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Uptake, retention, and repellency of a potential carrier of active ingredients in crack and crevice treatments for stored-grain beetles

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Abstract

Crack and crevice treatments are important in modern stored-beetle management, because: (1) pests are abundant in such microhabitats, (2) they potentially deliver insecticides to insect pests without contaminating food products, and (3) they can significantly reduce the need for broadcast spray applications and/or fumigations. As part of on-going research into the development of novel insecticide delivery systems to be used in processed and unprocessed food environments, we examined uptake and behavioural responses of *Oryzaephilus surinamensis* to an electrostatically chargeable powder, Entostat™. Entostat is a processed plant wax and has been identified as a potential carrier for active ingredients to be delivered to cracks and crevices in food facilities. Depending upon the initial Entostat concentration in rolled oats, 0.03–0.26 μg powder was extracted from individual beetles 72 h after being transferred from treated to untreated food. SEM images showed that Entostat adhered to all body parts, including joints, between body segments, and at the insertions of body hairs. Choice experiments showed that *O. surinamensis* individuals were repelled, when rolled oats in cracks contained >5% Entostat. In a three-choice experiment cracks contained: (1) untreated oats, (2) oats mixed with 5% (w/w) Entostat, or (3) oats mixed with 5% (w/w) Entostat and a piece of filter paper containing a beetle attractant which was also inserted into the crack. The beetle attractant did not significantly increase the attractiveness of the crack in which it was applied, but the average powder uptake of beetles from cracks treated with the attractant was significantly higher than from the other cracks. Results presented here suggested that Entostat adhering to insects was retained over several days and that considerable amounts of Entostat were taken up even when beetles were offered a choice between treated and untreated cracks.

Keywords: Behavioural response; *Oryzaephilus surinamensis*; Powder retention; Powder uptake; Repellency

1. Introduction

With growing concerns about excessive use of insecticides, the overall purposes of many applied stored-grain insect research programs are to develop and test: (1) insecticides that have lower adverse effects on humans, non-target organisms and the environment, (2) decision-support tools to make insecticide applications more targeted in both space and time, and (3) novel formulations that enhance the efficacy of active ingredients to lower lethal dosages for target insect pests. At the same time, the number of reports of resistance of stored-grain beetles to insecticides is growing and raises concern about their efficacy (Beeman and Schmidt, 1982; Collins et al., 1992, 1993; Hagstrum et al., 1999; Phillips et al., 2000). With governments increasing restrictions and gradually eliminating use of many insecticides, pest control operators and food facility managers face a challenging task of controlling stored-grain insects. This study is part of a research program to develop the use of fungal spores to reduce chemical pesticide in consumable product environments. The main objective of this program is to develop a pathogen-based crack and crevice formulation to be used for empty bin treatment. Instead of applying fungal spores directly into cracks and crevices, this program investigates the prospects of using an inert powder, Entostat™ (Exosect Ltd, Winchester, UK), as carrier for the fungal spores. One of the initial steps in this program is to determine whether stored-grain insects are repelled by

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Entostat, as other studies have shown that insects may be repelled by inert dusts (Glenn et al., 1999).

Crack and crevice treatments with residual insecticides in food processing facilities, empty grain silos, and food warehouses are important in modern stored-beetle management, because: (1) pests are abundant in such microhabitats, (2) they potentially deliver insecticides to insect pests without contaminating food products, and (3) when done effectively, crack and crevice treatments can significantly reduce the need for broadcast spray applications and/or fumigations. However, the main challenges regarding crack and crevice treatments are that most food facilities contain an infinite number of cracks and crevices, so it is virtually impossible to treat them all. In addition, in many food facilities operational procedures are associated with considerable amounts of dust and/food debris, which accumulate in cracks and crevices. An insecticide applied to a crack or crevice is therefore likely to become covered with food debris or dust, which decreases the likelihood of insect pests acquiring a lethal dosage. There are four likely consequences of ineffective insecticide applications: (1) money being wasted, (2) complaints and law suits by consumers due to contaminated food products, (3) increases in use of insecticides, and (4) target insect pests developing resistance to active ingredients. It is therefore important to investigate delivery systems and potential carriers of insecticides, which, for instance through electrostatic forces, can enhance the uptake and retention of insecticides.

Entostat is currently being investigated as a carrier of sex pheromones in mating disruption systems (www.exosect.com); it is composed of highly refined carnauba wax obtained from the leaves of the Brazilian wax palm, *Copernica cerifera* Martius (Palmae). Recent studies (Armsworth et al., 2006; Barton et al., 2006; Nansen et al., in press) described uptake and retention of Entostat by Mediterranean fruit flies, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). It was shown that Entostat treatments temporarily suppressed flight behaviour and mating compared to untreated medflies. Entostat-treated males transferred small amounts (5.77 μg ± 0.66) to untreated females during mating and 5–10% of the initial dosage was retained for 48 h after treatment.

In this study, *O. surinamensis* was used as a model species to examine uptake and behavioural responses to Entostat. *Oryzaephilus surinamensis* is a secondary pest on unprocessed grain and processed cereal products and is commonly found in food processing facilities (Fraenkel and Blewett, 1943). We used regression analysis to model Entostat uptake and retention by extraction from beetles. SEM images were collected to investigate Entostat retention on different body parts. A behavioural experiment was conducted to establish at what level Entostat mixed with rolled oats appeared to repel *O. surinamensis* individuals. We also conducted a three-choice experiment in which cracks contained untreated oats, oats mixed with 5% (w/w) Entostat, or oats mixed with 5% (w/w) Entostat and a piece of filter paper containing a beetle attractant. The purpose of this experiment was to determine whether the beetle attractant would increase the attractiveness of the crack containing Entostat.

2. Materials and methods

2.1. Insects

Oryzaephilus surinamensis were obtained from the Central Science Laboratory (York, UK) culture and reared on rolled oats (95% w/w) and brewers' yeast (5% w/w). The culture was maintained at 28 °C, 50–70% r.h. and ambient light conditions. Adults used in all experiments were of unknown sex, mating status, and age.

2.2. Entostat uptake

A fluorescent dye, Glo-Brite AW Powder (Himar, Bradford, UK) (10% w/w) was dissolved in ethanol and formulated into Entostat. Throughout this article, "dyed Entostat" refers to this formulated mixture of Entostat, "food mixture" refers to rolled oats mixed with dyed Entostat, while "clean rolled oats" refers to oats without dyed Entostat. Using a scanning fluorometer (Perkin Elmer Luminescence Spectrometer LS5OB), we found that Glo-Brite has an optimum excitation wavelength of 385 nm and emission wavelength of 450 nm, respectively. A calibration curve was constructed testing the emission from six concentrations of powder in ethanol: 0.1, 0.2, 0.4, 0.8, 1.5 and 1.9 μg of dyed Entostat per 1 ml 95% ethanol. In order to quantify Entostat uptake, *O. surinamensis* individuals were transferred to Eppendorf tubes containing 95% ethanol and vortexed for 30 s before the solution was analyzed in the fluorometer.

2.3. Uptake and retention of Entostat in rolled oats

The purpose of this no-choice experiment was to determine to what extent uptake and retention of dyed Entostat was associated with the amount to which the beetles were initially exposed. We mixed rolled oats with dyed Entostat in the following percentages (w/w): 0% (clean oats), 2%, 5%, 10%, 20%, and 30%. For each concentration, we placed 0.5 g food mixture (rolled oats and dyed Entostat) in a 5 cm-diameter Petri dish and introduced 10 *O. surinamensis* individuals for 24 h. After 24 h, the 10 beetles in each dish were transferred to a new 5 cm-diameter Petri dish with 0.5 g clean rolled oats. Beetles were kept in the clean rolled oats for 24, 48, and 72 h before being transferred as a group to an Eppendorf tube with 1 ml 95% ethanol for quantification of Entostat uptake. All combinations of dosage of Entostat in food mixtures and time in clean oats after treatments were repeated 15–25 times.