Journal of Invertebrate Pathology 99 (2008) 312–317

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

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

Virulence of Hypocreales fungi to pecan aphids (Hemiptera: Aphididae) in the laboratory

David I. Shapiro-Ilan [a,*], Ted E. Cottrell [a], Mark A. Jackson [b], Bruce W. Wood [a]

[a] USDA-ARS, SE Fruit and Nut Tree Research Laboratory, Byron, GA 31008, USA
[b] USDA-ARS-NCAUR, 1815 N. University Street, Peoria, IL 61604, USA

ARTICLE INFO

Article history:
Received 25 May 2008
Accepted 8 July 2008
Available online 13 July 2008

Keywords:
Aphid
Beauveria bassiana
Melanocallis caryaefoliae
Monellia caryella
Metarhizium anisopliae
Monelliopsis pecanis
Pecan
Isaria fumosorosea

ABSTRACT

There is need for efficacious biocontrol agents for aphids in commercial orchards. As a preliminary step to this end we determined the virulence of several Hypocreales fungi to pecan aphids. In the first experiment we tested the virulence of *Isaria fumosorosea* (ARSEF 3581) blastospores to three pecan aphids *Monellia caryella*, *Melanocallis caryaefoliae*, and *Monelliopsis pecanis* under laboratory conditions. Rates of 1×10^7 or 1×10^8 spores per ml were applied in 2 ml via a spray tower to 90 mm Petri dishes containing 10 aphids each. Mortality and mycosis were determined after 24, 48 and 72 h. Treatment effects were observed by 48 h post-application, and by 72 h the higher application rate caused >90% mortality and mycosis in *M. caryella* and *M. caryaefoliae*, whereas <70% was observed in *M. pecanis*.
We conducted two subsequent experiments (Experiments 2 and 3), using the same methodology, to compare the virulence of several Hypocreales species and strains against the aphid of primary economic concern to most pecan growers, *M. caryaefoliae*. In Experiment 2, we compared blastospores and conidia of two *I. fumosorosea* strains (ARSEF 3581 and ATCC 20874 [= strain 97]). The blastospores of ARSEF 3581 and conidia of ATCC 20874 showed higher virulence than other treatments and thus were included in Experiment 3, which also compared the virulence of conidia of *Beauveria bassiana* (GHA strain) and *Metarhizium anisopliae* (F52 strain). Results in Experiment 3 indicated the highest virulence in *I. fumosorosea* 3581 blastospores and *M. anisopliae* (F52) followed by *I. fumosorosea* (20874) conidia. The detection of pathogenicity to pecan aphids establishes the potential for commercial usage and additional study. Results reported here will narrow treatments to test in future greenhouse and field trials.
Published by Elsevier Inc.

1. Introduction

Pecan is economically the most important nut crop native to North America (Wood, 2003). The pecan aphid complex consists of three species: the black pecan aphid, *Melanocallis caryaefoliae* (Davis), the blackmargined aphid, *Monellia caryella* (Fitch), and the yellow pecan aphid, *Monelliopsis pecanis* Bissell. These aphids feed and reproduce during the foliated growing season, and during early autumn lay eggs that overwinter in bark crevices (Hudson, 2007). All three species are serious pests of pecan because of their potential to reduce current and subsequent season nut yield and quality due to direct and indirect damage that diminish tree energy reserves (Tedders and Wood, 1985; Wood et al., 1987). Generally, *M. caryaefoliae* is considered the most economically important aphid in pecans (Cottrell et al., 2002; Wood, 2003).

Current control recommendations for pecan aphids rely on usage of chemical insecticides once populations reach economic thresholds (Dutcher et al., 2003; Hudson et al, 2006). A negative side-effect is that chemical pesticides can lead to pesticide resistance by the aphids and/or the destruction of beneficial natural enemies (Dutcher et al., 2003; Ellington et al., 1995; Pickering et al., 1990), hence, research toward developing alternative pest management tactics is warranted.

Entomopathogenic fungi may have promise as an alternative control measure for aphid management in commercial orchard enterprises. Although certain entomopathogenic fungi, (i.e., several species in the order Entomophthorales) have been reported as natural pathogens that may regulate pecan aphid populations (Ekbom and Pickering, 1990; Pickering et al., 1990), no studies have investigated direct application of fungi to aphids for assessment of inundative or inoculative approaches. Several Hypocreales fungi, such as *Beauveria bassiana* (Balsamo) Vuillemin, *Isaria fumosorosea* (Wize), and *Metarhizium anisopliae* (Metschnikoff) Sorokin have been shown to be virulent to certain aphid species (Mesquita and Lacey, 2001; Pell and Vandenberg, 2002; Poprawski et al., 1999; Vandenberg, 1996), as well as to certain other soft bodied Hemiptera (e.g., Wraight et al., 1998, 2000). The objective of this study was to determine the pathogenicity and relative virulence of several Hypocreales fungi to pecan aphids under laboratory

* Corresponding author. Fax: +1 478 956 2929.
E-mail address: David.Shapiro@ars.usda.gov (D.I. Shapiro-Ilan).

0022-2011/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.jip.2008.07.001

conditions. Initially, we determined pathogenicity and virulence of a single *I. fumosorosea* strain (ARSEF 3581) to *M. caryaefoliae*, *M. caryella*, and *M. pecanis* under laboratory conditions. This strain is being developed as a biocontrol agent for various insect pests (Dunlap et al., 2007). Subsequently, focusing on *M. caryaefoliae*, we compared blastospore and conidia virulence of the 3581 strain with that of the commercially available strain *I. fumosorosea* (ATCC 20847), and then compared the most virulent *I. fumosorosea* treatments to conidia of commercially available *B. bassiana* and *M. anisopliae* strains.

2. Materials and methods

Isaria fumosorosea ARSEF 3581, *I. fumosorosea* ATCC 20847 (= Strain 97), *B. bassiana* (GHA strain), and *M. anisopliae* (F52 strain), were used in this study. Blastospores of *I. fumosorosea* NRRL 3581 and ATTC 20847 were produced in a 3 day liquid culture fermentation according to Jackson et al. (2003) using basal media supplemented with 25 g/L acid hydrolyzed casein (Hycase M, Kerry Biosciences, Kerry, Ireland) and with 50 g/L glucose (Difco, Detroit, MI). Briefly, blastospores were harvested by mixing whole cultures with diatomaceous earth, dewatered by vacuum filtration, and air dried to less than 5% moisture as previously described (Jackson et al., 2003). Air dried blastospores were vacuum-packaged and stored up to 44 d at 4 °C prior to rehydration and spray application. Conidia of all fungal isolates tested were produced on Potato Dextrose Agar (PDA) plates incubated at 25 °C, stored up to 16 d at 4 °C, and formulated in water via mechanical suspension (using a micropestle) according to methods described by Goettel and Inglis (1997). Aphids were reared on the foliage of greenhouse-grown pecan seedlings, germinated from open pollinated 'Curtis' nuts, at approximately 25 °C and 14:10 (L:D)h (Cottrell et al., 2002). All aphids were less than 5 d old when used in experiments.

Virulence assays were conducted based on procedures described by Vandenberg (1996). Blastospores were applied to aphids using an Automatic Potter Spray Tower (Burkard Scientific Limited, Uxbridge, Middx, UK) (Potter, 1952). The spray tower was equipped with an intermediate atomizer and delivered 2 ml of treatment at 69 kpa with an allowance of a 5 s settling period. Prior to the bioassays, spore viability was determined by spraying 2 ml of suspension onto Petri dishes containing ¼ strength Sabouraud dextrose agar (16.25 g per liter rather than 65 g per liter) with 1% yeast extract (SDAY) and counting the percentage of spores that had germinated after 6 h of incubation at 25 °C (Goettel and Inglis, 1997). Based on three replicate counts the viability estimates ranged from 65.4% (*I. fumosorosea* ATCC 20874 conidia in Experiment 2) to 85.0% (*M. anisopliae* conidia in Experiment 3), the viability estimates were taken into account in calculating application rates of viable spores.

Prior to the bioassays, the actual number of spores deposited for each rate of application was estimated by applying 2 ml of suspension and then counting the number of spores per cm^2 (at 200× magnification) on two cover slips that were placed on a Petri dish (90 mm) containing 1.5% water-agar, three replicate (dishes) per application rate yielded estimates of 5.7×10^3 and 1.49×10^4 per cm^2 spores for the 10^7 and 10^8 application rates (see below), respectively.

In the first experiment (Experiment 1), we measured the virulence of *I. fumosorosea* ARSEF 3581 blastospores to all three pecan aphid species. Blastospores were applied to 10 alate aphids, which were on five 2.5-cm-diameter leaf discs placed on 1.5% water-agar in a single 90-mm-diameter Petri dish. Two milliliters of *I. fumosorosea* suspension were applied at a rate of 1×10^7 or 1×10^8 viable blastospores per ml. Control dishes received 2 ml of water only. After inoculation the dishes were incubated at 25 °C for 72 h.

After 24 h of incubation, insects were moved to fresh leaf discs on Petri dishes without fungus. Mortality was checked daily. Dead insects were removed and placed on 1.5% agar plates at 25 °C for an additional 2 d to check for signs of mycosis. The experiment was set up as a factorial (main effects = fungus rates and aphid species) with four replicates per treatment in a completely randomized design.

In Experiment 2, we compared blastospores and conidial preparations of *I. fumosorosea* ARSEF 3581 and ATCC 20874 for virulence to *M. caryaefoliae*. The procedures were identical to those described above except that mortality was recorded after 24, 48, 72 and 96 h, mycosis was determined after 7 d rather than after 2 d, and the experiment, organized in a completely randomized design, was not analyzed as a factorial (since there was only one aphid species). In Experiment 3, using *M. caryaefoliae* as the target pest, we compared the virulence of blastospores of *I. fumosorosea* ARSEF 3581 and conidia of *I. fumosorosea* ATCC 20874 (because these treatments showed the highest virulence in Experiment 2) with conidia of *B. bassiana* and *M. anisopliae*. The procedures and design in Experiment 3 were identical with those of Experiment 2. All three experiments were repeated once after the first trial was completed.

For the analysis of each experiment, data from both trials were combined, and variation among trials was accounted for as a block effect. Additionally, percentage data were arcsine transformed prior to analysis (SAS, 2001; Steel and Torrie, 1980). Non-transformed means are presented. Treatment differences in aphid mortality (regardless of putative cause) and mycosis (exhibiting signs of fungal infection) were analyzed separately.

In Experiment 1, due to a lack of independence (i.e., a significant interaction reported below), the main effects in the factorial experiment were analyzed separately using ANOVA (Cochran and Cox, 1957; SAS, 2001; Steel and Torrie, 1980). The fungus effect was analyzed within each aphid species by comparing mortality and mycosis observed in the two fungus rates to each other and to the control. The aphid species effect was analyzed for each fungus rate, in this case, however, to avoid potential bias due to unequal control mortality, Abbott's formula (Abbott, 1925) was applied to the data prior to analysis. In Experiments 2 and 3, two-way ANOVA was applied (SAS, 2001). In all analyses, the Student–Newman–Keuls' test was used to elucidate treatment effects when a significant F value ($P \leqslant 0.05$) was detected in the ANOVA (SAS, 2001).

3. Results

In Experiment 1, a significant interaction between aphid and fungus effects was detected 3-d post-application in aphid mortality and mycosis ($F = 4.21$, df = 4, 62, $P = 0.0044$, and $F = 6.75$, df = 4, 62, $P = 0.0001$, respectively). Analysis of the fungus effect on aphid mortality indicated that *I. fumosorosea* (ARSEF 3581) is pathogenic to all three aphid species (Fig. 1). Although no difference in mortality was observed 1-d post-application, the higher application rate of *I. fumosorosea* caused higher aphid mortality than the control by 2-d post-application for *M. caryaefoliae* and by 3-d post-application in the other two species (Table 1, Fig. 1). Three-day post-application mortality in the higher fungal rate reached 100% in *M. caryella* and *M. caryaefoliae*, and (±SE) 69.9 ± 5.1% in *M. pecanis* (Fig. 1).

Analysis of mycosis by aphid species in Experiment 1 also confirmed pathogenicity. No mycosis was observed 1-d post-application, at 2-d post-application mycosis was observed in the high fungal rate applied to *M. caryella* and *M. pecanis*, and at 3-d post-application both application rates of *I. fumosorosea* caused significant mycosis in all species (Table 1, Fig. 2). Also 3-d post-application the higher fungal rate caused greater mycosis than