Table 1. Pest species of slugs collected in California

Scientific Name	**Common Name**
Arion ater	European Black Slug
Arion hortensis	Garden Slug
Deroceras reticulatum	Gray Garden Slug
Limax flavus	Tawny Slug
Limax marginatus	Banded Slug
Limax maximus	Great Gray Garden Slug
Milax gagates	Greenhouse Slug

Molluscicidal nematodes for biological control of pest slugs

Harry K. Kaya

Abstract:

Slugs (Mollusca: Gastropoda) are serious pests of home gardens, landscapes, nurseries, greenhouses, and field crops. The main control tactic is the use of baits containing metaldehyde. These baits do not directly kill the slugs but paralyze them with the slugs dying from dehydration. In Europe, the nematode *Phasmarhabditis hermaphrodita* is commercially available as a molluscicidal agent. In the United States, there are no biological control agents for use against slugs. *P. hermaphrodita* does not occur in the United States, and therefore, this nematode cannot be used here. We conducted a survey to determine whether *P. hermaphrodita* occurred naturally in slug populations. During our survey of 23 different sites throughout California, more than 5,000 slugs representing seven species were collected. Although some of the dead slugs produced nematodes, none of them was pathogenic or proved to be *P. hermaphrodita*. We also tested entomopathogenic nematodes against slugs. Some species of entomopathogenic nematodes, which are effective biological control agents of insects, have been shown to be effective biological control agents of some mollusk species. Our initial bioassays showed that *Steinernema longicaudum* and *Heterorhabditis marelatus* were two species that showed potential for killing the gray garden slug, *Deroceras reticulatum*, the most common and serious pest in California. Although the nematodes killed the slugs, they were unable to use the slugs as a nutritional resource for reproduction. Three isolates of *S. longicaudum* (California and two from Korea) and one isolate of *H. marelatus* (Point Reyes) were tested further at 0 (control), 500, and 1000 nematodes/cm^2. At 14 days post-treatment, average mortality by all three isolates of *S. longicaudum* ranged from 50 to 62.5% at 1000 nematodes/cm^2, 52.5 to 70% at 500 nematodes/cm^2, and 35 to 42.5% at 0 nematodes/cm^2. With *H. marelatus* at 14 days post-treatment, the average mortality was 82.5% at 1000 nematodes/cm^2, 72.5% at 500 nematodes/cm^2, and 32.5% at 0 nematodes/cm^2. The high control mortality is not unusual with slugs as other researchers have reported similar results in their controlled experiments. In larger arenas, *H. marelatus* and *S. longicuadum* were not effective against the slugs at 1000 nematodes/cm^2. We conclude that the entomopathogenic nematodes offer potential as a biological control agent, but a more virulent isolate is needed to obtain effective, sustained control of slugs. The isolation of *P. hermaphrodita* from slug populations in California will enhance its use as a biological control agent of pestiferous slugs.

Introduction

As one of the most destructive pests of home gardens, landscapes, nurseries, greenhouses, and field crops, slugs (Mollusca: Gastropoda) are a major concern of horticulturists throughout the United States (Ohlendorff, 1998; DeAngelis, 1993). California has been infiltrated by numerous problematic slug species including the gray garden slug, *Deroceras reticulatum*, the banded slug, *Limax marginatus*, and the greenhouse slug, *Milax gagates* (Ohlendorff, 1998). 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 may 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 capacity, slugs become inactive during dry conditions, when they move deep into the ground and do not cause crop damage. Similarly, during cold weather they hibernate in the topsoil. However, California's relatively mild climate, especially in coastal regions and southern California, enables slugs to be active throughout 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 and gardens, 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 refuge sites, frequent handpicking, non-chemical baits, and barriers (Ohlendorff, 1998). Chemical baits, usually containing metaldehyde, have proven to be effective when properly used. Metaldehyde does not normally cause slug mortality directly. Rather, metaldehyde paralyzes slugs, and the slugs consequently die from dehydration. However, under cool and wet conditions when slugs are most active and troublesome, the slugs can often recover (Ohlendorff, 1998). In addition, these chemical baits are toxic, posing health concerns for children, pets, and wildlife. At present, there are no biological products for the control of slugs in the United States.

Molluscicidal nematodes (Rhabditida: Rhabditidae) possess exceptional potential as biological control agents for pest slugs. In Europe, *Phasmarhabditis hermaphrodita*, isolated from gray garden slugs in England (Wilson et al., 1993), has successfully been developed as a biological control 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 mollusks. However, as there are no published records of its occurrence in the United States, regulatory issues prohibit the marketing of this nematode in the USA.

The development of a molluscicidal nematode, such as *P. hermaphrodita*, as a biological control agent for pest slugs will be of immense benefit to horticulturists in the United States. As part of this study, a survey for molluscicidal nematodes was conducted in California. In addition, the molluscicidal activity of entomopathogenic nematodes was explored.

Materials and Methods

California survey for molluscicidal nematodes - A survey for molluscicidal nematodes was conducted throughout Northern and Central California. Slugs were collected throughout slug-prone areas of the Central Valley, Sierra Nevada foothills, Central Coast, Bay Area, and North Coast. Slug collection was accomplished mainly through sight inspection of groundcover, although refuge traps were used in limited instances. Where used, refuge traps simply consisted of lengths of damp wood placed adjacent to slug infested areas. The slug species collected are shown in Table 1.

Slugs were collected, kept in 500 cc plastic containers with moist paper towels inside coolers maintained below 20°C, and transported back to the laboratory where they were identified to species. Captured slugs were placed in large plastic containers (38 x 27 x 15 cm) lined with sterile loam soil, kept under high humidity at 15°C, provided with lettuce as food, and observed for signs of nematode infection (swollen mantle) for a period of two weeks. Moribund slugs were placed in White traps (Kaya and Stock 1997) to collect any emerging dauer juvenile nematodes. Apparently healthy slugs were maintained in the containers for future use in bioassays.

Nematodes isolated from the survey were tested with Koch's Postulates to determine pathogenicity. The relative virulence and infectivity of the isolated nematodes were studied by exposing economically important slug species to the isolates.

Molluscicidal activity of entomopathogenic nematodes - Entomopathogenic nematodes were screened for molluscicidal activity against two common pest slug species, *Deroceras reticulatum* and *Limax marginatus*. The following experimental design was used for all bioassays. Plastic petri dishes (60 x15 mm) were lined on the inside walls with copper tape to prevent the slugs from crawling and remaining on the petri dish lids, out of contact with the nematodes. Three small ventilation holes were made in the lids, and the dishes were filled with 6 g of sterile sand. Nematode infective juvenile (IJ) suspensions were concentrated to the desired application rates, and applied directly to the soil surface in 1.5 ml of water. To insure optimum pathogenicity, only less than 4 weeks old IJ cultures were used in the bioassays. The control treatment consisted of 1.5 ml of deionized water. A single, healthy slug was added to each petri dish. Slugs were fed one lettuce disc (2 cm diameter) per day, and moisture was adjusted as needed. The experiment was maintained at room temperature (22ºC), and slug mortality was monitored daily for 14 days. Slug cadavers were placed in emergence (i.e. White) traps to recover any

Table 2. Collection of slugs from various localities in California

Location	Site Description	Predominant Species	Number Collected
American Canyon	Nursery	*L. marginatus*	54
Arroyo Grande	Nursery	*D. reticulatum*	37
*Auburn	Residential	*L. flavus*	88
Berkeley	Nursery	*L. marginatus*	201
Cayucos	Residential	*D. reticulatum*	19
*Davis	Residential	*D. reticulatum*	2109
Fairfield	Roadside	*L. marginatus*	64
Fremont	Residential	*L. marginatus*	478
Fresno	Roadside	*D. reticulatum*	43
Merced	Commercial	*L. marginatus*	16
Mill Valley	Roadside	*D. reticulatum*	46
Monte Rio	Residential	*D. reticulatum*	44
Monterey	Commercial	*D. reticulatum*	27
Newcastle	Orchard	*L. maximus*	36
Oakland	Commercial	*D. reticulatum*	4
*Petaluma	Nursery	*D. reticulatum*	311
Richmond	Residential	*D. reticulatum*	22
Riverside	Residential	*L. marginatus*	58
San Jose	Residential	*L. flavus*	22
*San Luis Obispo	Nursery	*D. reticulatum*	157
*UC Davis	Garden	*D. reticulatum*	1107
Vacaville	Park	*D. reticulatum*	303
Winters	Residential	*L. marginatus*	279
		Total:	**5525**

* Nematodes isolated from slugs at these locations. None of the nematodes from these slugs was pathogenic to other slugs (i.e., Koch's Postulates were not fulfilled).

emerging nematodes.

Initial nematode screenings were conducted at three concentrations. Briefly, nematode IJs were applied to pest slugs at a high rate (1000 IJs/cm^2), medium rate (500 IJs/cm^2), and a control (0 IJs/cm^2). Each screening consisted of five replicates per application concentration. The most promising entomopathogenic nematode species were selected for further investigation.

The promising nematode species and strains were selected for further study using more intensive bioassays. In addition to the high, medium, and control nematode rates, a low rate of 100 IJs/cm^2 was also included in subsequent experimental trials. A minimum of 4 replicates, consisting of 10 slugs per nematode application rate, were conducted for each nematode species or strain.

Results and Discussion

California survey for molluscicidal nematodes - Over 5,500 slugs, encompassing seven species, were collected from slug-prone areas of California (Table 2). Collections were made from 23 sites throughout the Sierra Nevada foothills, Central Valley, North Coast, Bay Area, and South Coast. Multiple slug species were collected at any given site, but one species was usually dominant. By far, the most common slug collected was the gray field slug, *Deroceras reticulatum*.

Captured slugs were observed for signs of nematode infection (swollen mantle). Many slug cadavers from Auburn, Davis, UC Davis, Petaluma, and San Luis Obispo placed on White traps produced nematodes. Koch's Postulates were followed to determine the pathogenicity of these nematode isolates, but the postulates remained unfulfilled and therefore, it was concluded that the nematodes were non-pathogenic. Nematode isolates from UC Davis and Davis were identified to genus level. These particular isolates belong to the Family Rhabditidae, genus *Cruznema*. Although it has been postulated that these nematodes may belong to a new species, further study was not pursued due to the original objectives of our proposal.

Our survey failed to recover the molluscicidal nematode, *P. hermaphrodita*, that is common in Europe. Likewise, Grewal et al. (2000) did not recover *P. hermaphrodita* from a broader survey of mollusk-associated nematodes of North America. *P. hermaphrodita* has, however, been isolated from slugs in Chile (France and Gerding, 2000), suggesting that this molluscicidal nematode or a close relative may someday be isolated in the United States.

Table 3. Initial screening of entomopathogenic nematodes (*Heterorhabditis* and *Steinernema* spp.) for molluscicidal activity against *Deroceras reticulatum* or *Limax marginatus*

Nematode species	% mortality		
	1000 IJs/cm^2	500 IJs/cm^2	0 IJ/cm^2
H. bacteriophora	40	40	20
*H. marelatus**	100	80	40
S. carpocapsae	20	40	20
S. glaseri	40	60	40
S. kushidai	20	20	20
*S. longicaudum**, #	100	80	0
S. oregonense	0	0	0
S. riobrave	60	20	20
S. siamkayai	60	80	20

* Species selected for further evaluation
# *L. marginatus* was used with *S. longicaudum* and *D. reticulatum* was used with all other nematode species.

Molluscicidal activity of entomopathogenic nematodes - There have been reports of *Steinernema* and *Heterorhabditis* species infecting slugs and snails (Jaworska, 1993; Wilson and Gaugler, 2000). We screened nine species of entomopathogenic nematodes for molluscicidal activity (Table 3). Two species, *Steinernema longicaudum* and *Heterorhabditis marelatus*, showed promising results by killing 100% of the slugs at an application rate of 1000 IJs/cm^2 and 80% of the slugs at a rate of 500 IJs/cm^2, whereas control mortality was 0% and 40%, respectively (Table 3). These two species were selected for further investigation for their molluscicidal activity.

Three strains of *S. longicaudum*, one from California (Stock et al., 1999) and two from Korea, were subsequently tested for optimal molluscicidal activity. Although mortality in the control groups was generally lower than within the *S. longicaudum*-treated groups, mean mortality percentages varied among treatments of the same nematode strain and among nematode concentrations (Table 4). Bioassays using *H. marelatus* produced the highest slug mortality percentages that corresponded with the highest nematode application rates. There is an indication that this nematode species may have potential for reduction of pest slug species. However, the high control mortality in our bioassay system masks the impact of the nematode. Apparently, the high control mortality is not unusual for slugs as Wilson et al. (1993) reports 40% death at 11 days for *D. reticulatum*. In addition, when larger arenas were used, the nematodes were not as effective (data not shown). This may be, in part, due to the ability of the slugs to "avoid" areas treated with the nematodes.

Interestingly, no entomopathogenic nematode reproduction was observed within the slug cadavers. In general, entomopathogenic nematodes usually proceed through up to 3 generations within the insect cadaver before IJs emerge to seek out a new host. However, no pathogenic nematodes were recovered from White (emergence) traps of slug cadavers. Believing that reproduction may have taken place but IJs were being restricted from emerging by the slug's thick mucus, slug cadavers were dissected. Upon examination, no reproduction was evident. In fact, few nematodes were recovered from these dissections. We were initially concerned by these results because the lack of reproduction prevents the selection of virulent individuals to build up optimal molluscicidal activity. However, this is an excellent short-term biological control trait as nematode applications will have no long-lasting effects on the environment and have minimal effects on non-target mollusks.

The portal of entry for the nematode remains undetermined. Wilson et al. (1993) believed that *P. hermaphrodita* enters the slug via the shell sac where it infects and multiplies. Crude dissections of moribund slugs recovered no nematodes from this region, although nematodes were recovered around the pneumostome, the respiratory opening in the slug's mantle. Perhaps more accurate histological studies can pinpoint the infection area of these nematodes.

Conclusion

We feel that nematodes are ideal biological control agents for pest slugs. Slugs and nematodes are both soil dwelling organisms with a requirement for relatively high moisture levels. However, our field survey failed to isolate a pathogenic nematode. We found one entomopathogenic nematode, *H. marelatus*, that may have potential for controlling slugs. Although *H. marelatus* is an insect pathogen, it does cause slug mortality without replicating in the host. Unfortu-

Table 4. Molluscicidal activity of *Heterorhabditis marelatus* and three strains of *Steinernema longicaudum* against *Deroceras reticulatum*

Nematode/strain	Week	Mean Percent Mortality*			
		1000 IJs/cm^2	500 IJs/cm^2	100 IJs/cm^2	0 IJ/cm^2
H. marelatus/Point Reyes	1	60	50	32.5	20
	2	**82.5**	**72.5**	**50**	**32.5**
S. longicaudum/California	1	35	55	22.5	20
	2	**50**	**70**	**50**	**42.5**
S. longicaudum/Gongju	1	42.5	37.5	42.5	20
	2	**62.5**	**52.5**	**65**	**35**
S. longicaudum/Nonsan	1	32.5	40	25	22.5
	2	**57.5**	**62.5**	**40**	**40**

* Mean percent mortality represents the average of four replicates with 10 slugs in each replicate. Slugs were checked daily for mortality but only data for 1 and 2 (in bold) weeks are reported. Each replicate was conducted on different days.

nately, the high control mortality and the reduced mortality of *H. marelatus* in larger bioassay arenas suggest that it may not be the ideal candidate for slug biological control. Our initial investigation offers considerable promise that molluscicidal nematodes do occur and that a greater effort to isolate an efficacious nematode species for use against slug pests is needed.

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Harry K. Kaya is a Professor in the Department of Nematology at the University of California, Davis.