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Dissemination of entomopathogenic fungi using *Busseola fusca* male as vector

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ABSTRACT

The stem borer, *Busseola fusca* (Fuller), is an important pest of maize *Zea mays* L. and sorghum *Sorghum bicolor* (L.) in eastern and southern Africa. To control this pest, biological control methods including the use of entomopathogenic fungi are being considered. The pathogenicity of one isolate of *Metarhizium anisopliae* (Metsch.) Sorok. and one isolate of *Beauveria bassiana* Bals. (Vuill.) were first tested on different developmental stages of *B. fusca* including eggs, neonate, 2nd and 3rd-instar larvae. Both fungal isolates were pathogenic to all the stages tested. However, differences in mortality were observed among larvae that hatched from treated egg masses. Experiments were conducted thereafter to test whether *B. fusca* males could serve as a vector for fungal conidia to contaminate *B. fusca* females and subsequently eggs and larvae. Results demonstrated that *B. fusca* males successfully transferred inoculum to females during copulation, which in turn transmitted it to the eggs they laid on maize plants, resulting in the decrease of leaf damages.

1. Introduction

The stem borer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), is an important pest of maize [*Zea mays* L. (Poaceae)] and sorghum [*Sorghum bicolor* (L.) Moench (Poaceaea)] in eastern and southern Africa (Kfir et al., 2002). Biological control using parasitic wasps and entomopathogenic fungi is among the methods being considered for the control of this pest (Maniania, 1992; Kfir et al., 2002). The potential of the entomopathogenic fungi to control stem borers was demonstrated against the maize stem borer, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), following inundative and granular applications of *Metarhizium anisopliae* (Metsch.) Sorok. (Hypocreales: Clavicipitaceae) and *Beauveria bassiana* (Bals.) Vuill. (Hypocreales: Cordycipitaceae) (Maniania, 1993a,b; Maniania et al., 1994). In recent years, another strategy called autodissemination is being considered (Vega et al., 2000; Baverstock et al., 2010). This strategy consists to attract insect pests into a focus of entomopathogens in a contamination device (Cd), from which the pathogen can be disseminated to their conspecifics before returning to the environment. Such devices have been developed for different groups of insects (Vega et al., 2000) including the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Yponomeutidae), whereby the pheromone trap was modified for the dispersal of *Zoophthora radicans* Brefeld. (Zygomycetes: Entomophthorales) (Pell et al., 1993). Pheromones, kairomones and visual cues are generally used to attract the target insects to the autoinoculator. The objective of this study was to test in the laboratory and screenhouse whether male of *B. fusca* contaminated with entomopathogenic fungal conidia can transfer the inoculum to female moths during copulation and subsequently transmit it to the eggs laid on maize plants. Since the contamination of neonate by fungus may be caused either by fungal penetration through the egg integument before hatching or by the conidia on the egg cuticle serving as inoculum for emerging larvae, which feed upon the chorion, we also investigated the susceptibility of different developmental stages of the insect to fungal contamination.

2. Materials and methods

2.1. Insects

The different developmental stages of *B. fusca* (eggs, larvae and adults) were obtained from the ICIPE's Mass Rearing and Quarantine Unit (Nairobi, Kenya). Larvae were reared on artificial diet of Onyango and Ochieng'-Odero (1994) in a rearing room at 25 ± 3 °C and 55 ± 5% r.h. and a L12:D12 reversed photoperiod with the scotophase lasting from 7.00 to 19.00 h, herewith referred to as night. To regenerate the colony, new insects collected from the

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field were added thrice a year. Adults were reared in plastic boxes (21 cm long × 15 cm wide × 8 cm high) and maintained as described above. The reversed photoperiod allowed all experiments to be carried out during daytime. Egg masses of 40–50 eggs, neonates and larvae at 2nd and 3rd instars were randomly selected for the bioassays. Within larval stage experiments, one day-old moulting 2nd and 3rd instars larvae were used. Freshly emerging adults were used for the autodissemination experiments.

2.2. Plants

Maize (cv. 511, a non-pubescent cultivar) obtained from Simlaw Seed Company (Nairobi, Kenya) was used in this study. Maize seeds were planted in a screenhouse (6 m long × 3 m large × 1.9 m high) in plastic pots (13 cm diameter × 12 cm high) containing peat at 26 ± 5 °C and 50 ± 22% r.h. during the day and 19 ± 2 °C and 75 ± 11% r.h. during the night. After emergence, plants were watered three times a week and once with a complete nutrient solution (NPK, 15-15-15). They were thinned to three plants per pot. Since pre-tasseling plants were found to be highly preferred by *B. fusca* female for oviposition (Calatayud et al., 2008a,b,c), plants at this stage were used for the experiments.

2.3. Fungi

M. anisopliae sensu lato (s.l.) isolate ICIPE 30, isolated from *B. fusca* larva in 1989, and *B. bassiana* isolate ICIPE 279, isolated in 2005 from *Cyclocephala* sp. (Coleoptera: Scarabaeidae), were used in this study. The culture was maintained on Sabouraud Dextrose Agar (SDA) in Petri dishes at ambient conditions (25–27 °C and 50–60% r.h.). Conidia were harvested by scrapping the surface of 3 week-old culture with a sterile wire loop and suspended in 20 ml sterile distilled water containing 0.05% Triton X-100. The suspension was vortexed for five minutes to produce homogenous conidial suspension. The viability of conidia was then determined by spread-plating 0.1 ml of the suspension (titrated to 3.0×10^6 conidia ml^{-1}) on SDA plates. The suspension was spread evenly on the plate and a sterile microscope cover slip was placed on each plate randomly. Plates were incubated at 26 ± 2 °C and percentage germination was determined by counting 100 spores for each plate after 24 h. Germinated conidia were the ones that had germinating tubes. Four plates representing each a replicate were used in all the viability tests. For the autodissemination experiments, conidia of both *B. bassiana* and *M. anisopliae* were mass-produced on long grain rice following the technique described by Maniania (1998). Conidia were dried for 48 h in a desiccator containing active silica gel and stored in a refrigerator (4–6 °C) until required. In viability tests, 83–90% of conidia germinated after 24 h on SDA.

2.4. Pheromone

The synthetic pheromone blend used in the present study was formulated at the Institut National de la Recherche Agronomique (INRA, France) according to the published *B. fusca* pheromone identification of Félix et al. (2009). Each rubber septum cap (Sigma–Aldrich) was impregnated with 1 mg of the following pheromone mixture: (*Z*)-11-tetradecen-1-yl acetate, (*E*)-11-tetradecen-1-yl acetate, (*Z*)-9-tetradecen-1-yl acetate and (*Z*)-11-hexadecen-1-yl acetate (62: 15: 13: 10).

2.5. Bioassays

2.5.1. Susceptibility of different developmental stages of B. fusca to M. anisopliae and B. bassiana

Eggs and larvae were sprayed with 10 ml of conidial suspension titrated to 1.0×10^8 conidia ml^{-1} using Burgerjon's spray tower (1956) at 16 mm Hg pressure. The control insects were sprayed with sterile 0.05% Triton X-100. Each experiment consisted of egg masses of 40–50 eggs or 20 larvae of different developmental larval stages. Fungal treatments were randomized and replicated five times. After treatment, eggs were transferred into sterile Petri dishes and the percentage of eggs that hatched was recorded 20 days post-treatment by counting the open shells left by the neonate larvae after hatching. Fifty neonate larvae that hatched were randomly selected and transferred into sterile Petri dishes containing a piece of artificial diet free of ascorbic acid, vitamin E, methyl-p-hydroxybenzoate and formaldehyde, and were maintained at 25 ± 2 °C under the conditions described earlier. Egg masses that failed to hatch and dead larvae from fungal pathogen were transferred to a Petri dish containing moist filter paper to allow the development of mycosis on the surface. Larval mortality was recorded after seven days.

2.5.2. Horizontal transmission of dye/inoculum

Two sets of experiments were carried out to determine whether male moths can transfer inoculum to female during mating. The first experiment was conducted to demonstrate that moths that entered and exited from the Cd could carry dye and transfer it to female. The Cd was made from a 1 l clear water plastic bottle. A hole (5 × 5 cm) was made 5 cm from the bottom of the bottle on each side of the bottle. The synthetic pheromone was released from a rubber cap contained in a small cylindrical plastic vial with a cap (1.5 cm diameter × 4 cm high) perforated with several holes (approx. 3 mm diameter) placed in the middle of the bottle. The plastic vial was suspended to the cap of the bottle by a wire. A mixture of 100 mg of fine brilliant yellow dye powder (201375, Sigma–Aldrich) and 1 g of pure maize starch was prepared and sprinkled at the bottom of the Cd. The Cd was then placed in a Plexiglass™ (Nairobi, Kenya) wind tunnel (184 cm long × 60 cm wide × 40 cm high) equipped with a fan that pushed air through the wind tunnel and an extractor on the opposite side, thereby generating airflow. Illumination was provided by a 40 W incandescent red light bulb mounted 70 cm above the midsection of the wind tunnel. The Cd was located 20 cm away from the upwind end of the tunnel. One-day old virgin males were then introduced individually into the dark wind tunnel room at least 1 h before the start of the experiment. Males were released individually from the top of a 15 cm-high carton platform (release platform) 124 cm downwind from the Cd and observed for 10 min. Each male was considered as a single replicate, and 58 males were observed. The behaviour of each male was recorded and included the following: attraction to the pheromone source (i.e. the Cd) or not, after attraction, entering the Cd and staying inside or exiting the Cd. Each observation was carried out in room temperatures (22–25 °C). Thereafter, each male that entered the Cd within the 10 min observation was placed in a mosquito-net cage (40 × 40 × 63 cm) containing a one-day old virgin female and a single pot of maize plants. After two nights, the staining of male, female and eggs batches on plants was noted under light using a binocular.

In the second experiment, conidia of both *B. bassiana* and *M. anisopliae* were used to determine whether females mating with fungus-contaminated males can pick up the inoculum and transmit contamination to eggs during oviposition. One-day old virgin males *B. fusca* were contaminated using small plastic vials (1.5 cm diameter × 6 cm high). The males were introduced individually in each of these vials previously treated with 0.3 g of dry conidia and exposed for 3 min for contamination. In the controls, inactivated spores by autoclaving were used. All males were then transferred individually to bigger plastic jars (10 cm diameter × 20 cm high) containing a one-day old virgin female for mating. Oviposition substrates consisting of wax papers cut rectangularly (15 × 6 cm) and rolled helicoidally from top to