LETHALITY OF THREE SPECIES OF *PHASMARHABDITIS* NEMATODES TO GRAY FIELD SLUGS IN A MICROCOSM STUDY

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Introduction

The gray field slug (*Deroceras reticulatum*) is the most important slug pest of grass seed production in the Willamette Valley, OR. Control measures focus heavily on the use of chemical molluscicides, but growers report considerable variation in the efficacy of the most widely used active ingredients, and only 29.5% reported that they were satisfied with bait performance (Mc Donnell and Anderson, 2018). Hence, there is an urgent need to develop alternative management strategies for use by producers in the region.

Biological control is one such option, i.e., the use of a pest's natural biological enemies (e.g., nematodes) to control it. In Europe, the nematode *Phasmarhabditis hermaphrodita* was used as a commercial biocontrol product (Nemaslug) from 1994 to 2022 to manage slug pests in crops (Rae et al., 2023). In April 2022, *Phasmarhabditis californica* was also commercialized and is now available as Nemaslug 2.0 in the United Kingdom and some other European countries. Since 2017, surveys conducted by our team have resulted in the discovery of *P. hermaphrodita, P. californica,* and a third species, *P. papillosa,* in various locations throughout Oregon (Howe et al., 2020). These discoveries have resulted in increased interest in using these nematodes as biocontrol agents of pest slugs. The objective of this study was to compare the lethality of U.S. strains of *P. hermaphrodita, P. californica,* and *P. papillosa* to the gray field slug in microcosm studies.

Materials and Methods

Microcosms consisted of plastic containers 24 cm high, 39 cm long, and 30 cm wide with vented lids. Approximately 0.2 ft^3 of autoclaved (270°F, 10 psi, 90 minutes) SS#4 potting soil was added to each container. Annual ryegrass (var. 'Bounty') seeds were planted on November 8, 2021 and consisted of 2 rows of 100 seeds planted 5 inches apart. Microcosms were watered as needed throughout the experiment—about every 6 days, although more frequently at the start of the study. Test slugs were collected on November 3, 2021 from a grass seed production field located in Linn County. Ten gray field slugs were added to each container, and all treatments and controls were replicated three times.

Treatments were administered on November 19, 2021. Nematodes were applied at a rate of 30 infective juveniles (IJs) per cm^2 (the recommended application rate for Nemaslug). Slug-Fest (liquid metaldehyde) was used at the labeled rate (47 fl oz/acre). Treatments were compared to a negative control, i.e., annual ryegrass with slugs and no molluscicidal treatment.

Microcosm containers were maintained in the OSU research greenhouses for the duration of the study. Mortality was assessed daily from November 20, 2021 through January 20, 2022. Recording mortality was not always straightforward, as slugs often buried in the substrate, making them difficult to detect. Thus, both living and dead slugs were tallied daily, and measured mortality was often corrected by inference. For example, if on day 5 seven dead slugs and three living slugs were found in a container, and on day 6 only six dead slugs and three living slugs were found in the same container, slug mortality for day 6 would be recorded as seven.

For numerous periods, an ANOVA was calculated to assess whether significant between-group mortality variation existed in our data. For data sets where ANOVA analyses returned an F statistic greater than the F critical value, the Tukey-Kramer q statistic was calculated pairwise for each group. These statistics were compared using a studentized q table using an alpha value = 0.05 to reject the null hypothesis of no difference between groups.

Results and Discussion

Slug mortality commenced soon after the start of the study, with major mortality initiating in *P. papillosa* after 1 week and the other two nematode treatments following soon thereafter (Figure 1). Between-group mortality variation was tested (using Tukey-Kramer q statistic) every day from November 24 to December 8, 2021 (the period during which the vast majority of mortality in nematode treatments occurred, Figure 1), on December 20, 2021, and on December 31, 2021 (chosen to maximize the difference between Slug-Fest treatments and the negative control).

All differences in slug mortality among nematode treatments occurred between November 25 (6 days

after application) and December 4 (15 days after application). Mortality differences between *P. papillosa* and both *P. hermaphrodita* and *P. californica* were more common than differences between *P. hermaphrodita* and *P. californica* (Table 1). All nematode treatments resulted in greater mortality than Slug-Fest and negative control treatments throughout much of this timeframe. Surprisingly, mortality in the Slug-Fest treatment did not differ from the negative control throughout the entirety of this study.

In conclusion, all three nematode species caused 100% gray field slug mortality and outperformed Slug-Fest in this study. *Phasmarhabditis papillosa* was the first treatment to cause complete slug mortality, followed by *P. hermaphrodita* and *P. californica.*

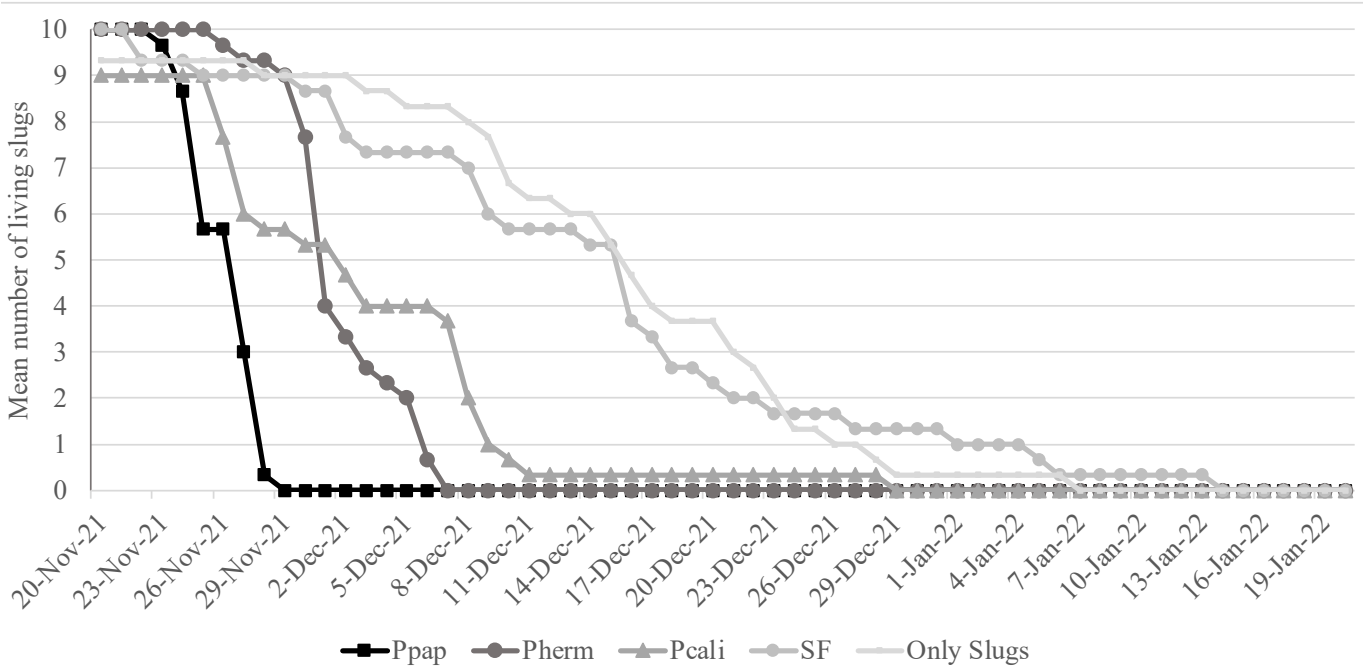

Figure 1. Mean number of living gray field slugs in microcosm containers treated with *Phasmarhabditis papillosa* (Ppap), *Phasmarhabditis hermaphrodita* (Pherm), *Phasmarhabditis californica* (Pcali), Slug-Fest (SF), and slugs only. Nematodes were used at a rate of 30 infective juveniles/cm^2 (recommended application rate for Nemaslug). Slug-Fest was used at a rate of 47 fl oz/acre (label rate). Error bars omitted for purposes of clarity.

Table 1. Gray field slug mortality among treatments and control across time.[1]

Treatments	Nov. 25	Nov. 26	Nov. 27	Nov. 28	Nov. 29	Nov. 30	Dec. 1	Dec. 2	Dec. 3	Dec. 4	Dec. 5	Dec. 6	Dec. 7	Dec. 8	Dec. 20
Ppap–Pherm	*	*	*	*	*	*	*	*	-	-	-	-	-	-	-
Ppap–Pcali	-	-	-	*	*	*	*	*	*	*	-	-	-	-	-
Ppap–SF	-	*	*	*	*	*	*	*	*	*	*	*	*	*	-
Ppap–only slugs	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Pherm–Pcali	-	-	-	*	*	-	-	-	-	-	-	-	-	-	-
Pherm–SF	-	-	-	-	-	-	*	*	*	*	*	*	*	*	-
Pherm–only slugs	-	-	-	-	-	-	*	*	*	*	*	*	*	*	-
Pcali–SF	-	-	-	*	*	-	*	-	-	-	-	-	-	*	-
Pcali–only slugs	-	-	-	*	*	-	*	*	*	*	*	*	*	*	*
SF–only slugs	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[1]Asterisks indicate a difference in slug mortality between treatments/controls on that date. Hyphens indicate that there was no difference in slug mortality between treatments/controls on that date. Differences are based on Tukey-Kramer *q* scores with a critical value of 4.654 using alpha = 0.05. Ppap = *Phasmarhabditis papillosa*, Pherm = *Phasmarhabditis hermaphrodita*, Pcali = *Phasmarhabditis californica*, and SF = Slug-Fest.

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