Table 1: Slug species found in California survey

Scientific Name	Common Name
Arion ater	Black Slug
Arion hortensis	Garden Slug
Deroceras reticulatum	Gray Field/Garden, Milky Slug
Deroceras lavae	?
Limax flavus	Tawny Slug
Limax marginatus	Striped Slug
Limax maximus	Spotted Garden Slug
Milax gagates	Greenhouse Slug

Molluscicidal Nematodes for Biological Control of Pest Slugs

Harry K Kaya and Darryl K. Mitani

Introduction

Slugs (Mollusca: Gastropoda) are major pests of horticultural plants throughout the world (South, 1992). They are destructive pests of home gardens, landscapes, nurseries, greenhouses, and field crops (DeAngelis, 1993; Ohlendorff, 1999). California has been infiltrated by numerous pestiferous slug species including the gray garden slug, *Deroceras reticulatum*, the striped slug, *Limax marginatus*, the greenhouse slug, *Milax gagates* (Ohlendorff, 1999), and the garden slug, *Arion hortensis.* As generalists, slugs feed on a variety of living plants and decaying matter. However, their preference for succulent foliage makes them serious pests of seedlings, herbaceous plants, and fruit ripening close to the ground (e.g., tomatoes and strawberries). Slugs also pose a health threat to humans, pets, and wildlife by serving as intermediate hosts for many vertebrate parasites (e.g., lungworm) (South, 1992).

California's slug problem is compounded by the state's mild climate. Restricted by their limited water retentive capability, slugs become inactive during dry conditions by moving deep into the ground and do not cause crop damage. They are also inactive in cold weather and hibernate in the soil. However, California's relatively mild climate, especially in coastal and valley regions and southern California, enables them to be active throughout much the year. Slugs reach maturity in about a year, but some species (e.g., the gray garden slug) are opportunistic breeders and can go through several generations in a year given optimum temperatures and moisture.

Homeowners' lawns, greenhouses, nurseries, and landscapes tend to create favorable conditions for slugs due to heavy mulching and watering. Most recommended non-chemical control measures for slugs are very labor-intensive, often impracticable, and their efficiency is questionable. Such control measures involve elimination of possible hiding places, frequent handpicking, non-chemical baits, and barriers (Ohlendorff, 1999). Although chemical control methods exist, they have limitations including poor efficacy, negative environmental impact, and human and veterinary health concerns. Under ideal conditions, chemical baits, containing metaldehyde, can be somewhat effective because this aldehyde paralyzes the slugs and they eventually die from dehydration. However, under cool and wet conditions when slugs are most active and troublesome, they can often recover (Ohlendorff, 1999).

Biological control provides an attractive alternative to traditional control practices. Molluscicidal nematodes (Rhabditida: Rhabditidae) possess exceptional potential as biocontrol agents for pest slugs. In Europe, *Phasmarhabditis hermaphrodita*, isolated from gray garden slugs in England (Wilson *et al.*, 1993a,b), has successfully been developed as a biocontrol agent (NemaSlug™, MicroBio Ltd, UK). Host range experiments have shown that *P. hermaphrodita* is effective against a wide array of economically important pest slug and snail species (Coupland, 1995; Wilson and Gaugler, 1997). Numerous field trials in various crops have shown that it can provide control equivalent to chemical standards without adverse effects on non-target molluscs. It would be a logical choice for introduction into the US, but there are no published records of its occurrence in the US. Thus, regulatory issues prohibit its introduction and marketing in the US.

Our initial objective was to generate baseline information that would allow the development of *P. hermaphrodita* as a biocontrol agent for pest slugs in California. However, because of the regulatory issues stated above, it will not be prudent to import this nematode species into California because of its potential impact on endangered molluscs (Ohlendorff, 1999). Therefore, our approaches are (1) to initiate a survey of California (emphasis in northern part of the state) for molluscicidal nematodes and to isolate ones that appear to be pathogenic and (2) to evaluate known species of entomopathogenic nematodes in the genera

Table 2: Two-week slug mortality of survey sites

Location	Predominant Species	Number Collected	Deaths	2 Week Mortality
Auburn	*L. flavus*	68	13	19%
Davis	*D. reticulatum*	1922	676	35%
UC Davis	*D. reticulatum, L. marginatus*	758	87	11%
Fremont	*L. marginatus, L. lavae*	443	51	12%
Monterey	*D. reticulatum; L. marginatus*	27	20	74%
Newcastle	*L. maximus*	28	0	0%
Winters	*L. marginatus*	279	0	0%
San Luis Obispo	*D. reticulatum*	43	7	16%
Vacaville	*D. reticulatum*	198	17	9%
Petaluma	*D. reticulatum, L. marginatus*	278	20	7%
Merced	*L. marginatus*	16	0	0%
Mill Valley	*D. reticulatum*	46	6	13%
Fairfield	*L. marginatus*	64	8	13%
San Jose	*L. flavus*	4	0	0%
Oakland	*D. reticulatum*	4	0	0%
Richmond	*L. marginatus*	22	0	0%
Berkeley	*L. marginatus*	201	13	6%
American Canyon/Vallejo	*L. marginatus*	54	0	0%
	Total:	4455	918	21%

Steinernema and *Heterorhabditis* against pest slug species.

Materials and Methods

Survey for molluscicidal nematodes

We have surveyed for *P. hermaphrodita* or other molluscicidal nematodes in natural populations of slugs in California. (If a natural population of *P. hermaphrodita* can be isolated and it proves to be pathogenic to slugs but nonpathogenic to snails and other nontarget organisms, it will be an ideal biological control agent for slugs). Emphasis has been placed on coastal regions where slugs are active throughout the year; slug prone areas of the Central Valley and Sierra Nevada foothills have also been surveyed. Slug collection was done through sight inspection of groundcover as well as the use of baited refuge traps. Traps were set up in promising locations and checked after 1-3 days. Collected slugs were kept in plastic containers with moist absorbent cotton wool inside coolers maintained below 15°C, and brought back to the lab where they were identified to species level. Captured slugs were placed in large plastic containers lined with sterile soil, kept under high humidity conditions at 15 to 23°C, provided with lettuce as food, and observed for signs of nematode infection (swollen mantle) for two weeks. Slugs that exhibited signs of infection were placed in emergence (White) traps (Kaya and Stock 1997) to collect any emerging dauer juveniles. Apparently healthy slugs were maintained in the containers for future use in bioassays.

Nematodes isolated from the survey were reared *in vivo* on slugs to maintain optimal pathogenicity to slugs. All isolates have been saved for taxonomic studies following standard procedures including preparation of permanent slides, microscopic measurements, and photography. Later, the type slides will be deposited in the UC Davis Nematology collection.

We have evaluated the relative virulence and infectivity of the isolated nematodes to various economically important slug species such as *D. reticulatum*, *L. marginatus*, and *M. gagates*. Slugs were placed individually in petri dishes lined with a layer of moist sterilized soil. Lettuce discs were provided as food. Nematodes were added at an initial rate of 0, 100, 500, or 1000 dauer juveniles per square centimeter. Mortality was assessed daily for two weeks and detected by loss of response to a thin stainless steel needle. There

were a minimum of 30 replicates per slug species and nematode strain combination.

Molluscicidal activity of *Steinernema longicaudum*

We evaluated the virulence and infectivity of *S. longicaudum* to slugs. Initial trials indicated that slug mortality occurred at high nematode concentrations, so bioassays were conducted to determine the lower thresholds for effective slug mortality. Slugs were placed individually in petri dishes lined with moist sterilized soil. Lettuce discs were provided as food. Nematodes were added at concentrations of 0, 100, 500, and 1000+ dauer juveniles/cm^2. Mortality was assessed daily for two weeks. There was a minimum of 30 replicates per slug species and nematode strain combination.

We examined ways to best apply *S. longicaudum* to obtain maximum effect on the slugs. In a preliminary trial, we used calcium alginate gels (Kaya and Nelson, 1985) and the slugs ate the gels containing sucrose. This approach seems feasible as the slugs occupy moist habitats, the nematodes require moisture for survival and infectivity, and the gels are most effective when they are hydrated. We added various types of feeding attractants (sugar, molasses, bran, etc.) to the gels to entice the slugs to consume the gels and the nematodes.

Results and Discussion

Survey for molluscicidal nematodes

Using baited traps and sight inspection of groundcover, 7 or 8 species have been collected (Table 1). Our survey has shown that there were two or three species at any given site, but one species was usually dominant (see Table 2). We observed a seasonal shift with *D. reticulatum* being predominant in cooler winter months, whereas *L. marginatus* was more common during the summer months.

Collections were made from 18 sites throughout the Sierra Nevada Foothills, Central Valley, Northern Coast, and Central Coast. Captured slugs were observed for 14 days for signs of nematode infection, and slug mortality was recorded over a two-week period (see Table 2). Slug mortality averaged 21% across all collection sites with a range from 0-74%. The high percent mortality of slugs was attributed to them being collected late in the season. These slugs were adults, laid eggs and then died.

Slugs that died were placed in White traps to collect any emerging nematodes. Although no indigenous *P. hermaphrodita* was found, we did isolate nematodes from slug specimens from several sites. So far, we have identified one genus belonging to the Family Rhabditidae, genus *Cruznema*. The isolated nematodes may represent a new species, but Koch's postulates were not fulfilled and therefore, we concluded that this genus was nonpathogenic to slugs. We will continue our survey in attempts to find pathogenic slug nematodes.

Molluscicidal activity of *Steinernema longicaudum*

Steinernema longicaudum is a known entomopathogenic nematode that has a wide host range infecting a number of different insect species (Stock et al., 1999). There have been reports of *Steinernema* species infecting slugs and snails (Wilson and Gaugler, 2000). We, therefore, tried several steinernematid species against slugs and found that *S. longicaudum* had molluscicidal activity. In further bioassays, we tested three strains of *S. longicaudum* against California pest slugs. Initial results of these trials were quite promising. Healthy slugs (*D. reticulatum* and *L. marginatus*), weighing between 0.7 and 1.0 grams, were exposed to graded concentrations of the Nonsan, Gongju, and California strains of *S. longicaudum*.

Slug mortality resulting from these treatments is shown in Table 3 with the Gongju strain showing the highest level of virulence. Greatest slug mortality, approaching 100 %, was achieved with the highest concentrations of *S. longicaudum*. These nematode concentrations are too high for practical purposes, but work is currently being done on increasing the molluscicidal activity of the nematode. We have confirmed the molluscicidal activity of *S. longicaudum*, but cannot complete Koch's postulates because the nematodes cannot reproduce in the slug cadavers. We were concerned about these results because the lack of reproduction prevents our selection of virulent individuals to build up optimal molluscicidal activity. However, this is an excellent short-term biocontrol trait as applications of the nematode will have no long lasting effects on the environment and minimal effect on nontarget molluscs. Moreover, these nematodes are easily produced *in vivo* and *in vitro.*

The use of calcium alginate capsules containing *S. longicaudum* was not successful. The addition of sucrose, molasses, or bran as an attractant to the gels increased feeding but did not result in slug mortality by the nematodes. The data suggest that ingestion of nematodes in calcium alginate by slugs is not the route

Table 3. Molluscicidal activity of *Steinernema longicaudum* against *Deroceras reticulatum* and *Limax marginatus.*

Strain	Concentration nematodes/cm²	1-Week Mortality (%)	2-Week Mortality (%)
California	1000	100	100
	500	60	100
	100	0	20
	0	0	0
Gongju	1000	60	80
	500	40	80
	100	60	100
	0	0	20
Nonsan	1200	100	100
	500	80	80
	100	20	60

of "infection." The nematodes may invade through the mantle as has been shown for *Phasmarhabditis* (Wilson and Gaugler, 2000). We cannot increase the biocidal activity of *S. longicaudum* through the calcium alginate formulation.

Conclusions

No pathogenic nematodes have been isolated from natural populations of slugs in California thus far. We will continue to collect slugs from the field and monitor those that have nematodes emerging from the cadavers. These nematodes will be tested for pathogenicity to slugs using Koch's Postulates.

Isolates of *Steinernema longicaudum* appear to be pathogenic to slugs. However, because no reproduction occurs in the slugs, Koch's Postulates cannot be fulfilled. We are still early in our study and will continue to work with this and other entomopathogenic nematode species to determine their pathogenicity to slugs. This approach seems feasible and we will continue to conduct bioassays to isolate pathogenic nematodes.

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