BECKER, G. 1942: Ökologische und physiologische Untersuchungen über die holzzerstörenden Larven von Anobium punctatum de Geer. Zeitschrift für Morphologie und Ökologie der Tiere 39 (2): 98–152.

BIEBL, S., AUER, J., 2017: The practical use of braconid wasps for the control of the common furniture beetle (Coleoptera: Anobiidae). In: Proceedings of the 9th International Conference on Urban Pests: 367–375.

GRAF, E.; 1992. Biologischer Holzschutz – Möglichkeiten und Grenzen. In: Deutsche Gesellschaft für Holzforschung (Hrsg.) DGfH 19. Holzschutz Tagung Rosenheim.

HAMMER, O., HARPER, D.A.T. AND RYAN, P.D. (2001) PAST: Paleontological Statistics Software Package for Education and Data Analysis, Version 2.14. Palaeontologia Electronica, http://folk.uio.no/ohammer/past

HAUSTEIN, T. 2010: Zur Diagnose und integrierten Bekämpfung Holz zerstörender Insekten unter besonderer Berücksichtigung der Buntkäfer. Fraunhofer IRB Verlag Stuttgart.

KASSEL, A., AUER, J. 2015: A new biological control method for the common furniture beetle, *Anobium punctatum.In:* Integrated Protection of Stored Products. IOBC-WPRS Bulletin Vol. 111, 2015 pp. 455–461.

LYGNES, R.; 1956: Zur Kenntnis der Biologie von *Spathius exarator* L. (Hym., Bracon.). Zeitschrift für angewandte Entomologie 38: 73–81.

PAUL, F., PROZELL, S., SCHÖLLER, M., 2008: Monitoring natürlicher Feinde des gemeinen Nagekäfers *Anobium punctatum* (De Geer, 1774) (Coleoptera: Anobiidae). Mitteilungen der Deutschen Gesellschaft für allgemeine und angewandte Entomologie 16: 323–326.

PINNINGER, D.B., CHILD, R.E., 1996: Woodworm – A necessary case for treatment? New techniques for the detection and control of furniture beetle. In: Proceedings of the second international conference of urban pests: 353–359.

OTT, R., 2005: Untersuchungen über die Entstehung von Bohrmehlhäufchen an Schlupflöchern des Gemeinen Nagekäfers (Anobium punctatum) De Geer. http://www.holzfragen.de/seiten/biol_holzschutz.html (Download: January 27, 2017)

QUERNER, P., 2017: Success and failure in biological pest control with the larval parasitoid *Lariophagus istinguendus* in museums and historic buildings. In: Proceedings of the 9th International Conference on urban pests: 359–366.

SCHMIDT, H., 1952. Holzschädlingstafeln: Anobium punctatum De Geer, Zeitschrift HOLZ als Roh- und Werkstoff 10. Jg., Heft 3: 119–120.

SCHÖLLER, M., PROZELL, S., FLORIAN, P., 2008: Versuche zur biologischen Bekämpfung des Gemeinen Nagekäfers Anobium punctatum. 2.N.i.Ke.-Workshop 2008. Berlin

SCHÖLLER, M., PROZELL, S., 2011: Biological control of cultural heritage pest Coleoptera and Lepidoptera with the help of parasitoid Hymenoptera. Journal of Entomological and Acarological Research Ser. II, 43 (2): 157–168.

Prospects of Entomopathogens in Post-Harvest Integrated Pest Management

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Abstract

In these exploratory experiments, entomopathogenic nematodes and fungi were investigated for the management of the populations of postharvest insect pests. Nematodes were screened for pathogenicity to *Plodia interpunctella* (Hübner), while nematodes and fungi were investigated for virulence to the maize weevil, *Sitophilus zeamais* (Motschulsky). Adults and larvae of *P. interpunctellea* were screened for susceptibility to the following six nematodes: *Heterorhabitis bacteriophora* Poinar (HP88, Lewiston and Oswego strains); *H. indica* Poinar, Karunakar and David (Homl strain); *H. marelatus* Liu and Berry (Point Reyes strain); *H. megidis* Poinar, Jackson, and Klein (UK211 strain); and *H. zealandica* Poinar (NZH3 strain). The nematodes that had the highest virulence to larvae and adults of *P. interpunctellea* were *H. indica*, *H. megidis*, and *H. marelatus*. Six strains of nematodes were studied, namely *H. bacteriophora*, *H. indica*, *H. georgiana* (K22), *Steinernema feltiae* SN and *S. carpocapsae.* All strains of fungi, *Beauveria bassiana* (GHA) and *Metarhizium brunneum* (F52) were evaluated for infectivity to adults of *S. zeamais*. The two strains of Steinernematidae nematodes and a strain of fungus, *B. bassiana* were found to cause significant mortality of the weevils compared to the rest of the entomopathogens and the control. To demonstrate the practical application of entomopathogens, wettable dust of *B. bassiana* were dispensed on jute bags after which weevils were exposed to the treated surfaces for 30 min. The exposed weevils recorded between 90 to 100% mortality 14-d after exposure. Additional study demonstrated that the parasitoid, *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae) could be integrated with entomopathogenic nematodes. These experiments demonstrate the potential usefulness of entomopathogens in the management of stored product Lepidopteran and Coleopteran pests.

Keywords: entomopathogens, nematodes, fungi, parasitoid, virulence.

1. Introduction

Stored product Lepidopteran and Coleopteran pests are cosmopolitan pests that cause severe postharvest losses of grains and processed commodities. In the past, chemical pesticides have been used to disinfest commodities of storage pests. Integrated pest management (IPM) strategies in postharvest systems based on chemical pesticides pose health, legal and financial risks due to pesticide residues that may occur in foods (Monaco et al., 2002). Furthermore, there are dramatic restrictions in the use of synthetic pesticides to control pest populations in storage facilities. Natural enemies present alternative methods to overcome the potentially harmful effects of chemical pesticides especially in the management of postharvest pests since natural enemies are mostly environmentally safe and do not pose any dangers to humans or the environment. Natural enemies employed in postharvest IPM have been mostly parasitoids. Other forms of biological control such as use of entomopathogenic nematodes and fungi have recently started to attract attentions. This study investigated the potential of entomopathogens in the management of stored product pests. *Plodia interpunctella (Hübner*) (Lepidoptera: Pyralidae) and *Sitophilus zeamais* (Motschulsky) (Coleoptera: Dryophthoridae) were the test insects in this study.

Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae spp.) have the potential to control a broad range of arthropod pests including stored product insects. Nematodes kill their insect hosts with their mutualistic relationship with bacteria *Xenorhabdus* for Steinernematidae and *Photorhabdus* for Heterorhabditidae (Gram-negative Enterobacteriaceae) that inhabit the intestinal lumen of nematodes as symbionts (Boemare, 2002). Free living and infective juveniles (IJs) or third stage juveniles of nematodes enter the hemocoel of the host insects through the natural openings such as mouth, anus and spiracles or respiratory system, and release their pathogenic bacteria that propagate rapidly and kill insects within 48 hours (Poinar, 1990). The nematodes develop and complete 2 to 3 generations before leaving the host insect. Mbata and Shapiro-Ilan (2010) investigated the pathogenicity of different spp. of entomopathogenic nematodes to *P. interpunctella* larvae and reported *Heterorhabditis indica* (HOM1) strain to be most virulent of all the strains tested. In a laboratory experiment, strains of Steinernematidae and Heterorhabditidae showed higher virulence for the larvae of *Ephestia kuehniella* Zeller, *Tenebrio molitor* (Linnaeus) and adults stages of *Acanthoscelides obtectus* (Say) compared to *Sitophilus zeamais* and *S. oryzae* (Barbosa-Negrisoli et al., 2013). In another study, *H. indica* was reported to be pathogenic to several stored product pests including *S. zeamais* (Maketon et al., 2011). Ramos-Rodriguez et al. (2006) reported that *T. molitor*, *Oryzaephilus surinamensis* (Linnaeus) and *Tribolium castaneum* (Herbst) were found to be susceptible to *Steinernema riobrave* Cabanillas, Poinar and Raulston.

Entomopathogenic fungi have also been demonstrated to be a promising alternative to chemical pesticides for biological control of arthropod pests. Pathogenicity of two strains of *Purpureocillium lilacinum* to *T. confusum, R. dominica* and *S. zeamais* has been reported (Barra et al., 2013). The pathogenic effect of ten strains of *Beauveria bassiana* and two of *Metarhizium brunneum (Metschnikoff) treated with 1 x* 10^9 *conidia/ ml against S. zeamais resulted in weevil mortality in the range of 53 to 93% (Ruelas-Ayala et al., 2013). In contrast, Trevisoli et al. (2015) reported that S. zeamais is less susceptible to the fungal strains of B. bassiana*, *M. brunneum and Isaria fumosorosea.* Thus, susceptibility of the maize weevil to *B. bassiana* and *M. brunneum* requires further elucidation.

The studies reported here comprised of results from exploratory investigation into the potentials of entomopathogenic nematodes and fungi for biocontrol of two stored product pests, P. interpunctella and S. zeamais. In addition, combined application of Braconid wasp, H. hebetor, with entomopathogenic nematodes were compared with applications of nematodes or wasp alone to determine if the two biocontrol agents could be integrated for the management of P. interpunctella populations.

2. Materials and Methods

Rearing of insects and natural enemies

Plodia interpunctella stock culture was originally obtained from USDA-ARS, Grain Marketing and Research Laboratory, Manhattan, KS in 2001 and had since been maintained on the artificial moth diet at 28 ± 1.5 °C, 70 ± 5% RH and a 16h: 8h photoperiod in Biology department of Fort Valley State University (FVSU), Fort Valley, GA.

Foundation culture of *S. zeamais* was obtained in August, 2006, from the University of Georgia's center for Invasive species and Ecosystem Health, Department of Entomology, Tifton, GA. Populations of *S. zeamais* have been maintained in the insectary of FVSU.

Wasp culture was originally collected from the Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK. Wasps were reared on 50 late instars of *P. interpunctella* in 1000 mL glass rearing jars kept in experimental chamber maintained at 28 ± 1.5 °C, 70 ± 5% RH and a 16: 8 (L:D) photoperiod.

Nematodes were reared at ~ 25ºC in last instar of greater wax moth, *Galleria mellonella* (Linnaeus) following a procedure described by 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 15 d or less before being used for experiments.

Cultures of *H. bacteriophora* (HP88) and *H. megidis* were obtained from the MicroBio Group of Becker Under Wood (West Sussex, UK), *H. bacteriophora* (Lewiston) and *H. indica* from Integrated BioControl Systems (Lawenceburg, IN), *H. bacteriophora* (Oswego) from Dr. Elson Shields (Cornell University, Ithaca, NY) and *H. marelatus* and *H. zealandica* from P. stock (University of Arizona, Tucson, AZ). *H. indica* Poinar, Karunakar, and David (Homl strain), *H. georgiana* (K22), *Steinernema feltiae* (SN), and *S. carpocapsae* (All) were obtained from USDA-ARS culture collection in Byron, GA.

Entomopathogenic fungi, *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (GHA strain) and *Metarhizum brunneum* Petch (F52 strain) were originally obtained from Stefan Jaronski (USDA-ARS) and cultured on Sabouraud dextrose agar supplemented with 0.2% yeast extract according to a procedure described by Goettel and Inglis (1997). Established cultures of fungi were stored at 4 ºC for one week before experimentation begins.

Pathogenicity of entomopathogenic nematodes to third and fifth instars of Indian meal moths

Virulence of the nematodes was tested at 150 and 480 dose rate of IJs per larva in plastic cups (3-4 cm internal diameter, 3 cm deep; Bioserv Frenchtown, NJ) at ~28 ± 1.5 °C and 70 ± 5% RH against 10-d third and 18-d-old fifth instars of *P. interpunctella*. One larva with 3.5 mL moth rearing medium was placed in each cup that was covered with plastic lid. Inoculum of IJs in 0.5 mL water was added to each cup 1-d after the introduction of larva and incubated until the emergence of adults and assessment of mortality. Moisture content of the medium was ~14% after adding 0.5 mL water. Four replicates of 10 cups per treatment of nematode strain and untreated control (water) of third and fifth instar of *P. interpunctella* were set up and two trials were conducted for both larval stage. Mortality of third and fifth instars was recorded after 10-d and 21-d respectively in treated and control cups.

Influence of combined application of nematodes and parasitoid versus single of either nematodes or parasitoid

This laboratory experiment assessed the virulence to *P. interpunctella* and interaction among biological control agents was conducted in 1 l rearing jars (7.4 cm diameter and16.8 cm height). A set of four jars were set up with two host larval densities, 20 and 40. Treatments consisted of *H. indica* and *H. hebetor*; 8000 IJs with 2 mL water (200 and 400 IJs/insect) were applied to larvae in one treatment, three pair (three males and three females) of adult parasitoids exposed to larvae in

second treatment, combination of exposure of 2 mL of water consisting of 8000 IJs and three pairs (2-d old males and females) of adult *H. hebetor* in treatment three and in treatment four, control was set up with 2 mL water. All jars were covered with filter paper and maintained at 28 ± 1.5 °C, 70 ± 5% RH and a 16: 8 (L:D) photoperiod. After 3-d period, mortality of host larvae and parasitoid was observed and afterwards all jars containing larvae with parasitoids, IJs or to both were transferred to incubator until the complete development of F_1 parasitoids. Each treatment was replicated four times and three runs were conducted.

Pathogenicity of entomopathogenic nematodes to *Sitophilus zeamais*

The protocol for inoculating the weevils with entomopathogenic nematodes followed a method used in screening *P. interpunctella* 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* Poinar (VS) and *S. carpocapsae* (All). Infective juveniles of nematodes in aqueous solutions were inoculated onto 7 cm filter papers (Whatman grade 40) placed in petri dishes (6 cm diameter) with 0.35 µL of nematode suspensions at the rates of 100 IJs/cm^2 (2400 IJs/0.350 uL), 200 IJs/cm^2 (4800 IJs/0.350 µL) or 400 IJs/cm^2 (27458 IJs/0.350 µL) to determine the rate of application that was infective to *S. zeamais*. Based on 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 d 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), consecutively. 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 °C and uncontrolled but high relative humidity due to the moist filter paper.

Pathogenicity of entomopathogenic fungi to *Sitophilus zeamais*

Beauveria bassiana (GHA strain) and *M. brunneum* (F52 starin) were investigated at three different concentrations designated as low (1 x 10^7 conidia), medium (1 x 10^8 conidia) and high (1 x 10^9 conidia) doses. The experimental design involved 7 treatments consisting of three different doses of each of the fungi and the control. Nine petri dishes for each of the strains, three for each dose, were prepared with filter paper and each petri dish was added with a different concentration of the fungal suspension from the cultures at the rates specified above that corresponded to 6857 (low), 13714 (medium), and 27482 (high) conidia/mL. One maize kernel, and 10 adult weevils were added to each petri dish and the dishes were sealed with parafilm. Observations of the weevils for mortality were carried out 7 and 14 ds post inoculation. The experiment was repeated over two consecutive trials with new generations of weevils.

Infectivity of fungal spores applied to jute bags against *Sitophilus zeamais*

The more virulent entomopathogenic fungus to *S. zeamais* determined in the test described above, *B. bassiana*, was investigated further for the protection of bagged maize grain by applying the wettable powder to jute bags used in the postharvest storage of maize. At this stage, it is reasonable to investigate the fungi further since they do not require moist surface for survival and dispersal[29]. Wettable powder of *B. bassiana* (GHA strain) 4.4 x 10^{10} conidia/g (Botanigard 22wp® WPO) was obtained from BioWorks (Victor, NY).

Fungal application during the experiment was carried out under a biosafety cabinet to contain the fungal powder and prevent contaminating the control weevils. The jute bags (surface area = 2.06 x 10^5 mm^2) were each treated with one of three rates of the wettable powder comprising of 2.13 x 10^7 conidia/mm^2, 1.07 x 10^7 conidia/mm^2, and 0.5 x 10^7 conidia/ mm^2. Control bags were not treated with any powder. Appropriate quantities of the wettable *B. bassiana* powder were weighed out for

each of the treatments and transferred into containers or bins (L 50.8 x W 47.8 x D 15.2 cm). Jute bags were placed in each of the containers dispensed with the wettable *B. bassiana* powder. Following the replacement of the lids of the containers, the containers were shaken vigorously to ensure even distribution of the powder through the surface of the jute bags. Each treatment and the control were replicated three times. Twenty weevils per bag were released to crawl on bags for a 30 min period and were transferred thereafter to 60 mm petri dishes lined with filter papers moistened with 0.35 mL of tap water. A kernel of maize that served as food for the weevils was placed in each of the petri dishes. The lids on dishes were taped down on to trays to prevent insects from escaping. Survival of insects was determined 7 and 14 d post-exposure. The entire experiment was repeated two times.

3. Results

Mortality of third and fifth instars of *P. interpunctella* exposed to entomopathogenic nematodes

Mortality differences in the 3rd and 5th instars of *P. interpunctella* exposed to nematodes were observed between all treatments and the control (Tab. 1; $F = 6.61$; df = 12, 67; $P < 0.0001$). Third instar larvae were found to be more susceptible at the rate of 480 IJs/insect than 150 IJs/insect. At the higher exposure rate of 480 IJs/larva, *H. marelatus* (Point Reyes), *H. megidis* (UK211), *H. indica* (HOM1) and *H. marelatus* strains caused higher larval mortality than the control whereas at the rate of 150 IJ/larva *H. zealandica* and *H. indica* caused greater mortality than the control (Tab. 2).

Influence of combined versus separate application of nematodes and parasitoid

Moth larvae exposed to nematodes at the rate of 200 (4000 IJs/2 mL) and 400 (8000 IJs/2 mL) IJs per larva had significantly different larval mortality (at 200 IJ/larva: F = 276.23; df = 3, 36; $P = 0.0001$; Fig. 1 and at 400 IJ/insect: F = 110.02; df = 3, 36; $P = 0.0001$; Fig. 1). Combined treatment of nematodes at the dose rate of 200 and 400 IJs per larva with *H. hebetor* (3 pairs of males and females) caused higher *P. interpunctella* larvae mortality.

Chi-square value (χ^2) indicated that combined effect of nematodes and parasitoids on the larval mortality were not antagonistic but could possibly be additive or synergistic because at both exposure rates of nematodes with *H. hebetor*, high mortality of host larvae was achieved (for 200 IJ/larva: $\chi^2 = 1.81$; M = 0.87; $P < 0.05$ and for 400 IJ/larvae: $\chi^2 = 2.26$; Me = 0.84; $P < 0.05$).

Pathogenicity of nematodes to *Sitophilus zeamais*

No significant differences were observed on the survival (%) of maize weevils following 3 and 5 dpi with nematodes compared to the control but survival of adult S. *zeamais* at 7 dpi was significantly lower than the control. (Tab. 3: $F = 2.78$; $df = 6, 41$; $P < 0.0001$). Maize weevils treated with *S. carpocapsae* (All) exhibited the highest susceptibility with survival rate at 28.3%. Pathogenicity of *S. carpocapsae* against weevils was significantly higher than the rest of the nematode strains except *S. feltiae*.

Pathogenicity of entomopathogenic fungi to *Sitophilus zeamais*

Survival of maize weevils was significantly reduced at 14 dpi ($F = 6.05$; df = 6, 41; $P \leq 0.0004$; Tab. 4) following exposure to fungi but at 7 dpi survival of weevils exposed to fungi was not significantly different from the control. Survival of weevils exposed to low dose of *M. brunneum* (6857 conidia/mL) was significantly different from the survival of weevils exposed to medium fungi doses (13714 conidia/mL) at 14 dpi. Significant reduction in the survival of maize weevils was obtained at low, medium and high rates of *B. bassiana* compared to *M. brunneum* and control.

Fungal strains infectivity to jute bags against *Sitophilus zeamais*

Wettable *B. bassiana* powder applied to jute bags at 25, 50 and 100 g highly affected the survival of weevils at 14 dpi (Tab. 5: F= 76.16; df = 3, 15; $P < 0.0001$) compared to 7 dpi (Tab. 5 : F= 40.75; df = 3, 15; $P < 0.0001$) and the control. No significant differences were observed on the percentage survival of maize weevils exposed to low dose (25 g) of *B. bassiana* and the control weevils. Higher rate (100 g) of *B. bassiana* generated 100% mortality of treated weevils at 14 dpi.

Tab. 1. Mortality of third instar of *Plodia interpunctella* after 10-d exposure to nematodes strains at rates of 480 and 150 IJs/larva.

	Mean ± S.E.	
Nematode strain	**480 nematodes/larva**	**150 nematodes/larva**
***H. bacteriophora* (HP 88)**	4.11 ± 0.68bc	2.78 ± 0.43cd
***H. bacteriophora* (Lewiston)**	4.22 ± 1.23bc	2.44 ± 0.88cd
***H. bacteriophora* (Oswego)**	4.00 ± 1.00bc	2.67 ± 0.60cd
***H. indica* (Homl)**	5.56 ± 1.12ab	3.78 ± 0.92c
***H. megidis* (UK 211)**	5.44 ± 1.20ab	2.11 ± 0.75d
***H.marelatus* (Point Reyes)**	6.22 ± 1.04a	2.00 ± 0.71d
***H. zealandica* (NzH3)**	3.22 ± 0.88cd	4.22 ± 0.92cd
Control	1.33 ± 0.44d	1.50 ± 0.46d

Mean ± SE (out of 10) within a column followed by same letter are not significantly different (Tukey's test, $P < 0.05$).

Tab. 2. Effect of strains of entomopathogenic nematodes on the mortality of fifth instars of *Plodia interpunctella*.

Nematode strain	Mean ± S.E.
***H. bacteriophora* (HP 88)**	3.6 ± 0.33a
***H. bacteriophora* (Lewiston)**	4.1 ± 0.43a
***H. bacteriophora* (Oswego)**	4.2 ± 0.36a
***H. indica* (Homl)**	4.1 ± 0.41a
***H. megidis* (UK 211)**	3.9 ± 0.31a
***H.marelatus* (Point Reyes)**	4.1 ± 0.46a
***H. zealandica* (NzH3)**	4.3 ± 0.42a
Control	1.4 ± 0.22b

Means within a column followed by same letter are not significantly different (Tukey's test, $P < 0.05$)

Tab. 3. Percentage survival of adult *Sitophilus zeamais* after exposure to entomopathogenic nematodes for 7-d. Control consisting of water only.

Nematode strain	**Mean ± S.E.**
Control	100 ± 0.00a
H. georgiana	78.33 ± 3.07b
H. indica	60.00 ± 10.95bc
***H. bacteriophora* (Lew)**	56.67 ± 2.10bc
***H. bacteriophora* (Osw)**	50.00 ± 7.30c
S. carpocapsae	28.33 ± 7.40d
S. feltiae	41.67 ± 4.01cd

Different letters within a column are significantly different (Student-Newman-Keul's test, $P < 0.05\%$).

Tab. 4. Survival (%) of maize weevils exposed to entomopathogenic fungi for 14-d. Control is with water.

Fungal strain	**Mean ± S.E.**
Control	90.00 ± 6.32a
Bb-lo	65.00 ± 12.58bc
Bb-med	61.67 ± 9.45bc
Bb-hi	38.33 ± 10.13c
Met-lo	75.00 ± 12.58ab
Met-med	78.33 ± 7.03ab

Met-hi	48.33 ± 6.00bc

Different letter (within column) is significantly different at 5% significant level (Student-Newman-Keul's test).

Tab. 5. Seven and 14 dpi survival (%) of maize weevils exposed for 30 min. to jute bags treated with different rates of wettable *B. bassiana* powder. Control was water only.

Dose of Fungi	7 dpi	14 dpi
Untreated control	98.33 ± 1.66a	76.66 ± 3.80a
Bb-25 g (low)	71.67 ± 2.10b	8.33 ± 4.01b
Bb-50 g (medium)	64.17 ± 5.83b	0.83 ± 0.83c
Bb-100 g (high)	72.50 ± 2.14b	0.00 ± 0.00d

Different letter (within column) is significantly different at 5% significant level (Student-Newman-Keuls test).

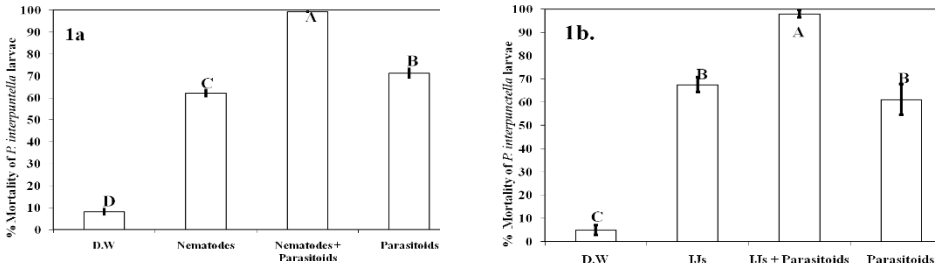

Fig. 1. Mortality (%) of Indian meal moth larvae at the exposure rate of 200 (1a) and 400 (1b) IJs of *Heterorhabitis indica/moth larva*, parasitoids (*Habrobracon hebetor*) or combination of *H. indica* and parasitoids. D.W. = Distilled Water for control, IJs= Infective juveniles nematodes. Different letter above bars indicate significant difference (Student-Newman-Keuls test, $P < 0.05\%$).

4. Discussion

Heterorhabditis marelatus (Point Reyes), *H. megidis* (UK211), and *H. indica* (HOM1) showed more pathogenicity and caused higher mortality in Indian meal moth larvae. These nematodes have unique characteristics that recommend them for consideration for year round management of *P. interpunctella*. *H. marelatus and H. megidis are considered to be cold tolerant (Grewal et al., 1994, Berry et al., 1997) while H. indica is heat tolerant (Shapiro and McCoy, 2000). This implies that these three nematodes could be used at different times of the year to regulate populations of P. interpunctella.*

Combination of nematodes and parasitoids enhanced the mortality of *P. interpunctella*. Dillon et al. (2008) observed that the interaction between the nematodes *H. downesi* or *S. carpocapsae* and the parasitoid *Bracon hylobii* enhanced the mortality of the pest host, *Hylobius abietis*. Interaction between entomopathogenic nematodes and the parasitoid could possibly be additive or synergistic.

Maize weevils were more susceptible to Steinernematid strains particularly *S. carpocapsae* (All) and *S. feltiae* (SN) compared to the Heterorhabditidae. Entomopathogenic fungi, *B bassiana*, exhibited strong virulence against adult maize weevils compared to *M. brunneum* (Vega and Hofstetter, 2014). High concentration of wettable powder of *B. bassiana* applied to jute bag surface caused 100% mortality in maize weevils 14 days after inoculation. This implies that a path exists by which entomopathogens could be integrated in the IPM of postharvest arthropods.

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References

BARBOSA-NEGRISOLI, C. R. C., NEGRISOLI, A. S., BERNARDI, D. AND M. S. GARCI, 2013: Activity of eight strains of entomopathogenic nematodes (Rhadditia:Steinermatidae, Heterorhabditae) against five stored product pests. Experimental Parasitology **134**, 384–388.

BARRA, P., ROSSO, L., NESCI, A., AND M. ETCHEVERRY, 2013: Isolation and identification of entomopathogenic fungi and their evaluation against *Tribolium confusum*, *Sitophilus zeamais*, and *Rhyzopertha dominica* in stored maize. Journal of pest science **86**, 217–226.

BERRY, R. E., LIU, J., AND E. GROTH, 1997: Efficacy and persistence of *Heterorhabditis marelatus* (Rhabditida: Heterorhabditidae) against root weevils (Coleoptera: Curculionidae) in strawberry. Environmental Entomology, **26**, 465–470.

BOEMARE, N., 2002. Biology, taxonomy, and systematic, pp. 35-56. In R.Gaugler (Ed.), Entomopathogenic Nematology. CABI, New York.

DILLON, A.B., MOORE, C.P., DOWNES, M. J., AND C.T. GRIFFIN, 2008: Evict or infect? Managing populations of the large pine weevil, *Hylobius abietis*, using bottom-up and top-down approach. Forest Ecol. Management **255**, 2634–2642.

GOETTEL, M.S. AND G.D. INGLIS, 1997: Fungi: hyphomycetes. In: LACEY (ed) Manual of techniques in insect pathology. Academic Press, San Diego, pp. 213–249.

GREWAL, P.S., SELVAN, S., AND R. GAUGLER, 1994: Thermal adaptation of entomoparhogenic nematodes: niche breadth for infection, establishment, and reproduction. Journal of Thermal Biology **19**, 245–253.

MAKETON, M., SOMSOOK, V., RATTANAKORN, P., AND D. HOTAKA, 2011: Pathogenicity and culture of a *Heterorhabditis indica* isolate from Thailand. Nematropica, **41,** 52-61.

MBATA, G.N., AND D.I. SHAPIRO-ILAN, 2005: Laboratory evaluation of Heterorhabditid Nematodes to *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae). Environmental Entomology **34**, 676–682.

MBATA, G.N., and D.I. SHAPIRO-ILAN, 2010: Compatibility of *Heterorhabditis indica* (Rhabditida: Heterorhabditidae) and *Habrobracon hebetor* (Hymenoptera: Braconidae) for biological control of *Plodia interpunctella* (Lepidoptera: Pyralidae). Biological control **54,** 75–82.

MONACO, J.T, WELLER, S.C. AND F.M. ASHTON, 2002: Herbicide registration and environmental impact. In Weed Science: Principles and Practices, 4th ed; MONACO, T.J., WELLER, S.C., AND ASHTON, F.M. (Eds.) John Wiley & Sons: New York, NY, USA.

POINAR, G.O. JR., 1990: Biology and taxonomy of Steinernematidae and Heterorhabditidae, pp. 23–62. *In* R. GAUGLER AND H.K. KAYA (Eds.), Entomopathogenic nematodes in Biological control. CRC, Boca Raton, Florida.

RAMOS-RODRÍGUEZ, O., CAMPBELL, J.F., AND S.B. RAMASWAMY, 2006: Pathogenicity of three species of entomopathogenic nematodes to some major stored-product insect pests. Journal of Stored Products Research **42,** 241–252.

RUELAS-AYALA, R.D., GARCÍA-GUTIÉRREZ, C. AND A. ARCHULETA-TORRES, 2013: Selection of *Beauveria bassiana* and *Metarhizium anisopliae* isolates tolerant to high temperatures for the control of *Sitophilus zeamais* (Coleoptera: Curculionidae). Southwestern Entomologist **28**, 313–324.

SHAPIRO, D.I. AND C.W. MCCOY, 2000: Virulence of entomopathogenic nematodes to *Diaprepes abbreviates* (Coleoptera: Curculionidae) in the laboratory. Journal of Economic Entomology **93**, 1090–1095.

TREVISOLI, A.T., TREVISOLI, A.L., TEIXEIRA, D.R., LINHARES, V.H.X., CLAUDIO, S., AND P.R. ANTONIO, 2015: Efficiency of entomopathogenic fungi to the control of *Sitophilus oryzae* L. (Coleoptera: Curculionidae) under laboratory condictions. Comunicata Scientiae, **6,** 90–96.

VEGA, F.E. AND R.W. HOFSTETTER, (Eds.) 2014. Bark beetles: biology and ecology of native and invasive species. London Academic Press.

WOODRING, J.I. AND H.K. KAYA, 1988: Steinernematid and heterorhabditid nematodes: a handbook of biology and techniques. Southern cooperative series (USA) bulletin **331**. Arkansas Agricultural Experimental Station, Fayetteville.

Chilled Aeration to Control Pests and Maintain Grain Quality During the Summer Storage of Wheat in North Central Region of Kansas

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Abstract

Chilled aeration allows to cool grain, independent of ambient conditions, to "safe" temperatures where insect, fungi, and spoilage is reduced to the minimum. The objective of this research was to evaluate the advantages of using grain chilling to preserve the quality of grain and reduce post-harvest losses, compared to conventional