*Short Communication*

# Horizontal transmission of the entomopathogenic fungal isolate INRS-242 of *Beauveria bassiana* in emerald ash borer, *Agrilus planipennis* Fairmaire

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**Abstract:** Emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, is an invasive and destructive beetle that causes extensive damages to ash trees in North America. The entomopathogenic fungus *Beauveria bassiana* is considered as an effective biological control agent for EAB adult populations. Using an assisted autodissemination with the fungal isolate INRS-242 of *B. bassiana*, our research aims to investigate the possibility of horizontal transmission of the fungal disease from infected to uninfected EAB adults during mating. Results show that the efficiency of fungal transmission is significantly related to the sex of EAB carrying the pathogen. EAB males are the promising vector to transmit INRS-242 isolate of *B. bassiana* to their partners during mating. Results strengthen the potential of the fungal autodissemination device as a powerful biological strategy to control EAB populations.

**Keywords:** Emerald ash borer; Horizontal transmission; Assisted autodissemination device; *Beauveria bassiana*

## 1. Introduction

Emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, is an extremely invasive beetle that is responsible for killing hundreds of millions ash trees, *Fraxinus* spp., in urban and forest ecosystems of North America. It was first detected in 2002 in Detroit, Michigan (USA) and in Windsor, Ontario (Canada) after its introduction from northeastern Asia [1]. To date, it is found in 35 American states and five Canadian provinces [2]. After hatching, EAB larvae tunnel through the bark and begin feeding the phloem and the cambium [3]. Abundance of larval galleries disrupts the flow of nutrients and water within the tree, resulting in tree death in 3-4 years [4].

Nowadays, various studies have been conducted in order to develop strategies to suppress EAB populations. These include the use of pesticides and biological control agents. However, management of this pest is still challenging because of its cryptic behavior. Fungal autodissemination device developed by Dr. Guertin and Dr. Lavallée have been shown its potential in controlling EAB [5]. Most EAB adults who contacted the autodissemination chamber died after five days. Under natural conditions, it is extremely difficult to demonstrate the horizontal transmission of the fungus between EAB adults. Based on the previous results, this study aims to test the hypothesis that infected adults that released from the assisted autodissemination device can horizontally transmit the fungal pathogen to uninfected partners during mating.

## 2. Materials and Methods

### *2.1. Emerald ash borer Collection*

During summer of 2017, six 12-funnel green Lindgren traps (Synergy Semiochemicals, Burnaby, BC, Canada) were installed in the upper third of the canopy of ash trees located in Laval, QC, Canada (Long. 45.541309; Lat. -73.718103). EAB adults captured in the collecting jars were daily harvested. In the laboratory, males and females of EAB were separated following morphological characters [6]. Insects were then reared in cages and feed with fresh ash leaves.

### *2.2. Fungal production*

The isolate INRS-242 of *Beauveria bassiana* (formerly named INRS-CFL), which was previously reported as an effective entomopathogen to *A. planipennis* adults [5] was used in this study. It is an endogenous fungus formerly isolated by Dr. Lavallée and Dr. Guertin from the pine shoot beetle, *Tomicus piniperda* L., near Cookeshire, QC, Canada. The fungal isolate is stored in 70% of glycerol at -80°C in the Fungal bank of the INRS-Institut Armand-Frappier, Laval, QC, Canada.

Fungal-coated pouches were introduced into an autodissemination chamber to contaminate EAB males and females. The production of coated-INRS-242 isolate pouches was performed in two steps [7]. First, the fungal isolate was produced in Yeast Extract Potato (YPD, Alpha Bioscience Inc., Baltimore, MD, USA) liquid media, in a rotary shaker (150 rpm) for four days at 25°C. The pouches containing of sterile pearled barley were then inoculated with the fungal conidial suspension prior their incubation for seven days in the growth chamber in controlled conditions (25°C, 70% of humidity and in the darkness).

### *2.3. Experimental design*

A randomized block design with three replicates was used to evaluate the transmission of the fungal disease from infected to uninfected EAB adults during the mating. Three treatments were randomly assigned to each block with 20 EAB couples per treatment. The first treatment referred as control, where one uninfected male and one uninfected female were introduced in a Petri dish (Petri Dishes 100 x 150 mm, Fisher Scientific, Ontario, Canada). For the second treatment, only the EAB male was inoculated with the fungus (IM+UF), whereas in the third treatment, just the EAB female was contaminated with the fungus (IF+UM). Fungal inoculation process using the autodissemination device was performed in this experiment [8]. Briefly, each EAB male or female was left walking on the fungal-coated pouch for 1 min. For all treatments, each EAB female was eventually glued on Petri Dishes surface using Evo-Stik (Evode Industry Ltd., Newtown, Ireland). This approach avoided the possibility of indirect horizontal transfer resulting from insect displacements inside the arena. Once installed in the Petri dishes, the insects were observed for 18 hours. Two parameters were measured during the assay: presence of mating and fungal transmission. The fungal transmission was recorded by the presence of conidia on the ventral side of uninfected males and the dorsal part of uninfected females. In addition to these parameters, the consecutive mortality of infected EAB was also reported. After 18 hours, EAB males and females of each arena were individually transferred to a new Petri dish in which a fresh ash leaf has been introduced as food substrate. All Petri dishes were then incubated in the growth chamber at 25°C with 50% of relative humidity and photoperiod 16:8 h (light:darkness). Mycosis symptom was confirmed by the appearance of white muscardine on cadavers. Both mortality of EAB adults and the presence of the muscardine were recorded daily for 14 days.

### *2.4. Statistical analysis*

All statistical analyses were performed using the software R, version 3.4.3. Data related to the effectiveness of horizontal transmission of INRS-242 isolate of *B. bassiana* among EAB adults were subjected to an analysis of variance (ANOVA), to show significant differences between treatments. Means associated with treatments were then compared using Duncan's multiple comparisons. For all statistical tests, the level of rejection was set at $\alpha = 0.05$.

## 3. Results

Looking for the potential horizontal transmission from infected to uninfected adults during EAB mating, the results showed that the transmission rate of INRS-242 isolate of *B. bassiana* was significantly different between treatments (ANOVA, n = 60, $p < 0.0001$) (Table 1). No mycosis symptom has been observed in the control groups. For the Treatment IM+UF, the percentage of infected EAB males transmitting the fungus to uninfected EAB females was about 73.3 ± 2.9% (mean ± SD). This percentage decreased significantly to 58.3 ± 7.6% when the fungal transmission was performed between infected females and uninfected males (Treatment IF+UM). All EAB adults infected died six days after the fungal contamination, and white muscardine was recorded on all contaminated individuals (data not shown).

**Table 1.** Horizontal transmission of *B. bassiana* isolate INRS-242 in *A. planipennis* adults. Control: EAB male and female was not in contact with the fungal isolate; IM+UF: fungal transmission between infected male and uninfected female; and IF+UM: fungal transmission between infected female and uninfected male. For each column, different letters indicate significant difference (Duncan, n = 60, $p < 0.05$).

| Treatments | Fungal transmission (mean ± SD %) |
|---|---|
| Control | 0.0 ± 0.0 [a] |
| IM+UF | 73.3 ± 2.9 [b] |
| IF+UM | 58.3 ± 7.6 [c] |

## 4. Discussion

Horizontal transmission by direct contact between fungal-infected and uninfected insects is a crucial phenomenon ensuring a natural dispersion of conidia in insect populations [9]. This fungal transmission could occur since their first contact during mating [10], as previously observed in various insect species such as the eastern larch beetle, *Dendroctonus simplex* LeConte [7], the European spruce bark beetle, *Ips typographus* L. [10], the Mexican fruit fly, *Anastrepha ludens* Loew [11], and the stem borer, *Busseola fusca* Fuller [12]. Under laboratory conditions, our results have demonstrated for the first time that an infected EAB adult can transmit the fungal isolate INRS-242 of *B. bassiana* to uninfected one during mating. For all EAB couples, the average duration of mating is about 42 min (data not shown). This observation is corroborated to that of Pureswaran and Poland [13]. This duration is long enough for fungal transmission between EAB. Following the mortality of EAB adults and the appearance of muscardine, it is possible to confirm that the fungal dose transmitted from infected to uninfected EAB adults during mating is strong enough to cause the death of insects.

The efficiency of the fungal transmission depends on the sex of insect that carries the pathogen. Transmission of entomopathogenic fungus *Metarhizium anisopliae* (Metschn.) Sorokin within the populations of the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann, was higher when males were carriers of the pathogen [14]. In *D. simplex*, horizontal transmission of INRS-242 isolate of *B. bassiana* was greatest when females were inoculated with the fungus [7]. Our results show that male-to-female transmission of the fungal isolate within EAB adults was higher than from female-to-male. During the assay, we also observed that some EAB males mated with at least two different females (data not shown). This observation suggests that the proportion of contaminated males would be increased by the fungal autodissemination device baited with lactones lure. In addition, because EAB males are more efficient to transmit the mycopathogen than females, this could lead to an increase of the mycosis into the EAB population. This finding suggests that using the assisted autodissemination device to release infected males in the new and small EAB population can be a valuable strategy to increase the mortality rate of the adult population during the endemic phase. Adding another mortality factor to other biotic and abiotic factors could contribute to slow or to keep the EAB population at a lower level, may eventually reduce oviposition on ash trees.

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**Conflicts of Interest:** The authors declare no conflict of interest.

**Data Availability:** The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request**.**

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