with beetle attacks, or the application of pesticides with long residual activity (Hudson and Mizell, 1999). Present control recommendations also include cultural methods: removal and burning of infested trees and retaining some infested trees to attract newly-arriving beetles and thus reduce total number of trees attacked in a nursery block (Mizell and Riddle, 2004).

We are currently evaluating the potential of microbial control agents to target exotic ambrosia beetles, including the brood inside galleries (c.f., Prazak, 1991). Laboratory studies on the ambrosia beetle *Xylosandrus germanus* Blandford have shown that it is susceptible to commercial strains of the entomopathogens *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium brunneum* Petch (Ascomycota: Hypocreales) (Castrillo et al., 2011). Sprayed beetles had significantly higher mortality compared with controls, resulting in reduced gallery formation and brood production in rearing tubes (Castrillo et al., 2011). Furthermore, some of the broods produced by treated females had up to 100% fungal infection, from inocula generated from the female cadaver, suggesting that the impact of treatments extends beyond the survival of treated females (Castrillo et al., 2011).

In this study we evaluated the susceptibility of *X. crassiusculus* to commercial strains of *B. bassiana*, strains GHA (Botanigard®, Laverlam international) and Naturalis (Naturalis-L®, Troy Biosciences), and *M. brunneum* strain F52 (Taenure®, Novozyme Biologicals). We also evaluated gallery formation and brood production among foundresses exposed to these control agents as well as to the mycoparasitic fungus *Trichoderma harzianum* Rifai (Ascomycota: Hypocreales) strain KRL-AG2 (RootShield®, BioWorks, Inc.) in a natural substrate – beech stems.

2. Materials and methods

2.1. Beetle collection and rearing

Beetles were collected from woodlands in the D.P. Flint Nassau County 4-H camp, Riverhead, NY, in May 2011, using ethanol baited traps as described in Castrillo et al. (2011). Beetles were reared on sawdust-based artificial diet reported by Peer and Taborsky (2004), modified from Norris and Chu (1985). The diet consisted of 75 g beech sawdust, 20 g agar, 10 g sucrose, 5 g starch, 5 g casein, 5 g yeast, 1 g Wesson salt mixture, 0.35 g streptomycin, 5 ml 95% ethanol, 2.5 ml wheat germ oil and 500 ml distilled water. Beech sawdust was prepared from logs collected in early April of 2011 and processed as reported in Castrillo et al. (2011). The diet mixture was autoclaved for 60 min at 121 °C and 25 ml poured into clear 50-ml polypropylene tubes. Diet tubes were loosely capped and kept in a laminar flow hood for 4 days to evaporate condensation. A flamed forceps was used to score the diet surface lightly prior to use. This provides a surface texture that facilitates tunneling activity by foundresses (Norris and Chu, 1985). Field collected beetles were surface-disinfected by washing for 10 sec in 70% ethanol, followed by two rinses in sterile distilled water and transfer to sterile Petri dishes lined with autoclaved filter paper. After $\geqslant$10 min, active beetles were transferred into individual diet tubes. Rearing tubes were placed horizontally in plastic bins (6 cm width $\times$ 28 cm length $\times$ 10 cm height) lined with paper towels and fitted with a lid and incubated at 25 °C in darkness. Bins were misted with distilled water weekly after the second week to minimize diet drying and optimize conditions for growth of the fungal symbiont. Following establishment of the beetle colony, adult female progeny were transferred to fresh diet tubes every 6–7 weeks to maintain the colony.

2.2. Fungal strains and mass production

Cultures or spore powders of entomopathogenic fungi *B. bassiana* strains GHA and Naturalis (= ATCC 74040) and *M. brunneum* strain F52 (= ARSEF 5198) were obtained from Laverlam International (Butte, MT), the American Type Culture Collection (ATCC, Manassas, VA), and the ARS Entomopathogenic Culture Collection (ARSEF, Ithaca, NY), respectively. Single spore isolates were established from cultures of each strain as described by Castrillo et al. (2004), and conidia were produced as reported in Castrillo et al. (2008). Dried conidia were stored at −20 °C until use. All strains are maintained at the USDA ARS Robert Holley Center, Ithaca, NY. A commercial formulation of the mycoparasitic fungus*T. harzianum* strain KRL-AG2 (RootShield® WP; recommended rate of 85–141 g per gallon for outdoor nursery crops, with 1.51% active ingredient or 1×10^7 colony forming units or CFU/g) was provided courtesy of BioWorks (Victor, NY).

2.3. Bioassay: virulence to adults

The comparative virulence of three entomopathogenic fungi against *X. crassiusculus* females was determined by conducting bioassays of laboratory-reared beetles. Adult females, emerged from galleries within rearing tubes, were collected from 6-week old broods and held in Petri dishes lined with moist filter paper at room temperature until use on the same day.

Fungal stock suspensions (25 mg conidia in 15 ml of 0.01% aqueous Tween 80 with 1 g of 2 mm diameter glass beads in 50 ml polypropylene tubes) were mixed using a wrist-action shaker (Model BT, Burell Scientific Inc., Pittsburg, PA) at 6.7 oscillations/sec for 15 min. The resulting conidial suspensions were quantified using a Neubauer hemocytometer and used to prepare a series of dilutions