

RESEARCH/INVESTIGACIÓN

CONTROL OF *CYLAS FORMICARIUS* USING ENTOMOPATHOGENIC NEMATODES ISOLATED FROM HAWAI‘I

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ABSTRACT

Wong, L. G. K., K.-H. Wang, R. Myers, and B. S. Sipes. 2025. Control of *Cylas formicarius* using entomopathogenic nematodes isolated from Hawai‘i. *Nematropica* 55:1-9.

Entomopathogenic nematodes (EPN) have potential to control the sweetpotato weevil (SPW; *Cylas formicarius*). Laboratory tests were conducted using *Steinernema feltiae* MG-14, *Oscheius tipulae* OA-12, and *Heterorhabditis indica* OM-160 isolated from Hawai‘i on larvae of SPW in infection courts. Subsequently, *S. feltiae* was tested for effectiveness at 0.5, 1.0, and 2.5 billion infective juveniles (IJ)/ha in a sweetpotato field. In another field experiment, monthly applications of *O. tipulae* were evaluated for SPW control. In the laboratory assays, *S. feltiae*, *O. tipulae*, and *H. indica* caused an adjusted mortality (mortality of treatment – mortality in control) of 50%, 30%, and 25% to SPW larvae, respectively. In a field experiment with *S. feltiae*, SPW damage and SPW densities were low (< 1 SPW/kg sweetpotato swollen root) across all application rates, resulting in an inconclusive study. In contrast, the field experiment testing *O. tipulae* experienced high SPW pressure (32 SPW/kg root in the untreated control). Sweetpotato treated with *O. tipulae* suffered less damage and had lower SPW densities than the nontreated control ($P \leq 0.05$). In conclusion, all three EPN species isolated from Hawai‘i showed promise in suppressing SPW in the laboratory and in the field.

Key words: Biological control, *Heterorhabditis indica*, *Ipomea batatas*, *Oscheius tipulae*, *Steinernema feltiae*, sweetpotato weevil

RESUMEN

Wong, L. G. K., K.-H. Wang, R. Myers, and B. S. Sipes. 2025. Control de *Cylas formicarius* mediante nematodos entomopatógenos aislados de Hawai‘i. *Nematropica* 55:1-9.

Los nematodos entomopatógenos (EPN) tienen potencial para controlar el gorgojo del camote (SPW), *Cylas formicarius*. Se realizó una serie de pruebas de susceptibilidad utilizando *Steinernema feltiae* MG-14, *Oscheius tipulae* OA-12 y *Heterorhabditis indica* OM-160 aislados de Hawai‘i en larvas de *Cylas formicarius* en fuentes de infección. Posteriormente, se probó la eficacia de *S. feltiae* a 0,5, 1,0 y 2,5 mil millones de juveniles infectivos JI/ha en un campo de camote. En otro experimento de campo, se evaluaron aplicaciones mensuales de *O. tipulae* para el control de SPW. En los ensayos de laboratorio, *S. feltiae*, *O. tipulae* y *H. indica* causaron una mortalidad ajustada (mortalidad del tratamiento – mortalidad del control) del 50%, 30% y 25% en larvas de SPW, respectivamente. En un experimento de campo con *S. feltiae*, el daño de SPW y las densidades de SPW fueron bajas (< 1 SPW/kg de raíz de camote) en todas las tasas de

aplicación, resultando en un estudio inconcluso. En contraste, el experimento de campo que probó *O. tipulae* experimentó una alta presión de SPW (32 SPW/kg de raíz de camote). El camote tratado con *O. tipulae*, sufrió menos daño y tuvo menores densidades de SPW que el control no tratado ($P \leq 0.05$). En conclusión, las tres especies de EPN aisladas de Hawai'i se mostraron prometedoras en la supresión de SPW en el laboratorio y en el campo.

Palabras clave: Control biológico, *Heterorhabditis indica*, *Ipomea batatas*, *Oscheius tipulae*, *Steinernema feltiae*, gorgojo del camote

INTRODUCTION

In warmer climates, sweetpotato is attacked throughout its cropping cycle by the sweetpotato weevil [SPW; *Cylas formicarius* (Coleoptera: Brentidae)], reducing marketable yields by up to 100% (Reddy *et al.*, 2014). Although several pesticides are effective and economical to manage SPW including imidacloprid, β -cyfluthrin, bifenthrin, dimethyl phosphorodithioate, and carbaryl (Richard, 2014), options for organic sweetpotato farmers are limited. However, SPW are susceptible to several parasites including entomopathogenic nematodes (EPN). Myers *et al.* (2020) evaluated several species of EPNs against SPW adult, pupae, and larval stages in laboratory conditions and found *Heterorhabditis indica* OM-158, *H. indica* KM-89, and *Heterorhabditis* sp. HM-108 caused 88%, 96%, and 4% mortality *in vitro* on larvae, pupae, and adults of SPW, respectively. In addition, efficacy of *H. indica* OM-160 against SPW in a field cage experiment using field soil was demonstrated (Myers *et al.*, 2020).

Due to restrictions on importing and releasing alien biocontrol agents in Hawai'i, commercial EPNs are not readily available in the state (State of Hawai'i, 2019). However, EPNs isolated and produced within the state may be used. An immediate challenge facing the use of EPNs in Hawai'i is finding effective locally occurring EPN isolates. Several strains of *Steinernema feltiae* and *H. indica* have been isolated in Hawai'i and have potential for pest management (Bisel, 2016; Hara *et al.*, 1991;). In addition, isolates of *Oscheius* spp. with entomopathogenic abilities have been isolated in Hawai'i (Bisel *et al.*, 2016) and may have potential to manage insects as well. This project focused on evaluating locally available indigenous EPN isolates for ability to control SPW under laboratory and field conditions.

EPNs have a variety of foraging strategies that may optimize them for different environments

(Campbell *et al.*, 2003; Grewal *et al.*, 1994). Heterorhabditids, in general, have cruising behaviors and are attracted to long-range volatile cues (Lewis, 2002). On the other hand, some Steinernematids wait for prey to wander within their vicinity and use an ambush strategy to attack insects (Campbell *et al.*, 2003). Whereas other Steinernematids, such as *S. feltiae*, may have intermediate cruising and ambushing behaviors (Campbell *et al.*, 2003; Grewal *et al.*, 1994; Wilson *et al.*, 2012) for foraging. The foraging behavior of *Oscheius* spp. is not well studied, but *O. tipulae* isolate OA-12 has demonstrated consistent entomopathogenic behavior in the laboratory (Bisel, 2016).

The objectives of this study were to evaluate the efficacy of indigenous EPNs, *Steinernema feltiae* MG-14, *Heterorhabditis indica* OM-160, and *Oscheius tipulae* OA-12, isolated from Hawai'i against the SPW 1) in laboratory conditions, 2) at different application rates, and 3) through basal stem spray application in sweetpotato fields.

MATERIALS AND METHODS

All EPN inoculum used in laboratory and field experiments was reared on laboratory-cultured *Tenebrio molitor* or *Galleria mellonella*. Colonies of *T. molitor* were maintained on rolled oats supplemented with carrots (Cotton, 1940). Colonies of *G. mellonella* were fed on Gerber baby cereal, honey, and glycerol (Eischen *et al.*, 1990). EPN used in these experiments were *S. feltiae* MG-14 isolated from Waikapu, Maui, *H. indica* OM-160 isolated from Waimanalo beach, Oahu, and *O. tipulae* OA-12 from a mixture of soils from Oahu, Hawai'i. The EPN cultures were maintained by placing infective juveniles (IJ) in a 100 mm \times 15 mm petri dish lined with Whatman filter paper #1 (an infection court) at 200 IJ/insect larvae delivered in 3 ml of water. Cadavers were collected and placed in White traps (White, 1927) 48 hours after

larvae were introduced into the inoculation courts. Infective juveniles were collected from the White traps, triple rinsed with tap water, adjusted to 1,000 IJ/ml, and stored in petri dishes at 15°C for use within 30 days after collection.

EPN efficacy in laboratory experiments

Efficacy of *S. feltiae* MG-14, *O. tipulae* OA-12, and *H. indica* OM-160 against SPW larvae was examined in petri dish infection courts in laboratory experiments. Third instar larvae of the SPW were collected from infested sweetpotato harvested from the field. The swollen roots were cut into 1-cm thick discs and SPW larvae were removed using soft forceps and immediately placed into infection courts for each of the experiments described below.

In the first experiment, *S. feltiae* MG-14 and *O. tipulae* OA-12 were introduced to five SPW larvae at 100 IJ/larvae delivered in 3 ml of water in a 60 mm × 15 mm petri dish (inoculation court) lined with Whatman filter paper #1. Inoculation courts were observed for larval mortality at 24, 48, and 72 hours after inoculation. Cadavers were counted and transferred onto individual White traps (White, 1927). White traps were observed 28 days after larval death to confirm the emergence of EPN. Larva exposed to 3 ml of water only in the infection court served as a negative control. Adjusted mortality was calculated by subtracting the mortality of the negative control from mortality in treatment groups. Each treatment was replicated three times, and the experiment was conducted twice. All larvae used in this experiment were collected in sweetpotatoes from the same field in Waialua, Oahu and used within one month of collection.

In the second experiment, five SPW larvae were exposed to *S. feltiae* MG-14 at 105, 210, or 525 IJ/larvae in a 60 mm × 15 mm infection court. These doses corresponded to 0.5, 1.0, and 2.5 billion IJs/ha. SPW mortality was recorded at 24, 48, and 72 hours after EPN inoculation. Cadavers were transferred onto individual White traps, and IJ emergence was noted 28 days after EPN inoculation. Sterile water without EPN served as the control. The experiment was conducted three times. In the first and second trials, each treatment was replicated three times, whereas in the third trial treatments were replicated six times. All larvae used in this experiment and trials were collected at

the same time from sweetpotatoes in a field at Poamoho Research Station on Oahu and used within one month of collection.

The third experiment was similar to the second experiment except that *H. indica* OM-160 was used at 105, 210, or 525 IJ/SPW larvae. Water without EPN served as a control. Each treatment was replicated five times, and the experiment was conducted twice. In the first trial larvae were collected at the same time from a sweetpotato field at the Poamoho Research Station on Oahu. In the second trial all larvae were collected two weeks after the collection for the first trial from a field at the Waimanalo Research Station on Oahu.

All data from the laboratory experiments were subjected to the Levene homogeneity of variance test using SAS 9.4 (SAS Institute Inc., Cary, NC) before being subjected to analysis of variance (ANOVA). Data from repeat trials were combined if data were deemed homogenous. Whenever appropriate, treatment means were separated using Tukey's honest significance test.

EPN efficacy in sweetpotato fields

Two field experiments were conducted to evaluate the efficacy of *S. feltiae* and *O. tipulae*. The first experiment examined different application rates of *S. feltiae*, whereas the second experiment compared the efficacy of applying *O. tipulae* monthly as a basal stem spray to conventional insecticide application.

In the first experiment, two sweetpotato field trials were established at the Magoon Research Facility, University of Hawai'i at Mānoa, HI (21.309° N, 157.807° W). The locality has an annual rainfall of 1,738 mm and an annual temperature of 23°C (Giambelluca *et al.*, 2014). *Steinernema feltiae* MG-14 was prepared as water suspensions at 0, 0.5, 1.0, or 2.5 billion IJs/ha and applied when tuberous roots were forming (5th and 6th month after planting) as basal stem sprays. Treatments were arranged in a randomized block design replicated five times. Each replicated plot was 1 m long by 0.5-m wide, planted on December 22, 2022, with four, 30-cm long slips of sweetpotato 'Okinawa' at 0.25-m spacing. Sweetpotato was irrigated weekly and fertilized with Nutricote®Total 13-13-13 (BFG, Burton, OH) at 425 kg/ha 2 and 3 months after planting. EPN basal stem sprays were applied at 5 months and again at 6 months after planting. Sweetpotatoes

were harvested 7 months after planting on July 31, 2023, from a 0.5-m section of a plot and weighed. EPN effectiveness was evaluated through sweetpotato yield, SPW damage on a scale of 0 to 5 (Fig. 1), and the number of SPW larva and adults/100 g roots. After sweetpotatoes were harvested, 10 arbitrarily selected sweetpotatoes from each plot were rated for SPW damage. After rating, the sweetpotatoes were cut into 0.5-cm thick discs and three, 100-g subsamples of the slices were sampled for SPW density. SPW adults or larvae were removed from each subsample using forceps and counted. The experiment was conducted twice in adjacent fields planted a week after the first trial (December 29, 2022) and harvested on August 3, 2023. All data from the *S. feltiae* field experiment were subjected to Proc Univariate using SAS 9.4 to check for normality of data. Data were transformed if needed prior to Levene homogeneity of variance test between the two trials and ANOVA. Means were separated using the Waller–Duncan k-ratio ($k = 100$) t-test whenever appropriate.

In the second experiment, two field trials were conducted in a commercial sweetpotato field in Waialua, Oahu, HI (21.580°N, 158.134°W, annual rainfall of 803 mm, and annual daily temperature of 23.64°C) (Giambelluca *et al.*, 2014). Sweetpotato ‘Okinawan’ was planted at 4 slips/m. Each plot was 1 m × 3 m in size with 0.5-m buffer between plots within a row. The following treatments were established: 1) EPN basal stem spray with *O. tipulae* OA-12, and 2) untreated

control. Each treatment was replicated four times per trial. Data were also collected from four additional plots following a conventional grower practice (GP) insecticide spray adjacent to the experimental plots. *Osccheius tipulae* OA-12 was applied monthly at 0.5 billion IJs/ha to the EPN plots, and water was applied to control plots commencing 1 month after planting. Application of *O. tipulae* basal stem spray was continued until harvest. GP plots were established in a nearby bed separated from the EPN and control plots per the farmer’s request. The GP consisted of monthly applications of Sevin® 4F at label rates (a.i. carbaryl, Bayer Crop Science, Research Triangle Park, NC). Sweetpotato plants were fertilized and irrigated following GP. Each treatment was replicated four times, and the EPN and control treatments were arranged in randomized complete block design. The experiment was repeated in an adjacent field on the same farm. A SPW pheromone lure (CYLFOR, Alpha Scents, Canby, OR) was installed to monitor SPW pest pressure and replaced once at three months after planting.

Sweetpotatoes were harvested five months after planting. Yield, SPW damage, and SPW population density in the tuberous roots were determined as described in the *S. feltiae* field experiment. All sweetpotatoes from a 0.5-m section in the center of each plot were collected and weighed. Ten arbitrarily selected sweet potatoes outside of the 0.5-meter section from each plot were rated for SPW damage on a scale of 0-5 (0 = no surface damage, 1 = less than 10% surface damage, 2 = less than 20% surface damage, 3 = less than 40% surface damage, 4 = less than 50% surface damage, 5 = greater than 50% surface damage).



Figure 1. Sweetpotato weevil damage rating scale of 0-5. 0 = no visible damage, 1 = less than 10% surface damage, 2 = less than 20% surface damage, 3 = less than 40% surface damage, 4 = less than 50% surface damage, 5 = greater than 50% surface damage.

damage, 2 = less than 20% surface damage, 3 = less than 40% surface damage, 4 = less than 50% surface damage, 5 = greater than 50% surface damage; Fig. 1). After rating, the 10 sweetpotatoes from each plot were sliced into 0.5-cm thick pieces, and 3 subsamples of root pieces (100 g each) were quantified for SPW larvae as described above. All data from the *O. tipulae* field experiment were subjected to Proc Univariate using SAS 9.4 to check for normality. Data were transformed if needed prior to the Levene homogeneity of variance test between the two trials and ANOVA. Means from grower practice were not included in the statistical analysis, but data were used as reference for efficacy.

RESULTS

EPN efficacy in laboratory experiments

In Experiment 1, *S. feltiae* MG-14 caused greater mortality of SPW larvae than *O. tipulae* OA-12 ($P \leq 0.05$). Nonetheless, both EPNs resulted in higher SPW mortality than the control ($P \leq 0.05$; Fig. 2). SPW exposed to *S. feltiae* MG-14 had an adjusted mortality of 83%, whereas SPW exposed

to *O. tipulae* OA-12 had an adjusted mortality of 33%. IJs emerged from SPW cadavers in both EPN treatments.

In Experiments 2 and 3, SPW mortality and emergence associated with *S. feltiae* and *H. indica* were higher ($P \leq 0.05$) than the control at all tested EPN dosages (Fig. 3A, B; Fig. 4A, B). Mortality in the untreated control associated with *H. indica* was high at nearly 60% compared to only 30% in the untreated control associated with *S. feltiae*. There were no additive effects of using a higher rate of *S. feltiae* compared to the lower rate in Experiment 2, but the intermediate rate of *H. indica* (210 IJs) outperformed the low rate (105 IJs) in Experiment 3.

Steinernema feltiae field experiment

SPW pressure at the Magoon Research Facility was low; only four males were trapped in the SPW pheromone trap placed in the nearby field throughout the two trials. SPW damage to the surface of the swollen sweetpotato root was not observed in either field trial. There were no differences between trials ($P > 0.05$), therefore, data were analyzed together. No difference in

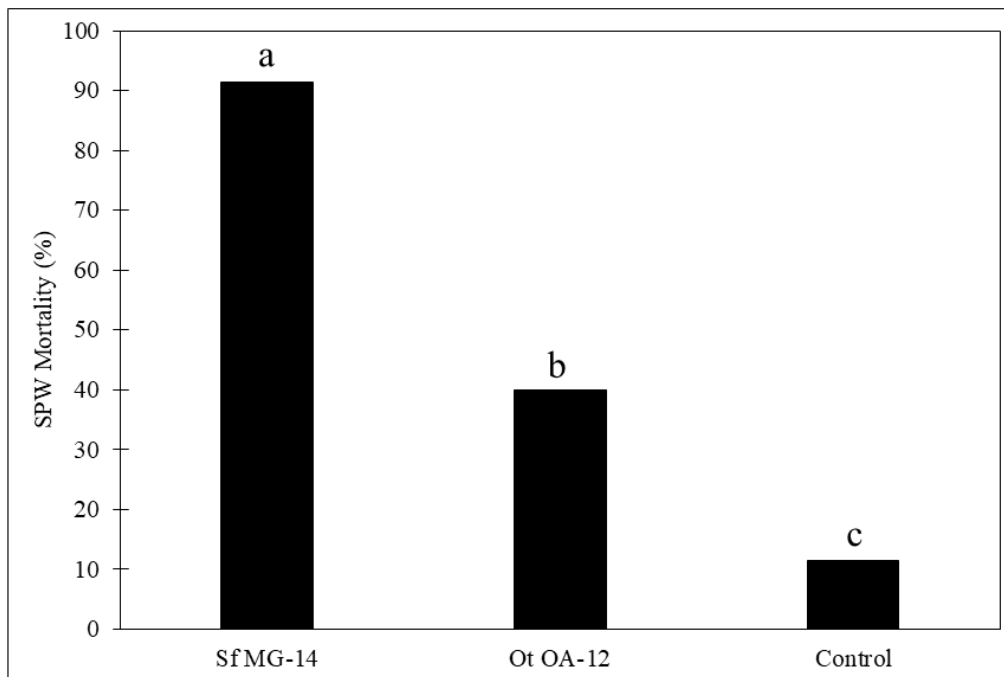


Figure 2. Mortality of sweetpotato weevil (SPW; *Cylas formicarius*) larvae treated with *Steinernema feltiae* MG-14 (Sf MG-14), *Oscheius tipulae* OA-12 (Ot OA-12), or sterile water (Control) in infection courts 48 hours after inoculation in the laboratory. Bars ($n = 9$) with different letters are different according to the Waller-Duncan k-ratio ($k=100$) t-test.

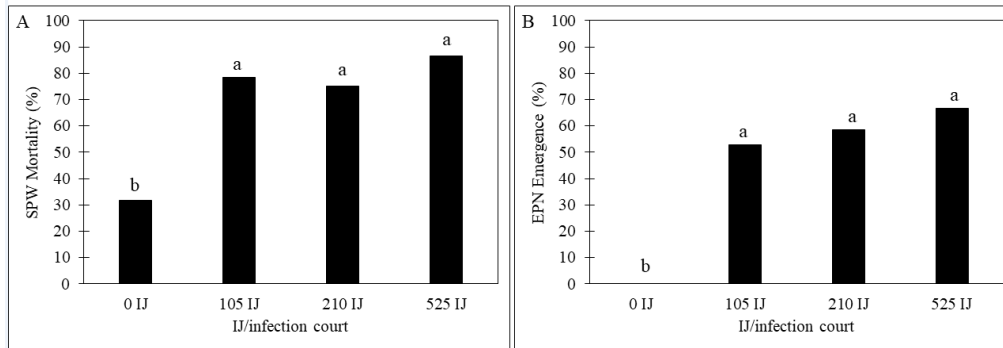


Figure 3. A) Sweetpotato weevil (SPW; *Cylas formicarius*) larval mortality, and B) percentage of SPW cadavers with infective juvenile (IJ) emergence as affected by different dosages of *Steinernema feltiae* MG-14 at 28 days after the entomopathogenic nematode (EPN) inoculation. Bars ($n = 12$) followed by different letters are different according to the Tukey-Honest significance test ($P \leq 0.05$).

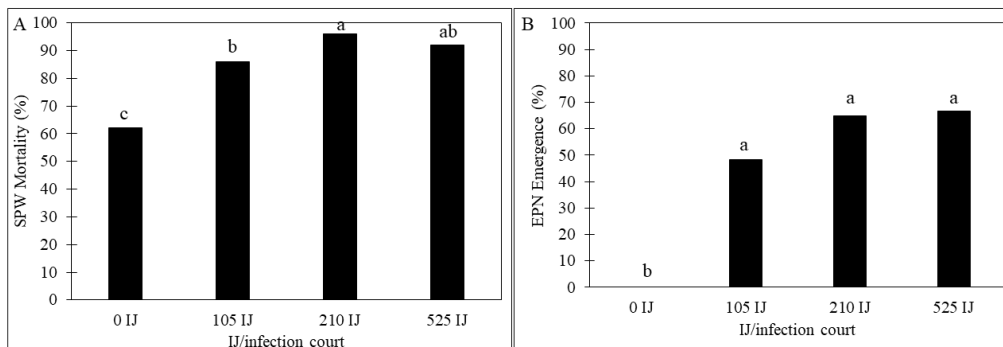


Fig. 4. A) Sweetpotato weevil (SPW; *Cylas formicarius*) mortality, and B) percentage of SPW cadavers with infective juvenile (IJ) emergence 28 days after entomopathogenic nematode (EPN) inoculation in the infection court receiving different dosages of *Heterorhabditis indica* IJs/larvae. Bars ($n = 10$) followed by different letters are different according to the Tukey-Honest significance test ($P \leq 0.05$).

sweetpotato yield was detected among treatments with averages of 31,425 kg/ha, 22,860 kg/ha, 21,526 kg/ha, and 27,885 kg/ha when *S. feltiae* was applied at 0, 0.5, 1.25, and 2.5 billion IJs/ha, respectively ($P > 0.05$). The SPW population density recovered in the tuberous roots was not homogenous ($P \leq 0.05$), thus data were analyzed separately for Trials 1 and 2. In Trial 1, SPW population densities were < 3 larvae/kg sweetpotato regardless of *S. feltiae* spray rate ($P > 0.05$). In Trial 2, no SPW were recovered in any of the treatments (data not presented).

Oscheius tipulae field experiment

The field trials at Waialua had high SPW pest pressure. The Alpha Scent SPW pheromone trap captured $> 2,609$ SPW males over the 5-month

growing period. Data from both trials were combined due to insignificant differences between trials in Levene homogeneity of variance Test. Although EPN basal stem spray did not affect sweetpotato yield and SPW damage rating on the tuberous roots, *O. tipulae* application did reduce SPW population density in the tuberous roots compared to the untreated control ($P \leq 0.10$, Table 1), this treatment still had numerically higher SPW population density than the grower practice, which was 0.

DISCUSSION

All three EPN species infected and killed larvae of *C. formicarius*. *Steinernema feltiae* MG-14 was most lethal to *C. formicarius* of the three EPN isolates tested in the laboratory. While SPW

Table 1. Sweetpotato yield, sweetpotato weevil (SPW; *Cylas formicarius*) density, and sweetpotato weevil damage after the application of *Osccheius tipulae* in the field at Waialua, Hawai'i in 2021 to 2022.

Treatment ^y	Sweetpotato yield (kg/ha)	Sweetpotato weevil	
		Number/kg sweetpotato	Damage rating ^x
EPN	8,290 a ^z	16 b	3.2 a
Control	9,865 a	32 a	3.2 a
GP	9,022	0	1.6

^xDamage rating on a scale of 0-5 where 0 = no visible damage, 1 = less than 10% surface damage, 2 = less than 20% surface damage, 3 = less than 40% surface damage, 4 = less than 50% surface damage, 5 = greater than 50% surface damage.

^yEPN = entomopathogenic nematode basal stem spray of *Osccheius tipulae*; Control = untreated. GP = grower practice with carbaryl monthly applications. GP plots were not part of the randomized complete block design. Therefore, data was not included in the analysis of variance (ANOVA) but was included as reference of efficacy.

^zMeans (n = 8 for yield, n = 24 for SPW density, n = 80 for damage rating) in a column followed by the same letter were not different based on ANOVA ($P \geq 0.10$).

exposed to *H. indica* had high mortality, effectiveness was obscured by high mortality in the untreated control. Based on adjusted mortality of EPN tested at ~100 IJ/ infection court (mortality subtracted from untreated control), *S. feltiae* MG-14, *O. tipulae* OA-12, and *H. indica* OM-160 caused mortality of 50-80%, 30%, and 25%, respectively, in the laboratory trials, suggesting that *S. feltiae* MG-14 was a more effective EPN strain against SPW in Hawai'i than *H. indica* OM-160. This finding deviated from that of Myers *et al.* (2020) who reported that *H. indica* OM-160 incited higher mortality against SPW in laboratory and field studies. The discrepancy in this laboratory trial could be because SPW larvae used in the current laboratory trials were collected from different fields several weeks apart. The SPW larvae used in Experiment 3 for *H. indica* OM-160 might have been malnourished, weakened, or injured during handling (used a few days after field collection) compared to those used in Experiments 1 and 2 (used within a few days after field collection). Based on the current laboratory results, *S. feltiae* MG-14 had the most potential to control SPW. Nonetheless, this corroborates a previous finding by Yu *et al.* (2012), who demonstrated that *S. feltiae* strains had high virulence against *C. formicarius*. A growing body of literature indicates that a direct mortality assessment in the lab may not be an accurate indicator of success in the field. Foye (2019) detected differences in effectiveness among EPN species in an *in vitro* mortality test but found that under simulated field conditions, all EPN species performed at similar levels of efficacy. Another study found that *S. carpocapsae*

outperformed *S. feltiae* and *H. bacteriophora* on *Tuta absoluta* in a petri dish bioassay, but when placed in a leaf bioassay the efficacy of all three EPN was not different (Saleh *et al.*, 2023).

Despite lower mortality compared to *S. feltiae* in the laboratory assays, *O. tipulae* applied via basal stem sprays was able to reduce SPW population densities in sweetpotato roots in the field trials conducted at Waialua. In this field experiment with *O. tipulae*, SPW pressure was extremely high (> 2,609 SPW captured in the pheromone trap) from the time of sweetpotato planting to harvest. Densities of SPW were reduced by half in *O. tipulae* treated plots compared to the untreated control ($P \leq 0.05$), though it was not on par with the grower practice of monthly carbaryl sprays. However, damage to the tuberous roots of sweetpotato remained high in the EPN plots. This is the first record of *O. tipulae* reducing *C. formicarius* densities in the field despite using a relatively low rate (0.5 billion IJs/ha). Abdisa *et al.* (2024) also demonstrated *O. tipulae* was more effective than *S. carpocapsae* against *Drosophila melanogaster*, *Aedes albopictus*, and *Delia platura* in laboratory experiments and effectively controlled *D. platura* in the field. In the field experiment with *S. feltiae*, SPW pressure was nearly absent. Consequently, pest damage was not observed in any plots, which obscured differences in SPW population densities among treatments. A subsequent soil bait for entomopathogenic organisms using *Galleria mellonella* indicated the presence of *Beauveria* spp., *Metarhizium* spp., and other EPN throughout the field (data not presented). The presence of these organisms may

have suppressed SPW throughout the field. The success of *O. tipulae* in the current field trials and the findings of Yu *et al.* (2012) that *S. feltiae* was effective in the field suggest some promising organic farming options for managing SPW in the field.

Overall, these studies provided more information that *S. feltiae*, *H. indica*, and *O. tipulae* isolated from Hawai'i are candidate EPNs for controlling SPW. While this study was inconclusive on whether using a higher dosage of EPN would be more effective in controlling SPW, it demonstrated that *S. feltiae* MG-14 and *O. tipulae* OA-12 have the potential to be viable tools for growers in Hawai'i to incorporate into their SPW management program.

ACKNOWLEDGMENTS

The authors thank Caitlyn Seaman, William Hearty, Roshan Paudel, Melanie Pitiki, Conrad Nelson, Nicco Sylvester, Justin Silver and Donna Meyer for technical assistance. This work was supported in part by USDA OREI Grant#20215130035225, USDA Hatch Project HAW09042-H and HAW9048-H.

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Received:

Accepted for publication:

9/IX/2024

5/XI/2024

Recibido:

Aceptado para publicación: