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Pestiferous slugs and their associated nematodes in agricultural fields, greenhouses, and nurseries in Alberta, Canada

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Abstract

Some slug species are considered a nuisance in agriculture and horticulture worldwide, causing economic losses to growers. *Phasmarhabditis* is a genus of bacteria-feeding nematodes that can parasitize slugs and snails and thus potentially serve as a biological control agent. Canada had no record of *Phasmarhabditis* until a survey conducted in 2019 reported a Canadian strain of *Phasmarhabditis californica* from a single *Arion rufus* slug. To build on this discovery, we surveyed three major agricultural sites, ten greenhouses, and nurseries in Alberta from June to September 2021 to collect pest slug species and investigate their associated nematodes, specifically *P. californica.* Slugs were collected from the field and returned to the laboratory to check for emerging nematodes on White traps. We collected 1331 slugs belonging to nine species, with *Deroceras reticulatum* being the most common. Only 45 (3.38%) slug samples were positive for nematodes, and the majority were identified to species level: *Alloionema appendiculatum, Caenorhabditis briggsae, Caenorhabditis elegans, Panagrolaimus subelongatus,* and *Mesorhabditis spiculigera.* We did not isolate *P. californica* from any of the slugs collected from these survey sites, which included the original site where *P. californica* was discovered. However, four *D. reticulatum* slugs retrieved from a residential garden sample were infected with *P. californica.* These findings suggest the possibility of a fragmented distribution of *P. californica* across Alberta. Future research should focus on extensively surveying agriculture and horticulture sites and residential gardens in different provinces across Canada.

Introduction

Certain slug species are considered pests of agriculture, horticulture, and floriculture worldwide (Barker 2002; Koslowski 2012). Agricultural and horticultural sites, commercial nurseries, greenhouses, and residential gardens provide ideal habitats for slugs to thrive as they often provide moist and shaded environments (Douglas & Tooker 2012; Koslowski 2012). When slug density is high, large-scale no-tillage crops such as wheat, barley, oats, rye, corn, canola, tobacco, soybean, alfalfa, and leguminous forages often suffer extensive damage (Douglas & Tooker 2012; South 1992). The damage is not limited to large-scale arable crops but may also include many other crops including strawberries, cabbage, leeks, potatoes, and carrots (Thomas 2010). In severe cases, complete germination failure is possible due to grain hollowing even before the seeds germinate. Further, herbivory on the seedlings of arable plants can lead to a reduced crop stand and reduced yield. Pestiferous slug species are often inadvertently introduced to new areas through poor quarantine practices during the transport of agricultural and horticultural commodities and human travel (Cowie *et al.* 2009; Darrigran *et al.* 2020; Howlett 2012; Schurkman *et al.* 2022b). Rapid adaptability and spread in new settings in the absence of pressure from natural predators and competitors have led to some pest slug species establishing in many new parts of the world, from the tropics to temperate regions (Howlett 2012). Pestiferous slug genera in temperate climates include *Deroceras*, *Milax*, *Arion,* and *Limax.* For example, *Arion vulgaris,* Moquin-Tandon, 1885 and *Deroceras reticulatum* are among the most pestiferous slug species in Europe (Howlett 2012). Certain European slug species have also been introduced to temperate regions such as New Zealand, Australia, and South Africa (Howlett 2012).

The dominant method for controlling slugs worldwide is the application of agrochemicals, e.g., methiocarb, metaldehyde, and iron phosphate (South 1992). However, growing public opposition to pesticides and appreciation of eco-friendly agricultural practices favor the use of biopesticides and biocontrol agents in controlling slug populations. These can provide higher specificity and sometimes are more efficient pest management tools than other practices (Jaffuel *et al.* 2019; Mc Donnell *et al.* 2020). Nematodes, some of which are parasitic, offer a potential solution due to their natural associations with terrestrial gastropods (Wilson & Grewal 2005).

Previous surveys conducted in Germany, France, Slovenia, Bulgaria, the USA, Australia, Africa, and the UK found nematodes associated with slugs belonging to seven families, namely Agfidae, Alloionematidae, Angiostomatidae, Cosmocercidae, Diplogasteridae, Mermithidae, and Rhabditidae (Ross *et al.* 2016). The most well-known is a facultative parasite of slugs, *Phasmarhabditis* (Family Rhabditidae), a genus of bacterial-feeding soil-dwelling nematodes (Wilson & Grewal 2005). *Phasmarhabditis* currently contains eighteen nominal species (Ivanova *et al.* 2022; Rae *et al.* 2023), four of which have been shown to be pathogenic to slugs (Holley 2020; Ivanova & Spiridonov 2021; Schurkman *et al.* 2022a; Wilson & Grewal 2005): *P. hermaphrodita* (Schneider, 1859); *P. papillosa,* (Schneider 1866), Andrássy, 1983; *P. neopapillosa,* (Mengert in Osche, 1954), Andrássy 1983; and *P. californica*, (Tandingan De Ley *et al.*, 2016). Among them, *P. hermaphrodita* has been widely used as a biocontrol agent since it was commercialized in 1994 (Rae *et al.* 2007). The product was launched under the trade name Nemaslug® and is currently only commercially available in 15 European countries, owing to the natural occurrence of *P. hermaphrodita* in those regions (Laznik *et al.* 2020; Mc Donnell *et al.* 2020; Rae *et al.* 2007). This nematode is lethal to many slug species (in the families Arionidae, Milacidae, Limacidae, and Vaginulidae), particularly to *D. reticulatum,* arguably the most damaging slug species throughout the world (Howlett 2012; Rae *et al.* 2007). Since its commercialization, this nematode has been discovered in geographic areas other than Europe, such as Iran, Egypt, New Zealand, the USA, Norway, and China (De Ley *et al.* 2016; Howlett 2012; Rae *et al.* 2007). Similarly, *P. californica* was recently discovered in California and Oregon (De Ley *et al.* 2016; Howe *et al.* 2020) and has recently been commercialized as Nemaslug 2.0 in the U.K. market (Mc Donnell *et al.* 2023).

Canada had no previous record of *Phasmarhabditis* in the region until a recent discovery of a Canadian strain of *P. californica,* which was reported from a single slug (*Arion rufus*) collected from the exterior grounds of a local nursery in Edmonton (Brophy *et al.* 2020a; Brophy *et al.* 2020b). However, isolating a Canadian strain of *P. californica* from a single slug specimen is not sufficient evidence to confirm that the nematode is established locally. Thus, the goal of our study was to complete a more comprehensive survey including agricultural sites, commercial nurseries, and greenhouses in Alberta, Canada for pest slug species and their associated nematodes. These results provide valuable information to agro-practitioners in the region about pest slug species that are of agricultural and horticultural importance (residential gardens were not considered economically important sites in this survey). Our second objective was to confirm if the novel Canadian *P. californica* strain naturally occurs in agricultural and horticultural sites in Alberta, Canada.

Materials and methods

Slug collection

Slugs were collected from field margins and the adjacent vegetation stripes of three major agricultural sites in Alberta (Figure 1) from August to September 2021, a period corresponding to peak slug activity (Brophy *et al.* 2020b). In addition, ten commercial greenhouses and nurseries in Alberta were surveyed from June to September 2021 (Figure 1). Surveys were conducted in potential slug microhabitats such as soil surface, leaf litter, crop foliage, near the roots, under nursery pots, rocks, and logs, and in the marginal vegetation; on certain occasions when the surface soil was dry, the slugs were collected 1–5 cm deep in the soil. Each site was searched for a maximum of 30 minutes/person (2–4 people), and the slugs were handpicked and transferred into plastic containers with a perforated lid and lined with damp paper towels to prevent desiccation. Every site was visited at least twice during the survey period. Slugs collected from different sampling sites were put into separate containers to avoid any cross-contamination. Each slug species was photographed and identified using available taxonomic keys (Grimm *et al.* 2009; Mc Donnell *et al.* 2009; Perez *et al.* 2008; https://idtools.org/mollusk). Molecular diagnosis for slugs was performed when we could not reliably make an identification using morphological traits, or if the samples contained immature slugs that were difficult to identify solely on external morphologies.

Slugs from residential gardens

In addition to the field-collected slugs, we occasionally received slugs from residential gardens in Alberta. Most of these samples were sterilized and used for slug colonies, except for 74 slugs (*D. reticulatum*) that were haphazardly chosen and placed on white traps to check for nematodes.

Nematode isolation

All slugs, except 55 (reserved for laboratory slug colonies) were rinsed thoroughly with distilled water to remove any external phoretic nematodes. The slugs were then decapitated to encourage the emergence of associated nematodes. The slug cadavers were kept in individual Petri dishes (6 cm diameter) lined with a damp paper towel, covered, and sealed with Parafilm™. The cadavers were incubated at 18 °C, 80% relative humidity, 12 h light; 12 °C, 60% relative humidity, 12 h dark for two weeks. After this time, cadavers were inspected under a stereomicroscope. Nematodes were collected into 95% ethanol or DESS (dimethyl sulphoxide, disodium EDTA, and saturated NaCl) for subsequent molecular identification (Brophy *et al.* 2020b; Yoder *et al.* 2006).

Molecular identification

Slugs

A piece of the tail tip was clipped from select slug specimens (n = 24) and stored in 95% ethanol for molecular identification. QIAGEN DNeasy Blood and Tissue Kit (Germantown, MD, USA) was used to extract slug DNA from a ~2 mm × 2 mm piece of slug tissue. Polymerase chain reaction cycling conditions followed Reich *et al.* (2015). Partial fragments of the mitochondrial cytochrome c oxidase subunit I (cox 1) gene were sequenced: Cox 1 primer set LCO-1490 (5′-GGTCA ACAAATCATA AAGATATTGG-3′) and HCO-2198 (5′-TAA ACTTCAGGGTGACCAAAAAATCA-3′) (Folmer *et al.* 1994). Geneious Prime® software (North America Biomatters Inc, San Diego, CA, USA) was used to trim and edit the resulting sequences. The latter was then blasted on GenBank (Benson *et al.* 2013) to identify the species. In all cases, query coverage was >99.5%; the E value was 0; and the highest percentage identity was >99.0%. Sequences were deposited in GenBank under accession numbers OQ642082-OQ642105.

Nematodes

Nematodes were transferred from either 95% ethanol or DESS to a proteinase-K-based lysis buffer for DNA extraction (Williams *et al.* 1992). Primer sets 18A (5′-AAAGATTAAGCCATGCATG-3′) and 26R (5′-CATT CTTGGCAAATGCTTTCG-3′) were used in PCR amplification and direct-end sequencing of the 18S ribosomal DNA, as previously described (Blaxter *et al.* 1998; Denver *et al.*

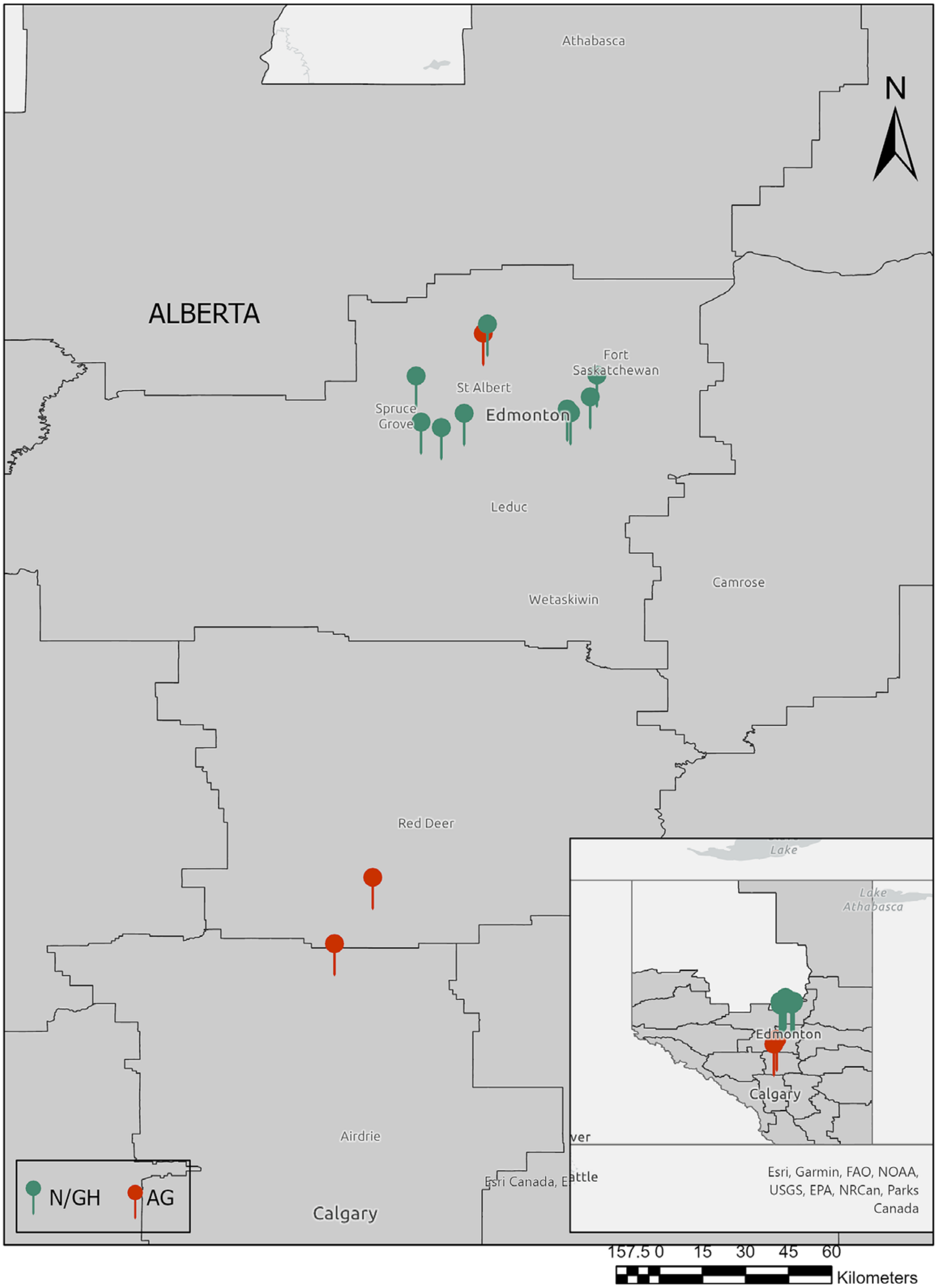

Figure 1. Slug survey (2021) locations. Slugs were collected from ten horticultural sites: i.e., nurseries and greenhouses (N/GH; green pins) and three agricultural sites (AG; red pins) in and around Edmonton, Alberta (main map). ArcGIS Pro software was used for visualization. (Esri, USGS | Sources: NRCan, Esri Canada, and Canadian Community Maps contributors. | Esri Canada | Northwest Territories, State of Alaska, Esri Canada, Esri, HERE, Garmin, FAO, NOAA, USGS, EPA, NPS, NRCan, Parks Canada

2003). The resulting 18S rRNA sequences were compared against GenBank's non-redundant (nr) database using Standard Nucleotide BLAST (Basic Local Alignment Search Tool), (NCBI Resource Coordinators. Database resources of the National Center for Biotechnology Information. Nucleic AcidsRes.2018;46(D1):D8-D13. doi:10.1093/nar/gkx1095. DNA sequences generated for this study

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were submitted to GenBank under accession numbers OQ645705 - OQ645739 (Supplementary Table S1).

Results

A total of 1331 slugs were collected from agricultural sites, greenhouses, and commercial nurseries in Alberta from June to September 2021. These comprised nine species: *Arion fasciatus*, *A. hortensis*, *A. rufus*, *A. subfuscus*, *Ambigolimax valentianus*, *Deroceras invadens*, *D. laeve*, *D. reticulatum*, and *Prophysaon andersonii*. Of the 551 slugs collected from the three agricultural sites, we identified four species: *A. fasciatus*, *D. invadens*, *D. laeve*, and *D. reticulatum* (the most abundant, Table 1). We collected a total of 780 slugs from ten greenhouses and nurseries, representing all nine slug species. The most prevalent slug species encountered in these sites were *D. laeve*, *A. valentianus, and D. reticulatum* (Table 1). Slug abundance rose gradually over the summer, peaking in August (Figure 2).

Out of 1331 slug samples collected during the survey, only 45 samples (3.38%) harbored nematodes (Table 2), but *Phasmarhabditis* was not isolated. The percentage of nematode-positive samples was high in June, followed by a gradual decline until August, but rose again in September (Figure 2). More nematode-positive samples were reported from nurseries and greenhouses (4.62%) than from agricultural (1.63%) sites (Table 2). Nematodes were isolated from four slug species: *A. rufus*, *A. valentianus*, *D. laeve*, and *D. reticulatum*, all of which were collected from two agricultural sites and four greenhouses/nurseries (Table 2). We identified 21 samples of nematodes to species level: *Alloionema appendiculatum*, *Caenorhabditis briggsae*, *Caenorhabditis elegans*, *Panagrolaimus subelongatus*, and *Mesorhabditis spiculigera*. Of all the nematode species, *C. elegans* and *P. subelonatus* were common to two agricultural sites and greenhouses/nurseries; the most prevalent nematode species was *C. elegans* (35.56%). By comparison, *A. appendiculatum*, *M. spiculigera*, and *C. briggsae* were only found in greenhouses and nurseries. *Alloionema appendiculatum* was isolated from a single *A. rufus* slug cadaver, while *C. briggsae* and *M. spiculigera* were isolated from an *A. valentianus* and a *D. laeve* slug cadaver respectively (Table 2).

Table 1. Abundance of the slug species collected in 2021 from Alberta agriculture sites, greenhouses, and nurseries. The following number of slugs were used to initiate laboratory slug colonies: *Arion fasciatus* (34), *A. hortensis* (4), *A. rufus* (3), *A. subfuscus* (3), *Ambigolimax valentianus* (3), *Deroceras laeve* (4), and *Prophysaon andersonii* (4).

Slug species	Agricultural fields	Greenhouses and Nurseries	Total
Family: Arionidae			
Arion fasciatus (Nilsson 1823)	41	2	43
Arion hortensis (Ferrusac 1819)		15	15
Arion rufus (Linnaeus 1758)		4	4
Arion subfuscus (Draparnaud 1805)		13	13
Family: Limacidae			
Ambigolimax valentianus (Ferussac 1821)		210	210
Family: Agriolimacidae			
Deroceras invadens (Reise *et al.* 2011)	2	21	23
Deroceras laeve (Müller 1774)	24	324	348
Deroceras reticulatum (Müller 1774)	484	180	664
Family: Anadenidae			
Prophysaon andersonii (Cooper 1872)		11	11
Total	**551**	**780**	**1331**

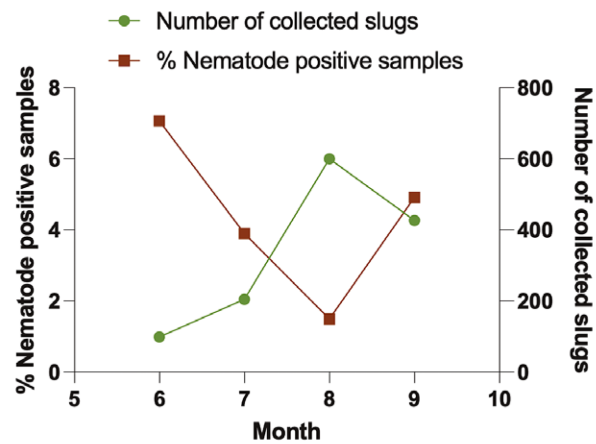

Figure 2. Temporal pattern of the total number of slugs collected from agriculture sites, nurseries/ greenhouses (green line), and nematodes isolated (%) from slug cadavers (brown line).

Of 74 slug cadavers, 22 samples (29.7%) from residential gardens were associated with nematodes. Of all positives, four samples were *P. californica* (18.18%). Other nematode species identified to the species level included *C. elegans, Choriorhabditis cristata, Pristionchus entomophagus,* and *P. subelongatus.* Other (n = 6) nematodes were only identified to the genus level: *Panagrolaimus* sp. and *Rhabditophanes* sp. (Table 2). The most common nematode species for both residential gardens and the survey sites were *C. elegans* and *P. subelongatus,* while *C. cristata, P. californica, P. entomophagus,* and *Rhabditophanes* sp. were only found in residential gardens.

Discussion

This study aimed to conduct a more extensive survey of agricultural and horticultural areas following Brophy *et al.* (2020a; 2020b), including nurseries where the nematode *P. californica* was previously isolated from a single slug. In total, 1331 slugs were collected, with peak slug collection in August, similar to what was reported by Brophy *et al.* (2020b). The slugs belonged to nine species from four families: Arionidae, Limacidae, Agriolimacidae, and Anadenidae. All slug species except *P. andersonii* (native) and *D. laeve* (exotic and native) are of European origin and were likely introduced to Canada via various means, e.g., trade and commerce of agricultural and horticultural commodities and/or travel (Araiza-Gómez *et al.* 2017; Grimm *et al.* 2009). Collectively, *D. reticulatum* was the most abundant slug species recorded during the survey (49.9%), the majority coming from agriculture sites (72.9%). The most common slug species found in greenhouses and nurseries were *D. laeve* (41.5%), *A. valentianus* (26.9%), and *D. reticulatum* (23.1%). Brophy *et al.* (2020a; 2020b) collected 2406 slugs belonging to nine slug species from 82 sites including natural green spaces (e.g., parks and ravines), seasonal nurseries, and greenhouses, with the majority coming from residential gardens in and around Edmonton, Alberta. In comparison, we only collected about half the number of slugs during the current survey, likely due to lower slug

Table 2. Slug-associated nematode species and their prevalence

Nematode	Agriculture sites	Nurseries/GH	Residential gardens	Total	Prevalence of nematodes	
					Survey	Residential
Family: Alloionematidae						
Alloionema appendiculatum		1(AR)		1	2.22%	
Rhabditophanes sp.			1(DR)	1		4.55%
Family: Rhabditidae						
Caenorhabditis briggsae		1(AV)		1	2.22%	
Caenorhabditis elegans	1(DR)	12(AV), 3(DL)	1(DR)	17	35.56%	4.55%
Choriorhabditis cristata			1(DR)	1		4.55%
Mesorhabditis spiculigera		1(DL)		1	2.22%	
Phasmarhabditis californica			4(DR)	4		18.18%
Family: Panagrolaimidae						
Panagrolaimus sp.			1(DR)	1		4.55%
Panagrolaimus subelongatus	1(DR)	1(DL)	2(DR)	4	4.44%	9.09%
Family: Neodiplogasteridae						
Pristionchus entomophagus			4(DR)	4		18.18%
A mix of nematodes/ Unidentified	7(DR)	17(AV/DL)	8(DR)	32	53.33%	36.36%
Total	9	36	22	**67**		

AR, *A. rufus*; AV, *A. valentianus*; DR, *D. reticulatum*; DL, *D. laeve*

emergence under elevated temperatures and unseasonably dry conditions during the survey period in 2021 compared to the year 2019 [i.e., 2019 July Olds college Agricultural Drought Monitoring Network (AGDM): maximum air temp ranged from 27.8°C–10.6°C, soil moisture at 005cm depth 39.4%–21%; 2021 July Olds college AGDM: maximum air temp ranged from 35.6°C–17.6°C, soil moisture at 005cm depth 19.1%–16.1%., Weather data provided by Agriculture and Irrigation, Alberta Climate Information Service (ACIS), https://acis.alberta.ca (February 2023)]. Further, unlike the previous survey by Brophy *et al.* (2020a; 2020b), our survey did not focus on slugs from residential gardens. We did not collect *Limax maximus*, despite its recovery during the previous survey (n = 2) by Brophy (2020a; 2020b). The failure to recover any would most likely be due to the extremely low population size of this slug species in and around the nursery where they were initially found.

Retail nurseries play a significant role as a focal point of terrestrial slug dispersal as they facilitate the passive transportation of exotic and native slugs with plant material to and from the nurseries (Bergey *et al.* 2014; Schurkman *et al.* 2022b). This could presumably cause the slugs to be transported over great distances, even provincial/ inter-state movements, with the primary destination being home gardens. Of the slug donations received from residential gardens, we identified two slug species, *D. reticulatum* and *A. fasciatus*. The latter was only received from a single residential garden and is the first report of *A. fasciatus* in a residential garden in Edmonton, Alberta. The dispersal of this slug species could have been a result of the horticulture trade (i.e., we collected *A. fasciatus* in greenhouses and nurseries in this study), with slugs being transported as adults, juveniles, and/or eggs residing in plant material and associated soil. Pest slug species including *A. fasciatus* and *D. reticulatum* are most likely to thrive in the absence of their natural enemies and are hard to eradicate once established (Kozlowski 2012; Robinson & Hollingsworth 2005). Their presence often results in persistent plant damage in home gardens and significant economic losses to large-scale crop farmers. Those may include yield loss and expenditures on labor, chemicals, and time required for the management of these pests. Further, these pest gastropods are ecologically important as they replace the detritivorous gastropod species that are mostly non-pests and native to the region, resulting in habitat alteration and reduced biodiversity (Kozlowski 2012). Therefore, we recommend regular monitoring and proper quarantine practices be in place, especially in retail nurseries.

In our study, *D. reticulatum* was the predominant slug species collected in agricultural sites. Besides *D. reticulatum,* only three others, *A. fasciatus, D. invadens,* and *D. laeve* were recovered but in fewer numbers. Douglas & Tooker (2012) listed *D. reticulatum, D. laeve, A. subfuscus,* and *A. fasciatus* as the most common slug species in field crops in the mid-Atlantic United States, and those species were not commonly associated with damage except *D. reticulatum.* In the Pacific Northwest region of the United States, *D. reticulatum* is thought to cause an estimated $60 million annual loss to the grass seed industry (Mc Donnell *et al.* 2020). During the current study, *D. laeve* was reported primarily from wheat field margins in Innisfail and around 5cm deep in the soil in one of the post-harvest fields in St. Albert. *A. fasciatus* and *D. invadens* were recovered mainly from wheat field margins in Innisfail and canola field margins in Olds, respectively. They were mostly seen associated with marginal vegetation with no visible direct damage to the cash crops. However, the damage (active feeding resulting in irregular holes and tears in foliage) was obvious with *D. reticulatum* in the fields in Innisfail. Marginal vegetation strips appear to provide ideal conditions for slugs, where they retain moisture and are in close proximity to crop fields. This observation aligns with those by Frank (1998), who found that *D. reticulatum* occurred in large numbers in both grass strips and adjacent rape

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fields in Belp, Switzerland. Further, it was reported that the marginal rape plants were more vulnerable to slug damage (defoliation), especially by *D. reticulatum.* A global survey recently revealed that slugs, and *Deroceras* species in particular, are serious pests of rapeseed (*Brassica napus*) production (Zheng *et al.* 2020). Canada is one of the major rapeseed producers in the world, yet little is known about the extent of slug damage in Canada. This highlights the need for more systematic surveys to assess the abundance and distribution of pest gastropods and their economic impact on crops across the country. Slug damage is often identified by the presence of slime trails on crop plants or the soil; however, certain symptoms could be similar to those of other organisms such as wireworms (Keiser *et al.* 2012) and black cutworms (Chandel *et al.* 2022). This might result in the overestimation or underestimation of crop damage by pest slugs. Thus, we also recommend robust quantitative assessments of damage caused by slugs to a variety of crops grown under different conditions (e.g., till v no-till) using different management strategies.

Nematodes were recovered from four slug species (*A. rufus, A. valentianus, D. reticulatum,* and *D. laeve*) collected from agricultural sites, greenhouses, and nurseries. We isolated nematodes belonging to four families: Alloionematidae, Rhabditidae, Panagrolaimidae, and Neodiplogasteridae. Twenty-one samples of nematodes were identified to five species; the rest remained unidentified due to mixed populations of nematodes or due to impurities. Nurseries— which have a more frequent exchange of plant material than crop fields—are more likely to support a higher diversity of slug-associated nematodes than agriculture fields with a single crop or a few crops cultivated with a less frequent exchange of plants. This was evident in our study by the higher prevalence of slug-associated nematodes in greenhouses and nurseries (4.62%) compared to the agricultural fields (1.63%). In greenhouses and nurseries, the nematodes were isolated from *A. rufus, A. valentianus,* and *D. laeve* (Table 2). Of the slugs collected, *A. rufus* only harbored *Alloionema appendiculatum*, a known parasite of *Arion* spp., including *Arion vulgaris,* a serious pest in central Europe (Nermut *et al.* 2019). Despite *A. appendiculatum* causing high snail mortality in heliculture (Nermut *et al.* 2019), research has shown that this nematode had no success causing mortality in *A. vulgaris* under laboratory conditions, suggesting that this nematode has limited potential as a biocontrol agent against *A. vulgaris* (Nermut *et al.* 2019). Surprisingly, *D. reticulatum*, the third most abundant slug species in greenhouses and nurseries, had no associated nematodes. However, among the agriculture sites, *D. reticulatum* was the only slug species to harbor nematodes (1.86%). We did not recover *P. californica* from any of the slugs in the agricultural or horticultural sites we surveyed. However, *P. californica* (accession number KM510210) was isolated from half of the *D. reticulatum* slug cadavers (4/8) from one of the residential garden samples in Lethbridge, Alberta. This nematode may have a patchy distribution in the province, and as such more extensive surveys are needed to determine its true range. Previously, *P. californica* has been isolated from greenhouses and nurseries in different parts of North America (Schurkman *et.al.* 2022b), including Alberta, Canada (Brophy *et al.* 2020a; Brophy *et al.* 2020b), but never from residential gardens. Therefore, this is the first report of *P. californica* being isolated from slugs in a residential garden in North America. Our results along with Brophy *et al.* (2020a; 2020b) demonstrate that the Canadian strain of *P. californica* can utilize both *D. reticulatum* and *A. rufus* as its host. We further suggest the possibility that the abundance/ presence of *P. californica* community dynamics in 2021 compared to 2019 may be due to the impact of the climatic variables (unseasonably dry conditions in 2021) and/or nursery substrate origins. Some *Phasmarhabditis* species, including *P. hermaphrodita, P. californica,* and *P. papillosa*, have been shown to cause mortality in *D. reticulatum* (Mc Donnell *et al.* 2020). Further, *P. californica* has a cosmopolitan distribution and has been isolated in Wales (Andrus & Rae 2019a; Andrus & Rae 2019b), Ireland (Carnaghi *et al.* 2017), the USA (De Ley *et al.* 2016; Mc Donnell *et al.* 2020), New Zealand (Wilson *et al.* 2016), and Canada (Brophy *et al.* 2020a; Brophy *et al.* 2020b). Given that *D. reticulatum* also has a global distribution and has been reported as a pest in Europe, North America, Australia, and New Zealand (Howlett 2012), there could be an opportunity to utilize *P. californica* as an alternative biocontrol agent against *D. reticulatum* in the absence of *P. hermaphrodita.* In fact, this species was commercialized (Nemaslug 2.0) for the UK market in 2022 (Mc Donnell *et al.* 2023). However, different strains of *P. californica* have shown striking differences in host preference to slug cues (Cutler & Rae 2021). Therefore, we recommend further research on the host preference, infectivity, and pathogenicity of the Canadian strain of *P. californica* to determine its efficacy as a potential biocontrol agent of pest slugs.

Other than *P. californica, Pristionchus entomophagus* was also isolated from residential slug samples during the current study. *Pristionchus* species show a species-specific necromenic association with beetles (Brown *et al.* 2011). However, they can hitchhike on other insects to reach their final hosts. Interestingly, we observed a non-insect host interaction of *Pristionchus* sp. during this study, which we found in association with *D. reticulatum* (n = 4). This aligns with the observations by Brophy *et al.* (2020b), who also found this taxon associated with *D. reticulatum.* This nematode is capable of producing dauer larvae under low food availability and infesting hosts (Brown *et al.* 2011). However, these necromenic nematodes cause no mortality to the host but wait until it dies to feed on the bacteria and resume development (Ishaq *et al.,* 2021; Sommer & McGaughran 2013). We suggest that the dauers we observed on slugs had a phoretic association, and when grown on *D. reticulatum* slug cadavers under laboratory conditions, *P. entomophagus* successfully propagated, suggesting that dead slugs are also a suitable growth substrate for this nematode taxon.

The introduction of a 'potentially exotic' nematode species as a biocontrol agent is restricted in many countries by legislation, and as such the importation and release regulations of those organisms are limited, often with the exception of those proven to be indigenous (Ehlers 2005; Wilson *et al.* 2016). Therefore, it is important to extend future survey efforts into the rest of Alberta, especially in and around the Lethbridge region, and into other provinces in Canada to verify the presence and distribution of *Phasmarhabditis* species. Further, there should be a thorough investigation of the infectivity and pathogenicity of this nematode on potential slug hosts to determine if this strain could be used as a biocontrol agent in Canada. Keyte *et al.* (2022) reported a higher prevalence of *P. californica* and *P. hermaphrodita* in snail hosts than in slugs. *P. californica* has been isolated from snail species such as *Theba pisana* (Tandingan De Ley *et al.* 2020), *Discus rotundatus* (Keyte *et al.* 2022), and *Oxychilus draparnaudi* in Germany and the UK (Grannell *et al.* 2021; Keyte *et al.* 2022). Therefore, future studies on nematodes associated with both terrestrial snails and slugs may reveal a wider distribution of *Phasmarhabditis* spp. in Canada. Relatively few studies have been conducted to determine the recent distribution and environmental impact of the introduced pest gastropods in Canada. There are even fewer studies on pest slugs in agriculture and horticulture habitats, which are of great economic importance. Here we report seven introduced and two native slug species in Alberta which are of agricultural and horticultural significance and the nematodes associated with those slugs. However, the economic damage and ecological impact of those slug species are poorly understood. *P. californica* has the potential to be used as a biological control agent in Canada due to its ability to cause mortality in pest slugs. Further studies including impacts on native non-target species are necessary to better understand the biological and ecological aspects of this nematode before an informed decision can be made on the use of this nematode as a slug control tool.

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Competing interest. RMcD declares that he is a co-inventor on a patent application entitled Mollusk-killing Biopesticide (U.S. application Serial No. 62/236,674).

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

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