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An investigation into the combination of the parasitic nematode *Phasmarhabditis hermaphrodita* and cedarwood oil to control pestiferous slugs

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ABSTRACT

Several slug species are voracious pests of agricultural crops in northern Europe and are difficult to control. The parasitic nematode *Phasmarhabditis hermaphrodita* has been developed as a slug control product, but there is little information about whether it could be combined with other control methods (such as essential oils) to enhance its efficacy. Here, we carried out experiments in propagators with lettuce at three different time periods (July, September and October), and tested the following treatments: water (untreated control), cedarwood oil, *P. hermaphrodita*, cedarwood oil and *P. hermaphrodita*, and Tween 80 (used as an emulsifier for the cedarwood oil solution). Lettuce was grown in propagators with either 10 *Deroceras reticulatum* or 5 *Arion vulgaris* and the percentage of lettuce eaten over 14 days (as well as weight, the number of live slugs and eggs produced) was recorded. Cedarwood oil reduced slug damage, slug numbers and slug eggs in the experiments with *D. reticulatum*, and *P. hermaphrodita* performed well in two out of three experiments. The mixture of *P. hermaphrodita* and cedarwood oil was superior in reducing the proportion of lettuce eaten compared to single doses of each treatment in one out of three trials. In propagators with *A. vulgaris* all treatments performed poorly. In summary, *P. hermaphrodita* and/or cedarwood can be used to reduce damage by *D. reticulatum*, but are ineffective at controlling *A. vulgaris*. Slugs from the genus *Arion* continue to be a difficult group to control.

1. Introduction

Several species of slugs (e.g. *Deroceras reticulatum*) are highly pestiferous in northern Europe and severely affect agricultural, horticultural and floricultural crops, by eating leaves, stems and roots, and contaminating plants with mucus and faeces (Barker, 2002). Slugs were commonly controlled by using chemical bait pellets, such as metaldehyde, but as they are toxic to non-target organisms, including mammals, (Bailey, 2002) metaldehyde has subsequently been banned in the U.K. This means the only realistic slug control methods available are ferric phosphate pellets, tillage or the parasitic nematode *Phasmarhabditis hermaphrodita*, which has been formulated and developed into a biological control agent called Nemaslug® by Becker Underwood BASF Agricultural Specialties, U.K, and used by farmers and gardeners for >25 years (Rae et al., 2023). Infective juvenile stage nematodes are applied to soil where they seek out slug hosts by detecting and responding to mucus and faeces (Rae et al., 2006a), enter the slug at the back of the mantle and reproduce prolifically, causing death to the slugs in 4–21 days (Wilson et al., 1993; Tan and Grewal, 2001). The nematodes reproduce on the slug cadaver and once the food supply is depleted they develop into infective juveniles and move into the soil to search for new slug hosts. *P. hermaphrodita* has been shown in numerous field trials to provide significant protection against slugs in arable, horticultural and floricultural crops (see Rae et al., 2007 for an overview), however, there are examples of where the application of nematodes has not been effective. For example, Wilson et al. (1995) reported no reduction in slug damage to lettuce after application of *P. hermaphrodita*. Similarly, Rae et al. (2009) reported a lack of reduction in slug numbers in lettuce in

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P. hermaphrodita treated plots. As the cost of nematodes can be prohibitively expensive compared to chemical pellets, such as metaldehyde, novel application strategies need to be sought to improve efficiency but reduce cost of nematodes (Grewal et al., 2005). For example, as slugs (*D. reticulatum* and *Arion ater*) have been shown to avoid areas where *P. hermaphrodita* has been applied (Wilson et al., 1999; Wynne et al., 2016) it has been suggested that *P. hermaphrodita* could be applied in bands, to deter slugs and reduce the number of nematodes applied and therefore cost. However, there have been mixed results using this approach, with Hass et al. (1999) showing application of *P. hermaphrodita* in bands instead of broadcast spraying had little effect on reducing slug damage in winter wheat. Alternatively, nematodes could be mixed with chemicals, or other compounds that could enhance slug control and reduce cost, but there has been little research on this using *P. hermaphrodita*.

Essential oils are organic pesticides that are promising, but poorly researched and are an attractive alternative to molluscicidal pellets as they are non-toxic to humans and several essential oils have been shown to kill slugs and snails. Klein et al. (2020) showed thyme, and spearmint were lethal to *D. reticulatum* and could be used in glasshouse trials to control slugs, to a level comparable to a metaldehyde control. Also, Mc Donnell et al. (2016) reported clove bud oil could be used to kill the eggs and juvenile stages of the pest snail *Cornu aspersum*. The combination of essential oils and *P. hermaphrodita* used to control slugs has never been investigated and could be promising. *P. hermaphrodita* is efficient at killing smaller susceptible slugs (Wilson et al., 1993; Tan and Grewal, 2001) but fails to kill larger slugs such as *Arion vulgaris* (Grimm, 2002), a highly invasive, problematic and pestiferous species that has spread across Europe and is difficult to control (Zając et al., 2019), perhaps essential oils could be used to target adult stages?

Therefore, the aims of our study were to 1) screen 13 essential oils for molluscicidal effects on two slug species and identify a candidate to use in further studies 2) investigate whether *P. hermaphrodita* could be combined with an essential oil to reduce slug damage in two pest species (*D. reticulatum* and *A. vulgaris*) in three propagator trials carried out at different times of the year (July, September and October 2020). Ultimately, the overall goal was to determine whether essential oils hold promise as a slug control tool in lettuce and whether their use with *P. hermaphrodita* could provide more efficacious slug control.

2. Materials and methods

2.1. Source of invertebrates

P. hermaphrodita strain DMG0001 (Nemaslug®) was supplied by BASF Agricultural Specialties, U.K. and stored at 10 °C until use.

Slugs (*D. reticulatum*, *A. vulgaris*, *Arion subfuscus* and *Ambigolimax valentianus*) were collected from local grassland in Maghull, Merseyside, U.K. (coordinates 53.506275, −2.963664). Adult slugs (length 2–3 cm, weight range: 0.20–0.70 g for *D. reticulatum* and *A. valentianus*, length 3–4 cm and weight range: 10–20 g for *A. subfuscus*) were collected in the early evening and stored in non-airtight containers and fed lettuce *ad libitum* and maintained at 10 °C until use. As these species are highly abundant in the area, we do not envisage any impact on the collection location. Slugs were identified using Rowson et al. (2014). Slugs that showed any signs of nematode infection e.g. swollen mantle, moribund, were not used in the trial.

2.2. Screening the effects of essential oils on A. valentianus slug eggs and A. subfuscus juveniles

The eggs of *A. valentianus* and the juveniles of *A. subfuscus* were exposed to 13 different essential oils. These two slug species were chosen because they are highly resistant to *P. hermaphrodita* infection (Dankowska, 2006; Ester et al., 2003), pestiferous, and are abundant and easy to collect or rear under lab condition. Slug eggs of *A. valentianus* were reared using a protocol developed by McDonald-Howard et al. (2021). Briefly, 50 ml universal bottles were filled with compost (10–15 % moisture content) to a depth of 3.5 cm. Two adult *A. valentianus* were added to each tube, a ball of cotton wool was placed on top and 0.5 ml of water sprayed into the tube, and the lid was loosely closed and incubated at 15 °C. After 8 days, clutches of eggs were counted and used in the essential oil experiment following protocols by Klein et al. (2020) and Mc Donnell et al. (2016). Five centimetre Petri dishes were lined with 5 cm diameter Whatman filter paper. To the paper in each Petri dish 1 ml of tap water, Tween 80, or one of 13 essential oil emulsions (pine, lemongrass, eucalyptus, birchtar, rosemary, cedarwood, garlic, thyme, peppermint, bitter orange, spearmint, clove and cinnamon) at a concentration of 0.5 % was added. Essential oils were purchased from Piping Rock (https://gb.pipingrock.com/). We chose these oils as they produced encouraging results in previous studies looking at their effect on *D. reticulatum* and *C. aspersum* (Klein et al., 2020; Mc Donnell et al., 2016). Oil emulsions were prepared by mixing the oil and Tween 80 at a ratio of 2:1 and then diluting with water. Tween 80 was used as a control on its own at a concentration of 1 %. Five *A. subfuscus* juveniles were placed into each 10 cm Petri dish and they were sealed with Parafilm® and stored in a non-airtight plastic box with moist, saturated kitchen roll at 10 °C. Every 24 h for 3 days the number of live *A. subfucus* juveniles were quantified. Slugs were recorded as dead if they did not respond to prodding with a pair of tweezers. This process was repeated four more times for *A. subfuscus*, (20 juveniles were tested in total for each treatment). The eggs of *A. valentianus* remained exposed up until the end of the hatching period of 14 days is developed through slug rearing protocol. The number of hatched eggs was then quantified and the other remaining eggs were checked under a dissecting microscope to confirm they were no longer viable. This process was repeated for *A. valentianus* but as there was a large founder population of these slugs more replicates could be set up and run in parallel. For each of the three replicates, 10 eggs were placed in separate Petri dishes and the experiment was repeated two more times in parallel.

2.3. Testing the effects of cedarwood and P. hermaphrodita on reducing slug damage in a propagator trial

Two separate trials were run to test the effects of *P. hermaphrodita* and essential oil treatments on reducing damage caused by *D. reticulatum* and *A. vulgaris*. For the first trial of *D. reticulatum*, twenty propagators (240 × 380 cm) were set up. For trials 2 and 3 of *D. reticulatum* and for all three trials of *A. vulgaris* 15 propagators were used. These were set up in a 90 cm wide polytunnel, filled to the top with peat-free Sylva compost and then three 14 day old little gem lettuce seedlings were planted per propagator. Lettuce seedlings were allowed to establish for 5 days in the soil before slugs and treatments were added to the propagators. Air vents in the propagator lids were covered in mesh netting to prevent the slugs from escaping but permitted airflow, and the rims of the trays were taped with copper tape to stop slugs from travelling onto the lids. Five treatments were applied to the propagators in each trial: 1. Water-only control; 2. Tween 80 control diluted in water to a concentration of 0.5 % for the trials with *D. reticulatum* and at 1 % for the trials with *A. vulgaris* (the emulsifier for cedarwood oil); 3. *P. hermaphrodita*, 4. Cedarwood oil; 5. Cedarwood oil and *P. hermaphrodita*. For treatments 3 and 5, *P. hermaphrodita* was applied at the recommended rate for Nemaslug® of 300,000 nematodes per m^2, and for treatments 4 and 5 cedarwood oil was mixed 2:1 with Tween 80 and then diluted in water to a concentration of 0.25 % for *D. reticulatum* and 0.5 % for *A. vulgaris*. We chose cedarwood oil as it killed slug eggs quickly in our previous experiment and has been shown to not negatively affect the survival or behaviour of *P. hermaphrodita* (Barua et al., 2020). Each treatment was applied in a volume of 560 ml per propagator. For each trial either 10 *D. reticulatum* or 5 *A. vulgaris* slugs were added per propagator (*D. reticulatum* weight range: 0.20–0.70 g, n = 100; *A. vulgaris* weight range: 10–20 g, n = 45). Slug shelters were placed in each tray using upturned flowerpot saucers and each propagator was sealed using masking tape to prevent slugs from escaping. Temperature and moisture was recorded by placing a Xiaomi flower monitor into random propagators for the experimental period. The moisture content of the soil was 20 %. If moisture decreased we added additional water to maintain the same moisture content across all propagators throughout each trial. Trials were carried out over 14 days, at which point the proportional damage to each leaf of the lettuce were recorded by taking photographs of the leaves and visually estimating the proportion eaten per leaf (Rae et al., 2009). The plants were then uprooted, weighed and the number of dead and live slugs was recorded. It was noted during the trial with *D. reticulatum* that slugs laid eggs in the soil and on leaves, so the numbers of eggs found in each propagator on day 14 was also counted.

To assess how season affected the efficacy of treatments, trials were repeated at three different time periods in 2020: 23rd July - 6th August, 16th - September 30, 2020, and 13th - 27th October.

2.4. Statistical analysis

To analyse the effects of essential oils on slug eggs, the number of slugs hatching from the water, Tween 80, and the essential oils was compared using a One Way ANOVA and Tukey's post hoc test. Data from the propagator trials with slugs were analysed with linear mixed models to measure the effects of treatment, time period and their interaction on proportion of lettuce leaves eaten, and on final lettuce weight. We ran Wald type 3 chi square tests to analyse the main effects and interactions, and propagator identity was fitted as a random factor within each model to account for non-independence of data on lettuce within each propagator. Data on lettuce weight were square root transformed and data on proportion of lettuce leaves eaten were rank transformed to meet model assumptions. Planned sets of seven pairwise contrasts per time period (21 contrasts in total) were run for each model to check differences within each time period between specific treatments: water vs. Tween 80; water vs. *P. hermaphrodita*; Tween 80 vs. cedarwood oil; Tween 80 vs. cedarwood oil + *P. hermaphrodita*; *P. hermaphrodita* vs. cedarwood oil; *P. hermaphrodita* vs. cedarwood oil + *P. hermaphrodita*; and cedarwood oil vs. cedarwood oil + *P. hermaphrodita*. A Holm-Bonferroni correction for multiple corrections was applied to the contrast results of each model to control family-wise error rate. Statistical analysis was carried out with R 4.2.3 (R Core Team, 2023) and RStudio v.2023.03.0, with the R packages 'lme4' (Bates et al., 2015), 'car' (Fox and Weisberg, 2019) 'emmeans' (Lenth, 2022) and 'performance' (Lüdecke et al., 2021). The numbers of surviving slugs and the numbers of eggs laid by the slugs on day 14 was compared using a One way ANOVA with Tukey's post hoc test.

3. Results

3.1. Susceptibility of A. vulgaris juveniles and A. valentianus eggs exposed to essential oils

Juveniles of *A. subfuscus* and eggs of *A. valentianus* were exposed to 13 different essential oils, as well as a water and Tween 80 control to assess molluscicidal effects. Over 72 h there was a strong molluscicidal effect of 11 essential oils on the survival of *A. subfuscus* juveniles compared to water and Tween (Fig. 1A–D; $P < 0.05$). However, there was no significant reduction in survival of *A. subfuscus* juveniles over 72 h exposed to birch tar ($F(3,12) = 0.44$; $P = 0.73$) and rosemary oil ($F(3,12) = 1$; $P = 0.42$). In contrast, all 13 essential oils killed the eggs of *A. valentianus* (Fig. 1E; $P < 0.05$). We chose cedarwood oil for further experiments as it killed both slug species efficiently, and had no effect on the health of *P. hermaphrodita* (Barua et al., 2020).

3.2. The effect of nematodes and cedarwood oil on the proportion of lettuce eaten by D. reticulatum

As seen in Supplementary Fig. 1 the mean temperature varied in July, September and October.

There was a significant interaction between time period and treatment on the proportion of lettuce eaten in the *D. reticulatum* propagator trials ($chi^2 = 28.890$, $p < 0.001$), a significant main effect of treatment ($chi^2 = 303.820$, $p < 0.001$) but no significant main effect of time period ($chi^2 = 2.795$, $p = 0.247$). The planned contrasts (Supplementary Table 1) showed that the cedarwood oil + *P. hermaphrodita* treatment significantly reduced lettuce damage in all time periods compared to the Tween 80 control or *P. hermaphrodita* alone ($p < 0.001$ – $p = 0.011$), and also compared to cedarwood oil alone in October ($p = 0.004$), but not in July and September (both $p = 1.00$; Fig. 2). The cedarwood oil treatment significantly reduced damage compared to Tween 80 control in July and September (both $p < 0.001$), with a trend to do so in October ($p = 0.051$). Cedarwood oil alone also significantly reduced damage compared to *P. hermaphrodita* alone in July ($p < 0.001$) and September ($p = 0.004$), but not October ($p = 1.000$). The *P. hermaphrodita* treatment significantly reduced lettuce damage compared to the water control in September ($p = 0.045$) and October ($p = 0.009$), but not in July ($p = 0.262$). There was no difference in lettuce damage between the water and Tween 80 controls in any time period (all $p = 1.000$).

3.3. The effect of nematodes and cedarwood oil on the weight of lettuce exposed to D. reticulatum

There was a significant interaction between treatment and time period on the weight of lettuce after 14 days in the *D. reticulatum* propagator trials ($chi^2 = 34.608$, $p < 0.001$), as well as significant main effects of treatment ($chi^2 = 128.954$, $p < 0.001$) and time period ($chi^2 = 59.675$, $p < 0.001$). The planned contrasts (Supplementary Table 2) showed that the cedarwood oil + *P. hermaphrodita* treatment resulted in heavier lettuce compared to Tween 80 control in all time periods ($p < 0.001$ – $p = 0.024$, Fig. 3). There was a trend for cedarwood oil + *P. hermaphrodita* lettuce to be heavier than *P. hermaphrodita* treated lettuce in September ($p = 0.056$), but not in July ($p = 0.244$) or October ($p = 1.000$), nor when compared with cedarwood oil only-treated lettuce in any time period ($p = 0.145$ – $p = 1.000$). Cedarwood oil-treated lettuce were significantly heavier compared to Tween 80 control in July ($p = 0.042$) and September ($p < 0.001$), but not October ($p = 1.000$). *P. hermaphrodita* treated lettuce were heavier compared to the water control in September ($p = 0.002$) and October ($p < 0.001$), but not in July ($p = 1.000$), and there was a trend for them to be heavier compared to cedarwood oil in October ($p = 0.056$), but not in July ($p = 0.488$) or September ($p = 1.000$). There was no difference in lettuce weights between the water and Tween 80 controls in any time period (all $p = 1.000$).

3.4. The survival and number of eggs produced by D. reticulatum exposed to nematodes and cedarwood oil

On day 14 of the experiment the number of slugs was quantified as well as the number of slug eggs found in soil (Supplementary Table 3). Data from the three replicates was analysed together. A single application of nematodes, cedarwood oil and the combination of cedarwood oil and nematodes significantly reduced the number of *D. reticulatum* compared to the untreated control (Supplementary Table 3; $P < 0.05$). There was no difference between the number of live slugs found in the propagators treated with nematodes, cedarwood oil and cedarwood oil and nematodes (Supplementary Table 3; $P > 0.05$).

The number of slug eggs found in the propagators in each treatment differed significantly (Supplementary Table 3, $P < 0.001$), however, the only treatments that were shown to have a significant effect reducing the numbers of slug eggs in the soil were cedarwood oil and cedarwood oil

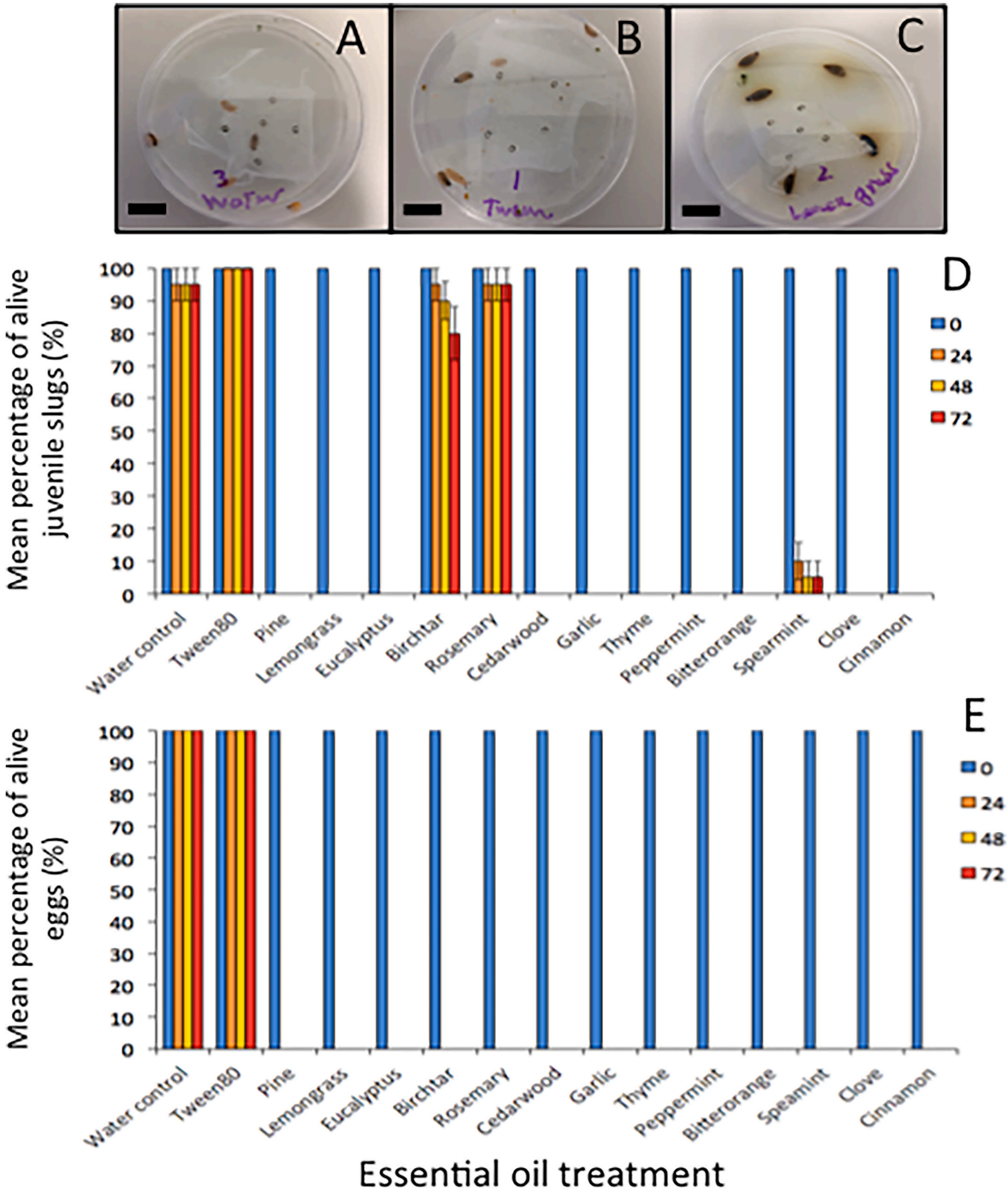

Fig. 1. *A. subfuscus* juveniles survived when exposed to water (A), and Tween 80 (B) but all slugs died when exposed to lemongrass (C) (pictured as an example). Scale bars represent 1 cm. Mean percentage of alive juvenile *A. subfuscus* (D) and alive *A. valentianus* eggs that hatched (E) exposed to water control, Tween 80 control and 13 different essential oils over 72 h. Bars represent ± one standard error.

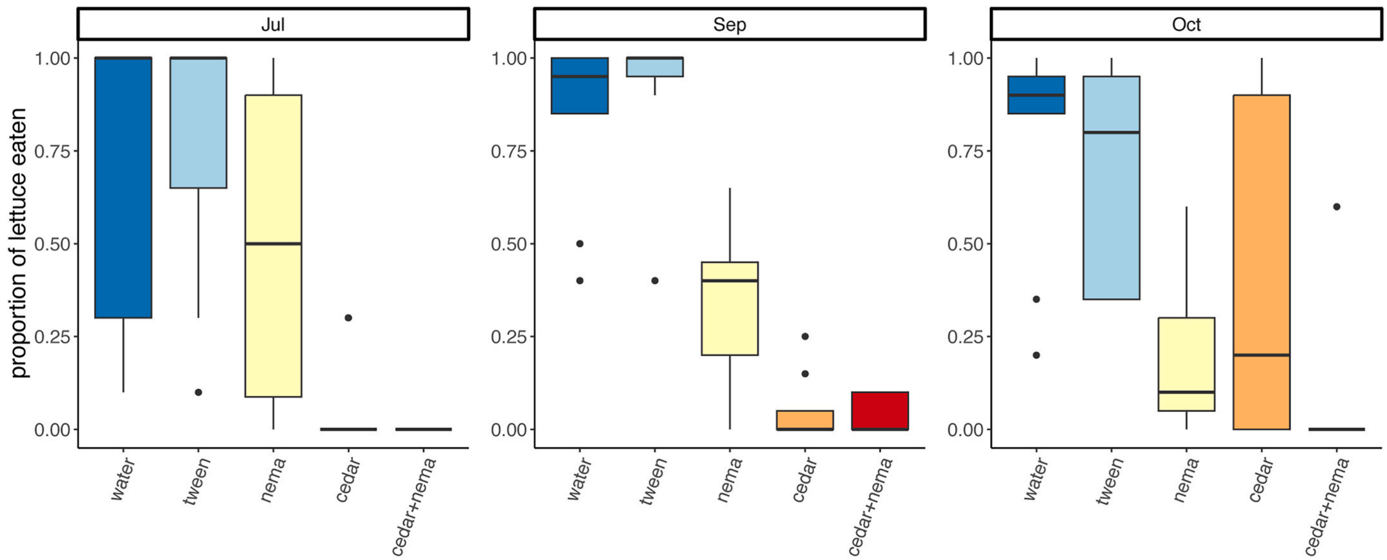

Fig. 2. Box and whisker plots showing proportion of lettuce leaves eaten during propagator trials with *D. reticulatum* in July, September and October 2020. Lettuce (n = 12 in replicate 1, n = 9 in replicates 2 and 3 per treatment per time period) were treated with either: water, Tween 80, *P. hermaphrodita* nematodes ('nema'), cedarwood oil ('cedar'), or *P. hermaphrodita* + cedarwood oil ('cedar + nema'). After 14 days, the proportion of damage to lettuce leaves was measured.

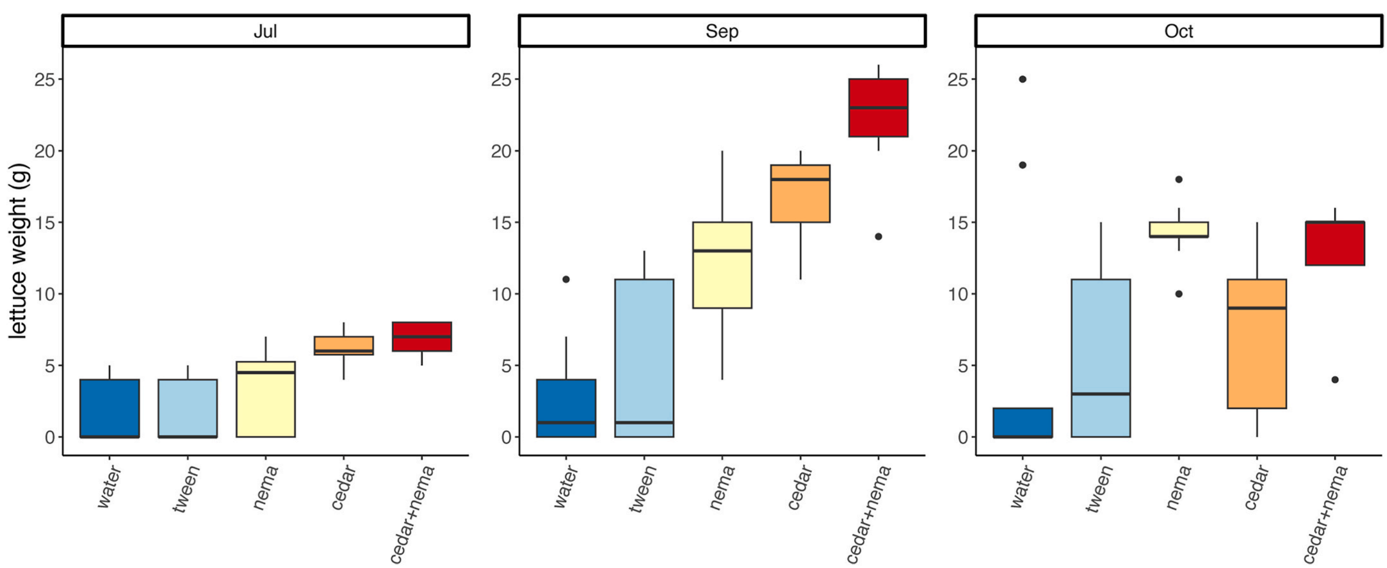

Fig. 3. Box and whisker plots showing weights of lettuce after 14 day propagator trials with *D. reticulatum* in July, September and October 2020. Lettuce (n = 12 per in replicate 1, n = 9 in replicates 2 and 3 treatment per time period) were treated with either: water, Tween 80, *P. hermaphrodita* nematodes ('nema'), cedarwood oil ('cedar'), or *P. hermaphrodita* + cedarwood oil ('cedar + nema').

and nematodes, compared to the untreated control (Supplementary Table 3, $P < 0.05$). These two treatments did not differ significantly in reducing numbers of slug eggs (Supplementary Table 3, $P > 0.05$). Nematodes failed to reduce the number of slug eggs found in the soil compared to the untreated control (Supplementary Table 3, $P > 0.05$).

3.5. *The effect of nematodes and cedarwood oil on the proportion and weight of lettuce eaten by A. vulgaris*

By day 4, *A. vulgaris* had eaten all of the lettuce in all three replicates (data not shown). There was no significant difference between the proportion (or weight) of lettuce eaten by *A. vulgaris* exposed to nematodes, cedarwood oil and the combination of cedarwood oil and nematodes or the untreated control in each replicate (data not shown; $P > 0.05$). There was no significant difference between the numbers of *A. vulgaris* or *A. vulgaris* eggs laid in any of the five treatments across all three replicates ($P > 0.05$).

4. Discussion

In the first experiment we screened 13 different essential oils to determine which oils could be used to kill two species of slug. All 13 essential oils severely affected the survival of *A. valentianus* and 11 caused significant mortality in *A. subfuscus*. As both slug species are resistant to *P. hermaphrodita* as adults (Rae et al., 2009; Ester et al., 2003), these essential oils show promise for slug control. Klein et al. (2020) tested the same 13 essential oils but on adult *D. reticulatum*, and found thyme and spearmint to be the most toxic, and went on to demonstrate that they could reduce slug numbers and damage in microcosms when applied to potted plants containing annual ryegrass infested with slugs. Furthermore, research by Mc Donnell et al. (2016) screened 11 essential oils and found clove bud oil was most toxic to eggs and juvenile stages of the snail *C. aspersum*. Undoubtedly, these essential oils (and the ones tested) show promise at controlling slugs and snails at the juvenile, egg and adult stages but it is unknown whether these oils could be combined with other organic pesticides, such as *P. hermaphrodita*, to provide superior protection against slugs. Crucially, we concentrated on cedarwood oil as we showed it was lethal to two species of slug and does not affect thrashing behaviour or survival of *P. hermaphrodita*, whereas several oils e.g. thyme, cinnamon, clove and garlic were lethal to these nematodes (Barua et al., 2020). Cedarwood oil or the combination of cedarwood oil and nematodes, were both highly effective at killing *D. reticulatum* and subsequently reduced crop damage quickly. The application of cedarwood oil and nematodes was superior to a single application of cedarwood oil or nematodes in October but not in July and September. Cedarwood oil could be a promising alternative control method for pest slugs, however field trials would be needed to confirm this. There is little information about the effect cedarwood oil has on other pest invertebrates. Flor-Weiler et al. (2022) found cedarwood oil was toxic and repellent to four species of tick, and had potential to be an eco-friendly acaricide. Also, cedarwood oil was found to be the most efficient essential oil (out of 26 tested) affecting activity and urease activity when exposed to the bacterium *Helicobacter pylori* (Korona-Glowniak et al., 2020).

A single application of *P. hermaphrodita* reduced slug damage in two out of three of the propagator experiments with *D. reticulatum*. The level of reduction in *D. reticulatum* damage provided by *P. hermaphrodita* was only comparable to cedarwood oil in the October trial. *P. hermaphrodita* has been successfully shown to protect agricultural, horticultural and floricultural crops against slug damage (see Rae et al., 2023), however, there are examples where it has not reduced slug damage or slug numbers (e.g. Wilson et al., 1995; Iglesias et al., 2003; Rae et al., 2009). The reasons for this could be due to the majority of nematodes dying upon application because of exposure to UV light, desiccation, exposure to parasites and pathogens and/or the physical properties of soil e.g. temperature, oxygen, moisture retention and texture (Griffin, 2015; Smits, 1996; Wilson and Gaugler, 2004). Out of all theses factors the most likely reason is temperature. *P. hermaphrodita* failed to provide any protection against slug damage in July, but the maximum temperature during this experiment was nearly 40 °C. The optimum temperature for growth of *P. hermaphrodita* is 17 °C and it can infect *D. reticulatum* at 5 °C but is killed rapidly at 35 °C (Glen et al., 1996; Andrus and Rae, 2019), hence the nematodes presumably perished. In trials in September and October, protection against slug damage occurred as mean temperatures were <20 °C and <15 °C, respectively, which are closer to optimal temperatures for slug infection and nematode survival.

There are only a handful of field trials that have looked at increasing the effectiveness of *P. hermaphrodita* by combing with other slug control methods. Hass et al. (1999) applied methiocarb and *P. hermaphrodita* as a furrow treatment and found the combination worked well to decrease slug damage in sugar beet. The rational of the study was that pellets could kill the slugs on the soil surface, while nematodes would hunt out the slugs in the subterranean soil layers. In a small experiment, Rae et al. (2006b) found the combination of *P. hermaphrodita* and iron phosphate pellets provided significant protection against slug damage by *D. reticulatum*, but this was akin to metaldehyde and iron phosphate applied on their own. In our experiments, the combination of cedarwood oil and nematodes outperformed a single dose of cedarwood oil or nematodes in just one out of three trials. This is in contrast to research that has shown entomopathogenic nematodes (EPNs) can be successfully combined with essential oils to control pests. For example, Monteiro et al. (2021) showed the combination of *Heterorhabditis indica* and the essential oil from *Lippa triplinervis* was 90 % efficient at killing cattle ticks (compared to 73 % using just the essential oil). Also, *Steinernema carpocapsae* and diallyl disulfide (an essential oil component of garlic) has a nematocidal effect and reduced numbers of the plant parasitic nematode *Meloidogyne javanica*, compared to nematodes applied on their own (Anastasiadis et al., 2011).

All treatments had no effect on *A. vulgaris*. This species is difficult to manage, and only the juvenile stages of *A. vulgaris* are killed by *P. hermaphrodita* and adults are unaffected (Grimm, 2002; Speiser et al., 2001). All lettuce was eaten within 4 days, and the nematodes did not cause any mortality or induce feeding inhibition. The inability of *P. hermaphrodita* to cause death to non-susceptible slugs has been problematic in field trials and the nematodes have failed to reduce slug damage in some studies (e.g. Rae et al., 2009). Interestingly, cedarwood oil also failed to reduce damage caused by *A. vulgaris*. Previous to the experiment, it was predicted the oil would be as efficient as in the experiment with *D. reticulatum* (and based on previous research by Klein et al., 2020; Mc Donnell et al., 2016), but this was not the case; however higher doses should be used in future research. It should therefore be emphasised that essential oils have potential to control certain pestiferous gastropods, but there are some slug species e.g. *A. vulgaris* that are still particularly problematic to control. This is unfortunate as *A. vulgaris* is a voracious pest currently rapidly spreading round northern Europe (Zemanova et al., 2017) and has recently been detected in North America (L'Heureux et al., 2023).

In summary, we have found cedarwood oil shows promise at killing juvenile slugs (*A. subfuscus* and *A. valentianus*) under laboratory conditions, as well as reducing slug damage in propagator experiments with *D. reticulatum*, and can outperform *P. hermaphrodita*. Another promising factor about the use of essential oils is they kill slug eggs, which *P. hermaphrodita* does not. Field trials are needed to confirm the utility of essential oils in slug control.

CRediT authorship contribution statement

Kerry McDonald-Howard: Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft. **William T. Swaney:** Formal analysis. **Archita Barua:** Data curation, Methodology. **Rory Mc Donnell:** Conceptualization, Resources, Writing – original draft. **Christopher D. Williams:** Supervision, Writing – original draft. **Hayley Jones:** Funding acquisition, Project administration, Supervision, Writing – original draft. **Robbie Rae:** Conceptualization, Formal analysis, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cropro.2024.106601.

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