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Lethality of *Phasmarhabditis* spp. (*P. hermaphrodita*, *P. californica*, and *P. papillosa*) Nematodes to the Grey Field Slug *Deroceras reticulatum* on *Canna* Lilies in a Lath House

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Abstract: The grey field slug, *Deroceras reticulatum*, is an agricultural pest causing damage to a wide variety of crops each year. The nematode *Phasmarhabditis hermaphrodita* has been shown to effectively kill this slug in field-simulated conditions, leading to its widespread use as a biological control agent in Europe. However, recently discovered isolates of *Phasmarhabditis* from California have not been tested in a field-simulated environment. The lethality of three local isolates of *Phasmarhabditis* (*P. hermaphrodita*, *P. californica*, & *P. papillosa*) as well as the molluscicide Sluggo Plus® was assessed on *D. reticulatum* in a lath house. Remaining leaf area on *Canna* lilies and slug mortality were recorded after 3 weeks of exposure to treatments. Local isolates efficiently killed *D. reticulatum* and protection from leaf damage was attained by treatment with *P. papillosa*. Further experimentation is required to assess plant protection afforded by *Phasmarhabditis* as plants in some trials may have been in poor health. The three tested *Phasmarhabditis* isolates are reasonable candidates for biological control within the United States but additional information, particularly on the lethality to non-target gastropods, is needed before an informed decision on their use can be made.

Keywords: biological control; *Phasmarhabditis*; virulence; slugs

Citation: Schurkman, J.; Dodge, C.; Mc Donnell, R.; Tandingan De Ley, I.; Dillman, A.R. Lethality of *Phasmarhabditis* spp. (*P. hermaphrodita*, *P. californica*, and *P. papillosa*) Nematodes to the Grey Field Slug *Deroceras reticulatum* on *Canna* Lilies in a Lath House. *Agronomy* **2022**, *12*, 20. https://doi.org/10.3390/agronomy12010020

Academic Editors: Ivan Hiltpold, Sergio Rasmann, Andrea C. Ruthes, Paul Dahlin and Ivo Toševski

Received: 2 November 2021
Accepted: 19 December 2021
Published: 23 December 2021

1. Introduction

Terrestrial gastropods (snails and slugs) serve a vital role in multiple ecosystems by consuming both live and dead plant matter, acting as herbivores and detritivores [1,2]. While some of these gastropods are charismatic, such as banana slugs belonging to the genus *Ariolimax* (Stylommatophora: Ariolimacidae), many terrestrial species are abhorred because they can be serious pests in agriculture and horticulture. In California, it is estimated that about 37 out of 279 terrestrial gastropods in the state are invasive species [3]. These invasives can thrive where they localize, often causing crop damage in the area. In agricultural and horticultural crops, gastropods can have a detrimental impact on sales and crop yields. In some cases, invasive species like *Cornu aspersum* (Stylommatophora: Helicidae) can reduce crop yields by 40–50% in normal climate conditions, and by 90–100% in years of high rainfall [4–6]. Gastropods can also spread plant and human pathogens or allow easy access points for pathogens to enter the plant from another vector source. Snails and slugs have been found to carry *Alternaria brassicicola* (Pleosporales: Plerosporaceae), the causative agent for black leaf spot, and other plant pathogenic fungi [7–9]. They may also contribute to recalls of leafy greens, as *Campylobacter* spp. (Campilobacterales: Campilobacteraceae) and *Escherichia coli* (Enterobacteriales: Enterobacteriaceae) have been reported in the feces of sampled gastropods [10,11]. Multiple gastropod species have also been identified with the parasite *Angiostrongylus cantonensis* (Strongylida: Metastrongylidae), the causative agent for rat lungworm disease in humans [12].

Developing an effective method of control for invasive and pestiferous snails and slugs is an ongoing need in the U.S. Currently the most popular means of control is the use of chemical molluscicides. One commonly used molluscicide is Sluggo Plus®. The main active ingredients in Sluggo Plus® are iron phosphate and Spinosad. Upon ingestion, iron phosphate damages the digestive tissue, causing the slugs to cease eating and seek shelter where they eventually die. Spinosad is a toxin which affects the nervous system. Upon consumption, it can cause muscle spasms, which eventually lead to paralysis and death [13–15]. However, Spinosad only affects insects, leaving snails and slugs unaffected. The addition of both active ingredients in Sluggo Plus® makes the molluscicide an effective choice to control both insect and gastropod pests. However, molluscicides are not an effective targeted method of pest control and can affect a range of organisms that encounter or eat the molluscicide. Molluscicides that use methiocarb and metaldehyde baits have been shown to be capable of causing harm to some birds, mammals, and other invertebrates [16,17]. Sluggo Plus® is a newer molluscicide which has not had a significant amount of non-target research performed on it, therefore the potential non-target effects are relatively unknown. However, it has been shown that iron phosphate has negative impacts on both the survival and growth of the earthworms *Lumbricus terrestris* (Haplotaxida: Lumbricidae) and *Eisenia fetida* (Haplotaxida: Lumbricidae), indicating that the iron phosphate in Sluggo Plus® likely affects these earthworms [18,19].

Biological control provides an alternative to chemical control, which can be safer and more targeted. One example of a successful biological control for invasive and pestiferous gastropods is Nemaslug® (BASF Agricultural Solutions, United Kingom) [20]. This product is a preparation of the nematode *Phasmarhabditis hermaphrodita* (Rhabditida: Rhabditidae), which was isolated in the UK and grown on *Moraxella osloensis*. It is applied on the soil to parasitize and kill a range of pestiferous slug and snail species. *P. hermaprhodita* is known to have greater efficacy against smaller pestiferous gastropods in both laboratory conditions and field simulated conditions and is safe to all other non-gastropod organisms that have been tested [21–25]. It is thought that *P. hermaphrodita* enters its gastropod hosts through the rear of the mantle as dauer larvae, where they form a lesion in the central opening of the mantle canal [20,26]. They eventually gain access to the shell region where they mature into hermaphroditic adults and reproduce. Fluid accumulates in the shell cavity throughout the infection causing a diagnostic swollen mantle phenotype. As the number of nematodes in the host grow, the gastropod host eventually dies between 4 and 21 days after exposure and the nematodes spread throughout the entire body of the gastropod, feeding on bacteria and the remains of the gastropod and reproducing. Eventually as resources begin to run low, the juvenile nematodes halt development, and enter an arrested state as dauer juveniles, searching for new hosts where the cycle continues [20,26]. The method in which *P. hermaphrodita* kills its host is not well understood. While *P. hermaphrodita* is known to associate with several different bacteria, it is unclear whether the bacteria are necessary for killing the host, or whether the nematodes can kill the host without the aid of the bacteria [27–29]. More studies, preferably utilizing modern sequencing technology, need to be performed in order to further understand *P. hermaphrodita* as a parasite.

Due to *P. hermaphrodita* not being found in the U.S. until recently [30], and without its registration for use in gastropod biocontrol, the product is not yet locally available. Therefore, there is a need for a successful biological control agent against invasive and pestiferous gastropods in the region. Recently, three different species of *Phasmarhabditis* were found in California. These are *P. hermaphrodita*, *P. californica*, and *P. papillosa* [31] and all three are lethal to at least one invasive gastropod species [32].

On the west coast of the United States, one of the most pestiferous slugs is the gray field slug, *Deroceras reticulatum* (Stylommatophora: Limacidae) [17,33]. It was first reported as an invasive species in the U.S. in 1843, where it arrived in Massachusetts from Europe. It was then reported on the West coast of the United States in 1891 [34] where it has caused extensive damage to various crops. One crop in particular which snails and slugs seem to target is the Canna lily. Canna lilies are tropical and subtropical perennial plants that

are often a result of many crosses. Hybrids are popularly grown for landscaping due to their striking banana-like foliage and showy flowers. In some countries, they are grown for their edible rhizomes. Grown from either seeds or rhizomes, they are quite hardy, however, above-ground foliage and flowers are susceptible to slug and snail damage. These slugs and snails are considered a minor pest of the ornamental crop and have been found to leave large holes in the leaves, often preferring the younger underdeveloped and unfurled leaves [35]. Controlled laboratory experiments using recently described U.S. isolates of *Phasmarhabditis* to kill *D. reticulatum* have been performed with success [36] but the efficacy of these nematodes needs to be confirmed in a more field-like setting, using crops that are known targets of the gastropods. Mesocosm field-like trials are vital in order to understand how a potential agent may work under the conditions of practical use. Here we report the lethality of *P. hermaphrodita* (isolated from *D. reticulatum*), *P. californica* (isolated from *Deroceras laeve* (Stylommatophora: Agriolimacidae)), and *P. papillosa* (isolated from *D. reticulatum*) to *D. reticulatum* in a field-like setting mimicking use in a typical plant nursery in addition to providing an assessment of Canna lily crop protection. We hypothesized that all three species would cause significant mortality in *D. reticulatum*, while also reducing plant damage.

2. Materials and Methods

Test arenas consisted of a tray (35 cm × 21 cm × 6 cm) provided with a copper wire (16 mesh/inch, 0.011″ wire diameter × 14.5 cm high copper wiring (TWP INC, Berkeley, CA 94710, USA)) barrier on the inside walls, filled with a layer of (a) 473.2 mL of pea gravel at the bottom, (b) lined with a fabric barrier (Dewitt 3′ × 100′ weed-barrier landscape fabric) that fitted the tray and (c) top layer of 950 g of autoclaved soil (75% SunGro Sunshine No. 4 mix and 25% UC soil mix 3 [37]. The copper wire was placed around the inside of the tray to discourage slug escape [32]. Soil was moistened by adding 650 mL of deionized water to each arena. Two 37-day old *Canna* 'Cannova® Bronze Scarlet Canna Lily' (Zingiberales: Cannaceae) of the same height and number of leaves were planted 7.5 cm from the center of the arenas. An 11 cm × 2.5 cm circular plastic potholder with pin stilts was placed in each arena between the two plants (Figure 1 (left)) and acted as a slug shelter. Each arena was placed inside a 61 × 61 × 61 cm Bugdorm (Bioquip Products, Rancho Dominguez, CA 90220, US, Cat #1462W) and the bugdorms were arranged in three rows of six in a randomized order within each replicate (Figure 1 right), on a west-to-east orientation in a lath house.

A lath house is an enclosed space which provides small sections of shade to an area by placing multiple spaced-out laths across the top of the structure to block out sections of sunlight. An additional 50% shade cloth was provided above the bugdorms to provide ample shading for the arenas. The lath house area was misted twice a day for about 2 min to maintain humidity and soil moisture was maintained with the daily addition of tap water via a squirt bottle. Tap water was added to the soil until the arenas were noticeably moist.

Nematode treatments were prepared using modified White traps [38] utilizing *Ambigolimax valentianus* (Stylommatophora: Limacidae) previously collected at various nurseries or from slug monitoring traps (LiphaTech® Inc., 3600 W Elm St., Milwaukee, WI 53209, USA) on UCR campus. Slugs were frozen at −20 °C, soaked for 1 min in hot water, cooled, inoculated with infective juveniles (Ijs) of each species isolate, and incubated at 17 °C. Slugs in the modified White traps were inoculated with *P. californica* (ITD726), *P. hermaphrodita* (ITD272) or *P. papillosa* (ITD510), previously grown on nematode growth medium (NGM) plates with *Ochrobactrum* spp. Ijs were collected into tissue culture flasks after 3 weeks. The concentrations of each nematode species in tissue culture flasks were measured by counting the number of nematodes in five 10 µL drops and calculating the average of the five drops. Prior to inoculation into the arenas, the total number of required nematodes were placed into 15 mL Falcon tube conicals, and the volume was adjusted to 10 mL with deionized water.

Figure 1. Potholders on pin stilts (**left**) which functioned as slug shelters between Canna 'Cannova® Bronze Scarlet Canna Lily'. The arenas were used to determine the mortality of *Deroceras reticulatum* exposed to three *Phasmarhabditis* species. The arrangement of bug dorms with arenas inside the lath house (**right**).

A five-fold increase (150 Ijs/cm^2) of the recommended rate of Nemaslug® was used for each nematode treatment due to space, logistical limitations, and the fact that this was our first field experiment utilizing all three species of local *Phasmarhabditis* strains. The higher recommended rate of Sluggo Plus® (4.88 kg/m^2) was used to compare with the nematode treatment efficiencies. We provided two controls, one with slugs and no nematodes, and the other with no slugs or nematodes.

D. reticulatum were collected from a field of perennial ryegrass (*Lolium perenne*) in Shedd, Oregon (44.438016, −123.120253) and were delivered to the University of California, Riverside under CDFA permit # 3449. Slugs used for the experiment ranged from 0.09 g to 0.981 g with an average of 0.290 g. The 3 repetitions of each trial were split into groups of small slugs, medium slugs, and large slugs based on their mass in order to account for potential size effects.

Ten pre-weighed slugs were introduced to the soil of the arena. All slugs used throughout the experiment were observed for one week before use in order to account for any slugs which may already be infected with *Phasmarhabditis*. Only slugs which appeared healthy were chosen for use. Immediately after slug introduction, the nematode inocula were slowly and evenly applied using an auto pipettor on the soil surface under each potholder. Inocula were applied only on this area as the slugs were likely to use the area under the potholder as a shelter, therefore increasing the likelihood of contact with the Ijs. This shelter application strategy uses far less inocula than application to the entire arena and is based on the findings of [39] using roofing shingles as shelter. The number of dead slugs was recorded daily for 3 weeks. Dead slugs were characterized by immobility and a failed response to stimuli (i.e., prodding with toothpick). Swollen mantles and the presence of mixed stages of nematodes on the cadaver characterized slugs that were killed by *Phasmarhabditis* treatments. Treatment arenas were replicated three times and the experiment was repeated thrice. The three experiments were conducted on 5 June 2019, 1 March 2021, and 1 April 2021. The second trial was not performed during 2020 due to campus restrictions during the COVID-19 pandemic.

At the conclusion of each experiment, plants were removed from the arenas and placed inside plastic bags for remaining leaf area measurements which were taken immediately

upon removal. This was performed to assess the protection each treatment provided for *Canna* plants. Individual leaves from each plant were scanned and the remaining leaf area was calculated from the scanned image using ImageJ version 1.8.0 [40]. In summary, scanned TIFF images with 600 dpi were uploaded to the software and were converted to 8 bit. A scale was created by creating a vector on an image of a ruler measuring 100 mm, and a conversion of pixels to mm was created. Each leaf image was then analyzed with ImageJ by adjusting the threshold and analyzing the particles within the highlighted regions of the uploaded image. The leaf area data represented the remaining leaf area following slug introduction and damage.

To assess nematode persistence, soil samples were collected at the conclusion of the third trial. Soil samples of approximately 38 g were obtained from underneath each slug shelter using 150 mL plastic conical tubes, rotating each tube into the soil in three spots until it reached the fabric liner. These samples were placed under misters in a Baerman funnel for 2 days to extract all living nematodes in the soil and to determine the presence and recovery of *Phasmarhabditis* and other nematodes after 3 weeks. After the observation of the persisting nematodes from the third trial, we decided to assess the possibility of nematodes surviving the autoclaving process at 220 °C and 15 psi for 20 min. Therefore, nematodes were extracted from about 30 g of freshly autoclaved soil. Extracted nematodes/mL suspension were counted through the stereoscope and identified morphologically using an Olympus BX51 DIC microscope (Olympus Corp., 3500 Corporate Parkway, Center Valley, PA 18034, USA). When needed, taxonomic keys were consulted [41,42]. Nematodes from the autoclaved soil were primarily IJs and hence could not be identified morphologically.

All statistical analyses were performed with GraphPad Prism 8.2.1 (GraphPad Software, San Diego, CA, USA). An individual log rank (Mantel–Cox) test was performed on all survival data comparing each treatment to each other, one at a time. A one-way ANOVA was performed on the remaining leaf area assays utilizing Tukey's multiple comparisons test.

3. Results

3.1. Local Phasmarhabditis spp. Kill Deroceras reticulatum

While comparing all three trials together, all treatments caused significant mortality compared to the control (Sluggo Plus® X2 (N = 84) = 8.304, $p = 0.004$, *P. californica* X2 (N = 84) = 36.29, $p < 0.0001$, *P. papillosa* X2 (N = 84) = 53.34 $p < 0.0001$, *P. hermaphrodita* X2 (N = 84) = 30.68, $p < 0.0001$) three weeks after application (Figure 2a). There was no significant difference in slug mortality between the nematode treatments ($p > 0.05$). The three *Phasmarhabditis* treatments caused similar mortality, reaching an average of 90.3% ± 0.05 mortality when combined. *P. papillosa* caused the highest mortality with an average mortality rate of 97.5% ± 0.01. All *Phasmarhabditis* treatments were significantly more lethal compared to the chemical molluscicide treatment Sluggo Plus® (*P. californica* X2 (N = 84) = 36.29, *P. papillosa* X2 (N = 84) = 53.34, *P. hermaphrodita* X2 (N = 84) = 30.68), which caused an average mortality rate of 68.6% ± 0.05. Each *Phasmarhabditis* treatment reached 50% mortality about 4 days after exposure to nematode treatment. Treatment with Sluggo Plus® resulted in 50% mortality about 6 days after exposure.

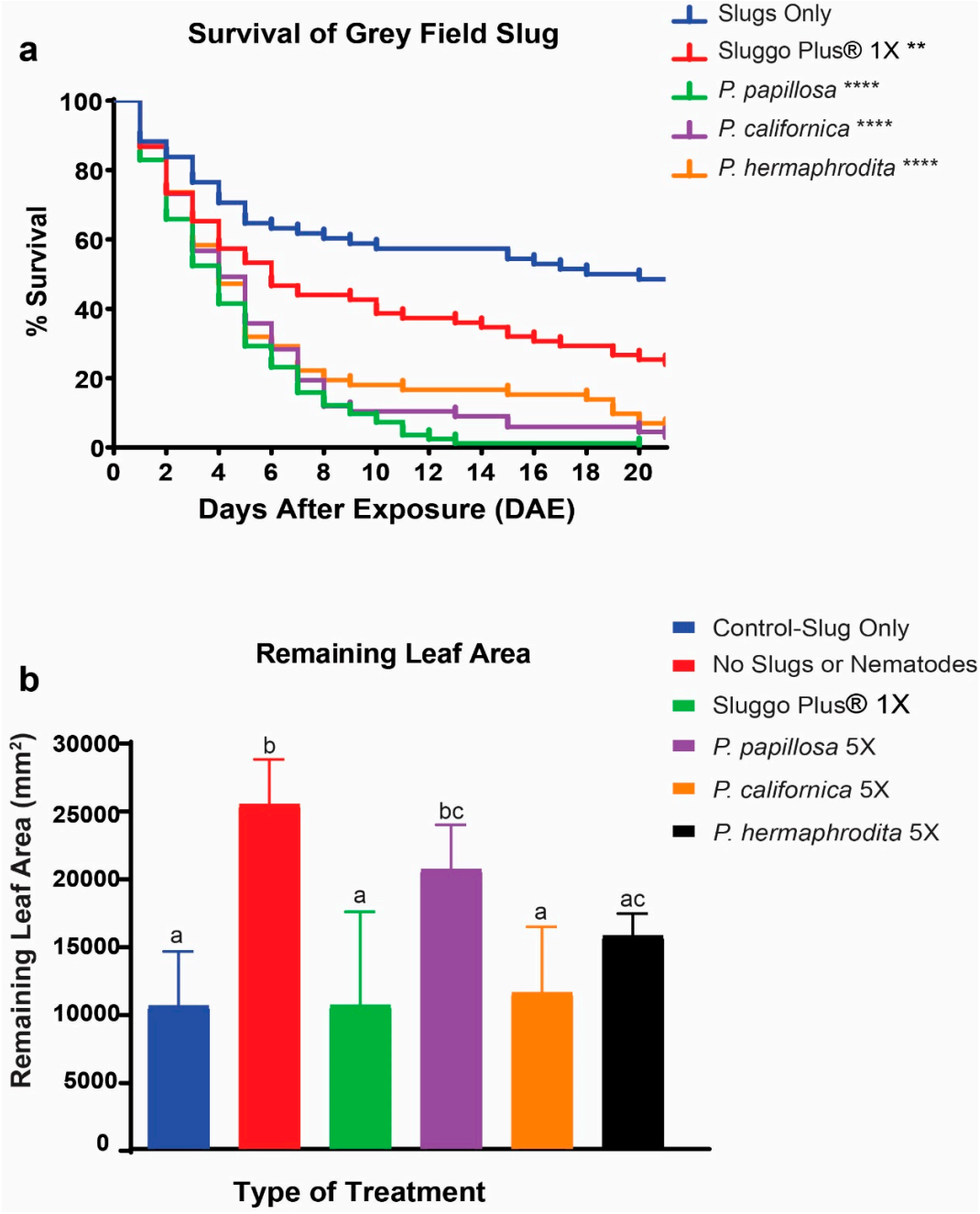

Figure 2. (**a**) Percentage survival of *Deroceras reticulatum* exposed to three species of *Phasmarhabditis* (*P. californica*, *P. hermaphrodita* and *P. papillosa*), compared with Sluggo Plus® (iron phosphate and spinosad). *P. papillosa* caused an average of 97.5% mortality (SEM = 0.01), *P. hermaphrodita* caused an average of 81.4% mortality (SEM = 0.09), *P. californica* caused an average of 77.2% mortality (SEM = 0.05), Sluggo Plus® caused an average of 68.6% mortality (SEM = 0.05), and the slug only control had an average of 41.9% mortality (SEM = 0.06). ** indicates a p value of < 0.01 and **** indicates a p value < 0.0001 compared to the slug only control (**b**). Average remaining leaf area (mm^2) of *Canna* 'Cannova® Bronze Scarlet Canna Lily' after 21 days after exposure (DAE) to *Phasmarhabditis* treatment, compared with Sluggo Plus® treatment and controls. Picture was taken 21 DAE in trial 1 of the lath house experiment. Shared letters indicate the absence of significant differences whereas treatments not sharing letters significantly differ from one another. Log rank analyses were used, comparing each treatment to one another in panel A. A one-way ANOVA utilizing Tukey's multiple comparisons test was used for panel B.

3.2. Remaining Leaf Area Is Affected by Treatment

During Trials 2 and 3, we observed that all plants were in relatively poor health throughout the experiment. Leaves were a brighter green color and wilted, with dark brown coloration at the tips. At the conclusion of both experiments, aphids were found on the plants. We therefore analyzed the remaining leaf area taking only Trial 1 into account. When Trial 1 was analyzed alone, the *P. papillosa* treatment resulted in the highest remaining leaf area of all treatments with an average remaining leaf area of 20,774.56 mm^2 ± 1350.644 (Figure 2b). The *P. papillosa* treatment also resulted in significantly higher remaining leaf area compared to the slug-only control (Mean diff = −10,131, 95% CI of diff = −17,756 to −2500, N = 6, q = 5.711, DF = 30, $p = 0.0042$), and was comparable to the control with no slugs or nematodes (Mean diff = 4823, 95% CI of dif = −2809 to 12,454, N = 6, q = 2.718, DF = 30, $p = 0.4090$). All other treatments had no significant effect, compared to the slug-only control. Treatment with *P. papillosa* resulted in visibly noticeable differences in plant health and remaining plant tissues, which were comparable to the control with no slugs or nematodes (Figure 3). Based on results of the first trial, *P. papillosa* can prevent significant damage from *D. reticulatum* within a three-week time period.

Figure 3. Plant stands of *Canna* 'Cannova® Bronze Scarlet Canna Lily' 21 days after exposure to *Deroceras reticulatum* (10 slugs/arena) and treatment with three *Phasmarhabditis* spp. at 150 infective juveniles/cm^2 duringtrial 1. From left to right the treatments are: Sluggo Plus®, *P. hermaphrodita*, *P. californica*, *P. papillosa*, No slug or nematodes control, and slug only control. Photo taken at the conclusion of the 1st lath house trial, on 6 June 2019.

3.3. Nematode Recovery Post-Inoculation

Nematodes were recovered from the soil under the potholders three weeks after soil inoculation (Figure 3). Prior to the virulence assay, the soil used in these arenas was autoclaved, leading to the expectation that only nematodes that were added to the experimental arena would be recovered. However, most nematodes recovered from the soil were cephalobids, mainly *Acrobeloides* and *Cephalobus*, some rhabditids, including *Oscheius* and *Mesorhabditis*, as well as the plant-associated genera *Hirschmanniella*, *Aphelenchus* and *Xiphinema*. *Phasmarhabditis* was recovered from two samples with *P. californica* treatment (1 mature female and 2 IJ), one with *P. papillosa* treatment (1 young adult female), and none from *P. hermaphrodita* (Table 1). In addition to surveying the soil from the experimental arenas, the unused autoclaved soil was also examined. Nematodes that were mostly newly hatched were also present in this soil. We also examined the same soil source which was not autoclaved about 3 months after the final experiment was performed. Similar newly hatched nematodes were also present in this soil.

Table 1. Nematode recovery from soil (38 g) extracted from arenas, three weeks after treatment application. Lath house experiment 3, April 2021.

Nematode Genera/Group	Treatments				
	Control (No Slug, No Nematode	Slugs Only	*P. californica*	*P. hermaphrodita*	*P. papillosa*
Acrobeloides	33	16	98	16	34
Cephalobus	9	8	14	3	12
rhabditid			6	4	3
diplogasterid		4	3	1	1
Oscheious		1	1		3
Mesorhabditis			7		
Hirschmanniella	1				
Aphelenchus		1			
Xiphinema		1			
Phasmarhabditis			2		1
Unidentified young juveniles	11	6	30	2	9
Total	54	37	160	26	63

4. Discussion

It is important to assess the use of novel pest control treatments to determine how they perform in field-like conditions, compared to laboratory-controlled conditions [43]. Practical use of biological control agents is influenced by a multitude of variables including soil type, temperature, sunlight exposure, and organismal interactions. These variables are often not accounted for in laboratory experiments. While controlled laboratory experiments are important to determine the effects of specific variables on pest control products, more field experiments must be conducted in order to assess the practicality of these products. The efficacy of *P. hermaphrodita* (Nemaslug®) has been assessed in multiple field trials, measuring the effects on non-targets and targets in practical scenarios [44–46]. In this paper, we assessed the efficacy of the local species of *Phasmarhabditis* (*P. californica*, *P. hermaphrodita*, and *P. papillosa*) under field-like conditions.

This research is the first mesocosm type experiment assessing the efficacy of local U.S. *Phasmarhabditis* isolates outside of laboratory conditions. Our second and third trials resulted in plants which were unhealthy and infected with aphids post experiment. However, our results showed that when zero arthropods were found on the leaves of the *Canna* lilies at the conclusion of the experiment, *P. papillosa* provided adequate protection from slug damage, comparable to the no slug and no nematode control (Figure 2b).

Local *Phasmarhabditis* species may be a better gastropod pest control option than the commonly used Sluggo Plus®. All local isolates of *Phasmarhabditis* caused higher mortality to *D. reticulatum* than Sluggo Plus® and control treatments in field-simulated conditions (Figure 2a). The molluscicide Sluggo Plus® was chosen for this experiment due to its increase in popularity, as metaldehyde baits cause serious off-target effects to various organisms, such as birds and mammals, resulting in a ban on their usage in multiple countries [16,17]. Iron phosphate baits (like Sluggo Plus®) are now more popular chemical molluscicides, showing less non-target effects. However, iron phosphate baits have been found to have non-target effects on the earthworm *Lumbricus terrestris*, making them potentially less desirable as a method for gastropod pest control [19]. The need for a gastropod control agent which has zero non-target effects is highly desirable. Biological control of gastropods with local *Phasmarhabditis* species may provide a safe, targeted gastropod pest control. More research is needed in order to verify that multiple non-target species will not be affected.

The presence of other free-living and plant-parasitic nematodes in the test arenas was unexpected. Their presence may suggest that the soil sterilization (220 °C for 20 min for one cycle) did not completely sterilize the soil when a large bag weighing about 7 kg was autoclaved. It is also possible that the nematodes were introduced into the arena from the plants, as the plants were only a few centimeters away from the slug shelter, under which the soil samples were collected. These seedlings are generally grown in commercially available soil mixes, and our extracts from similar non-sterilized soil mix as well as left-over plant plugs also showed nematodes 24 h after incubation. Nematodes also could have been carried within the arena upon introduction of *D. reticulatum* via a phoretic interaction [47], or the water with which the plants were misted/watered. Another possible source of nematodes could have been from the small arthropods (e.g., aphids and spiders) which were able to enter the arenas through the bug dorms [48]. Surprisingly, very few *Phasmarhabditis* were recovered from the soil, even though they were clearly reproducing on dead slug cadavers, most of which were observed on the soil surface of the arena with few under the potholder. Non-recovery of *P. hermaphrodita* does not necessarily mean the nematodes were not present, as the sampled soil was from a small area. It is possible that the nematodes were seeking hosts in other parts of the arena, or they were simply present in other parts of the arena. However, *P. hermaphrodita* has been found to have low persistence in some soils. Natural predators in the soil may lead to the nematode surviving in low numbers or not at all [49]. Due to these findings, Nemaslug® application is recommended at 4–6 weeks intervals at 30 Ijs/cm^2. However, our recent results show that these nematodes may require shorter intervals (Table 1). Further experimentation is needed to assess the pathogenicity of persisting *Phasmarhabditis* species, which have been present in the soil for longer periods of time. While the 3 *Phasmarhabditis* isolates may not persist well in soil, they are effective at controlling *D. reticulatum* upon application.

Author Contributions: Conceptualization, J.S. and I.T.D.L.; methodology, J.S. and I.T.D.L.; software, J.S.; validation, J.S., I.T.D.L., C.D., R.M.D. and A.R.D.; formal analysis, J.S.; investigation, J.S. and I.T.D.L.; resources, I.T.D.L., C.D., R.M.D. and A.R.D.; data curation, J.S.; writing, J.S., C.D., I.T.D.L., R.M.D. and A.R.D.; original draft preparation, J.S., I.T.D.L. and A.R.D.; writing—review and editing, J.S., C.D., I.T.D.L., R.M.D. and A.R.D.; visualization, J.S.; supervision, I.T.D.L. and A.R.D.; project administration, I.T.D.L. and A.R.D.; funding acquisition, I.T.D.L., R.M.D. and A.R.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the California Department of Food and Agriculture Specialty Crop Multi-State Program (CDFA SCMP) grant #12509488, a generous gift from the Plant California Alliance (PCA), and logistical contribution from and partnership with Altman Plants in Perris, California USA. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is available upon request from the senior author: jschu011@ucr.edu.

Acknowledgments: We would like to thank Timothy Paine and Mikael Roose for the use of lath house space and members of the Dillman laboratory for feedback on this project.

Conflicts of Interest: Irma Tandingan De Ley and Rory McDonnell declare that they are co-inventors on a patent entitled Mollusk-killing Biopesticide (WO2017059342A1).

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