Biological Control 103 (2016) 129–137

Contents lists available at ScienceDirect

Biological Control

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

Impact of acquired entomopathogenic fungi on adult *Drosophila suzukii* survival and fecundity

Joan Cossentine [a,*], Mairi Robertson [a], Rose Buitenhuis [b]

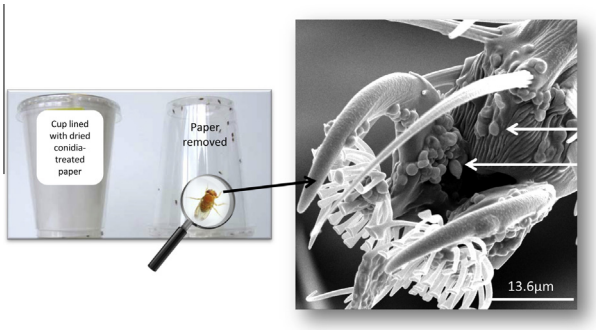
[a] Summerland Research and Development Centre, Agriculture and Agri-Food Canada, 4200 Hwy 97, Summerland, BC V0H 1Z0, Canada
[b] Vineland Research and Innovation Centre, 4890 Victoria Avenue North, Box 4000, Vineland Station, ON L0R 2E0, Canada

HIGHLIGHTS

- *Drosophila suzukii* is an invasive pest of fruit in North America and Europe.
- Adults acquired conidia on pretarsi through tactile exposure to dried treated paper.
- Exposure to each of four fungal isolates resulted in adult infection and mortality.
- The *M. brunneum* isolate resulted in the lowest LC_{50} and reduced fecundity.
- Transmission of acquired *M. brunneum* conidia was recorded between sexes.

GRAPHICAL ABSTRACT

Scanning electron micrograph of *Beauveria bassiana* spores (indicated by arrows) found on *Drosophila suzukii* (Diptera: Drosophilidae) pretarsus (magnification 2.2 K) acquired after exposure to spores dried on paper.



ARTICLE INFO

Article history:
Received 18 December 2015
Revised 25 August 2016
Accepted 1 September 2016
Available online 3 September 2016

Keywords:
Spotted wing Drosophila
Metarhizium
Beauveria
Isaria
Lecanicillium

ABSTRACT

Exposure of *Drosophila suzukii* adults to surfaces treated with *Metarhizium brunneum*, *Beauveria bassiana*, *Isaria fumosorosea* or *Lecanicillium lecanii* conidia under laboratory conditions resulted in fly infection and dose dependent mortality. Scanning electron microscopy confirmed that conidia accumulated on *D. suzukii* pretarsi after ≤48 h exposure to the dried conidia treated surfaces. Approximately 50% of the flies died post exposure to 1×10^8 conidia of each isolate distributed over approximately 148 cm^2 by 7, 10, 12 and 13 days respectively at 25 °C. Temperature had an impact on fungus-induced *D. suzukii* mortality when tested at 20, 25 and 30 °C, although control mortality was also significant at 30 °C. Fifty percent of the control *D. suzukii* adults died after being held at 30 °C for 11–13 days. Significantly lower oviposition, recorded as F1 pupae, was recorded from adult *D. suzukii* exposed to *M. brunneum* compared to the number of offspring produced by control flies indicating that the fungus had a negative impact on fly fecundity. Evidence of transmission of acquired *M. brunneum* conidia between sexes was recorded.

1. Introduction

The spotted wing Drosophila (SWD), *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), is a native of southeast Asia that was reported to have first established in both North America (California) and southern Europe (Spain) in 2008 (Hauser et al.,

* Corresponding author.
E-mail address: joan.cossentine@agr.gc.ca (J. Cossentine).

http://dx.doi.org/10.1016/j.biocontrol.2016.09.002
1049-9644/

2009; Calabria et al., 2012). The invasive pest has since spread in both continents where it has been found to infest a broad range of soft-skinned fruits. In North America it has been associated with many cultivated and wild hosts including commercially grown fruits such as sweet and sour cherries, blueberries, strawberries and raspberries (Walsh et al., 2011). Unlike other *Drosophila* species that infest harvested and fallen fruit, the *D. suzukii* female is able to insert eggs under the skin of ripening fruit before it is harvested. Larvae develop inside the fruit, fruit tissue begins to decompose around the feeding site and there is potential for extensive crop losses (Lee et al., 2011). The pest completes multiple generations in a season and vulnerable crops require protection particularly when their skins soften and the sugar levels in the fruits increase (Burrack et al., 2013). The insecticides that are being used to suppress *D. suzukii* damage in Canadian orchards negatively impact beneficial non-targets and their frequent applications facilitate potential development of resistance in the fruit fly (Boyd and Boethel, 1998; Williams et al., 2003; Jansen et al., 2010). An efficacious entomopathogenic fungus targeted against adult *D. suzukii* may offer a more environmentally sustainable suppression strategy.

A large number of adult dipteran species in numerous families have been found to be susceptible to various isolates of the entomopathogenic fungi, *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metchnikoff) Sorokin and *Isaria fumosorosea* Wize (*Paecilomyces fumosoroseus*) complexes. Adult *Aedes aegypti* (Diptera: Culicidae) have been recorded as susceptible to infection by isolates of all three fungi (Darbo et al., 2011) as have houseflies, *Musca domestica*, hornflies, *Haematobia irritans* (Diptera: Muscidae) (Kuramato and Shimazu, 1992) and tropical and temperate species of fruit flies, (Diptera: Tephritidae) (Sookar et al., 2008; Daniel and Wyss, 2009). In addition, adult pea leafminers, (Diptera: Agromyzidae) (Migiro et al., 2010) have been recorded as being susceptible to both *B. bassiana* and *Metarhizium* isolates, biting midge (Diptera: Ceratopogonidae) were found to be susceptible to infection by *B. bassiana* (Ansari et al., 2011) and adult blow flies, *Lucilia sericata* (Diptera: Calliphoridae) and tsetse fly adults, *Glossina* spp. (Diptera: Glossinidae) were demonstrated to be susceptible to *Metarhizium* isolates (Maniania, 1998; Wright et al., 2004). Kaaya (1989) has also shown adults of the tsetse fly *Glossina morsitans* to be susceptible to infection by *I. fumosorosea*.

Within the genus Drosophila, adult *Drosophila melanogaster* Meigen and the fig fly, *Zaprionus indianus* were found to be susceptible to superficially applied *B. bassiana* (Tinsley et al., 2006) and *M. anisopliae* (Svedese et al., 2012). More recently, *D. suzukii* adults have been recorded as being susceptible to infection after direct superficial treatment of the flies with isolates of *I. fumosorosea* (Naranjo-Lázaro et al., 2014) and to a lesser extent isolates of the *M. anisopliae* complex (Naranjo-Lázaro et al., 2014; Woltz et al., 2015).

Lecanicillium lecanii (*Verticillium lecanii*) (Zimmerman) Zare & W. Gams is generally known as a pathogen of Hemipteran and Homopteran insects. Although there has been little published evidence that *L. lecanii* causes significant Dipteran mortality, low natural *L. lecanii* infections have been found associated with pasture flies and stable flies (Muscidae) (Steenburg et al., 2001; Skovgård and Steenberg, 2002). In 2009, Mahmoud published evidence that a commercial formulation of *L. lecanii* was more efficacious versus the olive fly, *Bactrocera oleae* (Diptera: Tephritidae) than were formulations of *B. bassiana* or *M. anisopliae*.

Contamination with entomopathogenic fungi has been found to be an effective way to potentially suppress adult flies in the field (Maniania, 1998; Dimbi et al., 2003; Migiro et al., 2010). Given the substantial evidence that many Diptera, including *Drosophila* species, are susceptible to infection by isolates of *B. bassiana*, *M. anisopliae*, *I. fumosorosea* and to a lesser extent *L. lecanii*, the objective of our study was to determine in a laboratory evaluation the susceptibility of *D. suzukii* adults to isolates of each of these complexes. We used a conidia acquisition step, rather than a direct superficial treatment, to verify that the infection could be realistically achieved through tactile contact with a treated surface. The fly susceptibility was then assessed over a field season-appropriate range of temperatures for the three isolates that demonstrated reasonable impacts on fly mortality at 25 °C. The transmission between flies of the most efficacious isolate in these studies and its impact on *D. suzukii* fecundity were also evaluated.

2. Materials and methods

2.1. Insects

All *D. suzukii* were obtained from a laboratory colony (Summerland Research and Development Centre) established from wild locally collected insects and reared on Formula 4–24 Instant Drosophila Medium (Merlan Scientific, ON) at 24 °C, 50 ± 5% RH, 18 L: 6 D photoperiod. For trials, a subset of ≤24 h old *D. suzukii* flies were held in a separate cage before they were collected using a vacuum aspirator, cooled for 90 s and placed in treatment containers.

2.2. Fungus cultures

Beauveria bassiana isolate GHA was obtained from BotaniGard® 22WP (Laverlam International Corporation, Butte, MT), *Metarhizium brunneum* strain F52 from Met52® (Monsanto BioAg Inc., St. Louis, MO), *Isaria fumosorosea* strain FE9901from No-fly® (Natural Industries, Houston, TX) and *Lecanicillium lecanii* isolate 595 obtained from the Canadian collection of fungal cultures, AAFC-Ottawa. Each of the four fungal isolates was exposed to *D. suzukii* adults using the contact exposure described below. Post infection, flies were surface sterilized and plated on Potato Dextrose agar (PDA) (Lacey and Brooks, 1997) and the *D. suzukii* source of fungus was then cultivated on PDA for use in the trials. Spore germination was verified at over 87% for all spore preparations used in the study.

2.3. Treatment exposures

The technique used to distribute entomopathogenic spores over the interior surface of a cup of *D. suzukii* was adopted from an assay technique for mosquitoes (Farenhorst and Knols, 2010). Conidia were removed from the plates of isolates listed above and spore concentrations in sterile distilled water were determined using a haemocytometer. The suspension was then diluted in 2% mineral oil in water to equate to 1×10^9, 10^8, 10^7 and 10^6 conidia ml^{-1}. Two percent mineral oil in water was used as the control. One millilitre of each suspension and control was applied to the matte surface of proofing paper (Testing Machines Inc., New Castle, DE, USA) and the conidia distributed using a wired stainless steel K-bar No. 2 (K bars®, RK Print Coat Instruments Ltd., United Kingdom) with a diameter of 0.16 mm following methodology developed by Farenhorst and Knols (2010) that resulted in a coating thickness of 12 μm. This resulted in approximately 6.8×10^6, $\times 10^5$, $\times 10^4$ and $\times 10^3$ conidia cm^{-2} when spread over the 148 cm^2 area, for each of the suspensions listed respectively above. The treated sheets were air dried and cut to tightly fit the inner wall of 250 ml clear plastic cups, each of which rested on a 30 ml plastic cup. A cotton wick conducted 10% sucrose in dH_2O through a small hole in both the lid of the 30 ml cup and the bottom of each treatment cup. Twenty ≤24 h old *D. suzukii* adults (10 males and 10