Biological Control 125 (2018) 39–43

Contents lists available at ScienceDirect

Biological Control

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

The potential for using entomopathogenic nematodes and fungi in the management of the maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae)

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ARTICLE INFO

Keywords:
Maize weevil
Biopathogen
Heterorhabditidae
Steinernematidae
Biological control

ABSTRACT

Maize (*Zea mays* L.) is an important cereal crop that is cultivated globally. In storage, maize is infested by the maize weevil (*Sitophilus zeamais* Motchulsky). In sub-Saharan Africa where maize is an important staple crop, infestation by *S. zeamais* is severe. Chemical pesticides have been the key pest management tools for this pest but these practices come with consequences such as insect resistance to pesticides, food and environmental contamination, and depletion of non-target species. These challenges associated with use of chemical pesticides may be overcome by controlling this pest with natural enemies such as entomopathogens.

In the laboratory, this study evaluated the pathogenicity of six entomopathogenic nematodes (EPN) – *Heterorhabditis bacteriophora* Poinar (Lewiston, and Oswego strains); *H. indica* Poinar, Karunakar, and David (Homl strain), *H. georgiana* (K22), *Steinernema feltiae* (SN), and *S. carpocapsae* (All), and two fungi namely, *Beauveria bassiana* (GHA) and *Metarhizium brunneum* (F52) to adult weevils (*S. zeamais*). All nematodes used in the study were pathogenic to adult weevils. However, *S. carpocapsae* was the most virulent to the adult weevils. High doses (1×10^9 conidia/mL) of the fungi application caused significant weevil mortality compared to the control. Subsequently, in a novel approach, this study established the basis for effective storage of maize by treating storage bags (jute bags) with wettable powder of *B. bassiana* (2.13×10^7 conidia/mm^2) and then exposing adult weevils to the treated jute bags. The results showed that adult weevils that walked for 30 min on the treated jute bags recorded 100% mortality at 14 days post-inoculation. Thus, there is the potential for using a wettable powder of *B. bassiana* to protect maize from *S. zeamais* during storage in jute bags.

1. Introduction

Maize (*Zea mays* L.) is the third most important globally cultivated cereal grain after wheat and rice (Golob et al., 2004; Suleiman et al., 2013). This crop is even more important in sub-Saharan countries such as Ghana, Nigeria, Kenya, Zambia, etc, where it is part of the staple diet for millions of the population (FAO, 2017; Mwololo et al., 2013). Maize constitutes an export crop in several other countries such as Argentina where more than 60% of harvested maize is for export market (Barra et al., 2013). With some 4200 different uses for maize products, as well as an annual increase of 2.2% in global demand, many developing countries are growing maize on a large scale (Bbosa, 2014). Postharvest losses reduce both the quality and quantity of available maize for human and livestock consumption and for the export market. Global postharvest losses of agricultural commodities are estimated at about 40% (Loeck, 2002). Postharvest losses are even more severe in developing countries due to inadequate storage and processing facilities that predispose commodities to insect pests and fungi with concomitant development of mycotoxins (Hell and Mutegi, 2011; Anankware et al., 2013; Akowuah et al., 2015).

Postharvest insect pests, particularly the beetles and weevils, reduce the quality and quantity of stored maize via feeding and burrowing into the kernels. An average weight loss of maize resulting from infestation by insects has been reported to be between 20 and 30% (Rees, 2004) but could sometimes be up to 80% if maize is stored without insecticide application (Boxall, 2002). Among the insects that attack maize in storage, the maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae), is the most cosmopolitan (Vowotor et al., 2005).

Sitophilus zeamais is known to be one of the most destructive pests of stored maize (Costa et al., 2006). This pest develops internally in the maize kernel and feeds on the endosperm. By the time development is complete and adult weevils emerge from maize kernels, the kernels are hollowed with reduced nutritional value since the endosperm is consumed. The emergent holes created by the adult weevils pre-dispose

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https://doi.org/10.1016/j.biocontrol.2018.06.008
Received 8 April 2018; Received in revised form 14 June 2018; Accepted 20 June 2018
Available online 21 June 2018
1049-9644/

maize to fungi such as *Aspergillus flavus* Link and *Fusarium verticillioides* Sacc Nirenberg (Tefera et al., 2011). These fungi produce mycotoxins such as aflatoxin and fumonisin (Lamboni and Hell, 2009), which are carcinogenic substances that are also acutely lethal to poultry birds, farm animals and humans.

Management of *S. zeamais* populations with chemical pesticides is the strategy of choice among farmers and warehouse managers in most parts of the world due to its presumed effectiveness (Hell and Mutegi, 2011). Unmitigated applications of chemical pesticides have resulted in several consequences such as resistance of insect populations to pesticides, risks of pesticide poisoning, and pesticide residues in food, animal feeds and in the environment (Subramanyam and Hagstrum, 1995; Collins et al., 2002; Vadivambal et al., 2010). Cultural practices used in protecting maize from *S. zeamais* such as varietal screening, treatment with botanicals, wood ash, or cobs subjected to smoke and heat from the kitchen fire lit underneath the maize platform have produced varying degrees of protection of maize from *S. zeamais* (Golob et al., 2004; Hakeem et al., 2017). Research efforts are currently focused on finding alternative non-chemical, ecologically friendly strategies that can be used in the reduction or elimination of maize weevils in storage systems.

The current study explored the use of entomopathogenic nematodes and fungi in the control of the maize weevil. Entomopathogenic nematodes and fungi could have great potentials in the disinfestations of storage structures of residual populations of S. zeamais, or could be applied on the surface of sacks of maize to prevent re-infestation. Previous screening of *S. zeamais* for susceptibility to entomopathogenic nematodes (Maketon et al., 2011; Barbosa-Negrisoli et al., 2013) and fungi (Barra et al., 2013; Ruelas-Ayala et al., 2013; Agostini et al., 2015) found just a few to be virulent to the weevil. Thus, results so far from studies evaluating susceptibility of *S. zeamais* to entomopathogens have been mixed. Screening more entomopathogens is required to discover those that exhibit strong virulence to *S. zeamais*. Maize is stored in sacks, which include jute bags (burlap bag), by subsistent farmers in sub-Saharan African countries, such as Ghana, Kenya and Nigeria (Midega et al., 2016; Danson et al., 2018). A study in rural counties in western Kenya found that 76% of rural farmers store maize in sacks in their residences (Midega et al., 2016).

The research reported here screened both entomopathogenic nematodes and fungi for pathogenicity against the maize weevil. The entomopathogenic nematodes and fungi used in the current study were either similar or related to strains that have been previously demonstrated to be pathogenic to other stored product pests (Maketon et al., 2011; Barbosa-Negrisoli et al., 2013; Barra et al., 2013; Ruelas-Ayala et al., 2013; Agostini et al., 2015). Further, a novel study was done to establish the optimum dose of entomopathogens to apply on bagging material to protect disinfested maize in sacks against re-infestation by the maize weevil during storage.

2. Materials and methods

2.1. Rearing of the maize weevil

Sitophilus zeamais was obtained from the University of Georgia's Center for Invasive Species and Ecosystem Health, Department of Entomology, Tifton, GA, in August 2016 and has since been maintained at the rearing facility at the Department of Biology, Fort Valley State University, Fort Valley, GA 31030.

Protocol for rearing *S. zeamais* for this study followed procedures in a previous study (Beti et al., 1995). Cultures of the maize weevil were made by placing 100 adult weevils in 1-L wide-mouth, canning glass jars containing 250 mL of maize kernels. Pesticide-free maize kernels used for rearing maize weevil and for experimentation were obtained from Department of Entomology, University of Georgia, Tifton, GA. The wire mesh screen allowed for ventilation and prevented adult weevils from escaping. A filter paper (7 cm diameter) was placed on top of the wire mesh prior to placing the metal ring as extra care to prevent the weevils from escaping. The rearing jars containing maize kernels and the adult weevils were transferred to an environmental chamber maintained at 28 ± 2 °C and 65 ± 5% RH. The adult weevils were removed after 20 days to avoid using weaker and older generations for experimentation. Only adults that were less than 10 days old from the time of emergence from maize kernels were used in the study.

2.2. Nematode culture

Nematodes used in this study were reared at ~25 °C on last instar greater wax moth, *Galleria mellonella* (L.), according to established procedures (Woodring and Kaya, 1988). The larvae of *G. mellonella* were obtained from Webster's Waxie Ranch (Webster, WI). Nematodes were stored at 13 °C for less than 15 days before being used for experiments. Six strains of nematodes (five different species), namely *Heterorhabditis bacteriophora* Poinar (Lewiston, and Oswego strains); *H. indica* Poinar, Karunakar, and David (Homl strain), *H. georgiana* (K22), *Steinernema feltiae* (SN), and *S. carpocapsae* (All) were used in this study. All nematodes were obtained from the USDA-ARS culture collection in Byron, GA. The culture of *Heterorhabditis bacteriophora* (Oswego strain) was originally obtained from Dr. Elson Shields (Cornell University).

2.3. Fungi culture

Beauveria bassiana (Balsamo-Crivelli) Vuillemin (GHA strain), and *Metarhizium brunneum* Petch (F52 strain) originally obtained from Stefan Jaronski (USDA-ARS) were cultured on Sabouraud dextrose agar (SDA) with 0.2% yeast extract according to established procedures (Goetell and Inglis, 1997). *B. bassiana* and *M. brunneum* were stored at 4 °C for 1 week prior to starting the experiment.

2.4. Susceptibility of Sitophilus zeamais to entomopathogenic nematodes

The protocol for inoculating the weevils with entomopathogenic nematodes followed a method used in screening *Plodia interpunctella* (Hübner) for susceptibility to entomopathogenic nematodes (Mbata and Shapiro-Ilan, 2005). Dose-response evaluation of the nematodes was carried out with infective juveniles (IJs) of *H. bacteriophora* (VS) and *S. carpocapsae* (All). Infective juveniles of nematodes in aqueous solutions were inoculated onto 6 cm filter papers (Whatman grade 40) placed in Petri dishes (6 cm diameter) with 0.35 µL of nematode suspensions. Initial trials were carried out with the following nematode rates 100 IJs/cm^2 (2400 IJs/0.350 ul), 200 IJs/cm^2 (4800 IJs/0.350 ul) or 400 IJs/cm^2 (27458 IJs/0.350 ul) to determine the rate of application that was infective to *S. zeamais*. Based on the dose-response experiment, the application rate of 400 IJs/cm^2 was selected as the effective rate for screening of the six nematodes for virulence to *S. zeamais*. The controls were set up as described above but consisted of 0.35 µL of tap water sprayed onto 6 cm filter papers in Petri dishes. Ten *S. zeamais* adults (1–3 day old) and a kernel of maize were transferred to each of the Petri dishes. The experiment was organized in a completely randomized design with nine replicates of 10 weevils each per treatment and control that were grouped into three sets for examination of weevil mortality 3, 7 and 14 dpi (days post inoculation). The experiment was conducted over four consecutive trials with new generations of weevils. The Petri dishes were kept in a controlled chamber maintained at 25 ± 1.5 °C and high relative humidity (> 75%) maintained with moist filter paper.

2.5. Susceptibility of Sitophilus zeamais to entomopathogenic fungi

Beauveria bassiana and *Metarhizium brunneum* were investigated at three different concentrations designated as low (1×10^7 conidia/mL), medium (1×10^8 conidia/mL) and high (1×10^9 conidia/mL) doses. The experimental design involved 7 treatments consisting of three different doses of each of the fungi and the control. Nine Petri dishes for