

Birch tar oil is an effective mollusc repellent: field and laboratory experiments using *Arianta arbustorum* (Gastropoda: Helicidae) and *Arion lusitanicus* (Gastropoda: Arionidae)

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Populations of two molluscs, the land snail *Arianta arbustorum* and the Iberian slug *Arion lusitanicus*, have increased substantially in many places in the northern Fennoscandia in recent years. This has resulted in considerable aesthetic and economic damage to plants in home gardens and commercial nurseries. Birch tar oil (BTO), is a new biological plant protection product, and was tested against these molluscs. In this study we examined whether 2 types of BTO, used either alone, mixed together, or mixed with Vaseline[®], could be applied as 1) a biological plant protection product for the control of land snails by direct topical spray application, 2) as a repellent against snails when painted on a Perspex[®] fence, and 3) as a repellent against slugs when smeared on pots containing *Brassica pekinensis* seedlings. Both the fences and the pots with seedlings were placed in each field with a high population of the target organism.

When applied as a spray on snails, BTO did not act as a toxic pesticide but rendered the snails inactive for a period of several months. The BTO barriers were effective in repelling both snails and slugs. However, the repellent effect of BTO alone against the molluscs was short-term. Repeated treatments were required to keep the slugs away from the plants and we found that the interval between treatments should not exceed two weeks. A collar fastened around the rim of the pots, combined with the BTO treatment, did not give any additional benefit in hindering slugs from invading the plants. Most noticeably, the BTO+Vaseline[®] mixture prevented the land snails from passing over the treated fences for up to several months. The results of these experiments provide evidence that BTO, especially when mixed with Vaseline[®], serves as an excellent long-term repellent against molluscs.

Key-words: birch tar oil, biocontrol, biological plant protection product, *Arianta arbustorum*, *Arion lusitanicus*, land snail, Spanish slug, Iberian slug, repellent, mollusc.

Introduction

Birch oil distillate or birch tar oil (BTO), is a by-product of processing birch (*Betula sp.*) wood to produce charcoal. Although anecdotal evidence in Finland suggests that BTO has been an effective repellent against burrowing rodents and moose, the use of BTO as a biological plant protection product or biocide is a new innovation. However, due to its novelty as a repellent and because of the chemical complexity of BTO, no comprehensive information on the active compounds of BTO is available. Preliminary analyses suggest phenols, comprising 20–30% of BTO (Czernik 2002), are among the most interesting compounds of BTO having a repellent effect on molluscs (Hagner 2005), but various other volatile compounds can also play a role. Among the phenolic compounds, cresols, allylphenol, guaiacol, 4-methyl- and 4-ethyl guaiacol, eugenol, isoeugenol, vanillin, and ethylvanillin have been identified in birch biomass pyrolysis (Murwanashyaka et al. 2002). Despite its potential value as a biological plant protection product, we are aware of only one publication in which the applicability of birch/pine oil has been tested as a repellent against mosquitoes (Thorsell et al. 1998).

This study focuses on two molluscs, the land snail *Arianta arbustorum* L. (Gastropoda: Helicidae) and the Iberian slug *Arion lusitanicus* Mabilie (Gastropoda: Arionidae). The species *A. arbustorum* belongs to the local snail fauna in Finland, but before 1990 its distribution was restricted to the southern coast of Finland with some sporadic establishments in fertile deciduous forests in the northern part of Finland (Terhivuo 1978, Valovirta and Heino 1994). In recent years the snail has increased its distribution dramatically in many urban/semi-urban areas in southern and central Finland. The species *A. lusitanicus* is a major pest of European horticulture and a newcomer to Fennoscandia (Weidema 2006). Originally from the Iberian Peninsula, *A. lusitanicus* reached the Åland Islands in Finland in 1990 and it is now widely distributed from the west coast to the south-eastern areas of the country (Valovirta 2001). The northernmost population was found in Oulu (65 02' N; 25 31' E) in

2005 (Valovirta personal communication). Despite its southern origin the slug has established itself in Finland's northern climate and Fennoscandia (Hofsvang and Haukeland 2006), highlighting the ability of eggs and immature stages to acclimatize to the cold. The distribution of *A. lusitanicus* or other *Arion* slugs has not been studied in Finland, but the occurrence of *A. circumscriptus* (Johnston), *A. fasciatus* (Nilsson), and *A. fuscus* (O.F. Müller) [syn. *Arion subfuscus* (Drap.)] have been reported (Valovirta 1968).

Both *A. arbustorum* and *A. lusitanicus* are omnivorous, feeding on a wide range of living and dead plant material, mushrooms and dead fauna (Hägele and Rahier 2001, Valovirta 1964). As these molluscs are hermaphroditic, a population can begin from a single individual which can produce up to 400 eggs (*A. lusitanicus*). Although the distribution is still sporadic in Finland, it is not uncommon to find hundreds of individuals of *A. arbustorum* per square meter. Both species have spread with an alarming rate, often by the trade of produce and plants. As such, these molluscs have rapidly become an increasing problem with severe impacts, particularly in home gardens (Speiser and Rowell-Rahier 1991, Valovirta 2001). *A. lusitanicus* also threatens larger professional horticultural operations and would have serious economic consequences in, for example, fields with perennial crops.

Control of *A. arbustorum* and *A. lusitanicus* has hitherto been tedious, usually collecting and killing being the most common methods in home gardens. To some extent, chemical control is useful but can be harmful to other organisms and ecosystems. Methiocarb (Mesuro), for example, is one of the two pesticides currently permitted for use as a molluscicide in Finland, is known to pose a threat to non-target biota (Purvis and Bannon 1992, Shore et al. 1997) and to ground waters (Garcia de Llasera and Bernal-González 2001). Of the less harmful methods of chemical control, iron phosphate (Feramol) has been effective against species of slugs and is relatively non toxic (Procop 2005). However, high numbers of iron phosphate pellets may increase earthworm mortality (Langan and Shaw 2006). Biological control using the nematode *Phasmarhabditis hermaphrodita* Schneider (Nematoda:

Rhabditidae), has been successfully used, not only against slugs (Grimm 2002), but also to some extent against snails (Coupland 1995). Grimm (2002) and Speiser et al. (2001) also reported that this nematode only killed immature stages of the slug *A. lusitanicus*. This is in agreement with the results from a small-scale experiment conducted at MTT Agrifood Research Finland. (MTT pesticide efficacy studies 2005, unpublished data). Consequently, the possible repelling effect of BTO on *A. arbustorum* and *A. lusitanicus* warrants further investigations to complement the current methods of control used in horticultural operations and home gardens.

This study is part of a larger research programme in which the effects of BTO as a molluscicide (the current study) and their ecotoxicological effects on both terrestrial (Hagner et al. 2010a) and aquatic (Hagner et al. 2010b) organisms were investigated. Here we aimed at 1) studying the toxic effect of BTO on populations of *A. arbustorum* in the laboratory by direct spray application, 2) exploring the efficacy of the distillate to repel *A. arbustorum* outdoors when painted on a Perspex® fence, and 3) demonstrating the repelling effect (and duration) of a BTO barrier in a field with a high frequency of *A. lusitanicus*.

Material and methods

Birch tar oil (BTO)

Two types of BTO from pyrolysed birch wood and bark were supplied by Charcoal Finland Ltd.: BTO1, a more soluble form resulting from the early phase of the distillation process i.e. at temperatures less than 380 °C, and a viscous form (BTO2) resulting at the end of the process when the temperature reaches 400 °C. A mixture (BTOm) of the two forms was also used in the experiments. Birch tar oil (BTO) has been assigned with a CAS number (8001-88-5) in the worldwide substance database (American Chemical Society 2007). Thus the same abbreviation, BTO, has been used for the closely related product in our studies.

Experiment I: Toxic effect of BTOs on *A. arbustorum* – direct spray application

Experimental setup

An experiment using different ages (adults, eggs and the newly hatched young) of the snails was established as a laboratory study at the Department of Ecological and Environmental Sciences in summer 2003. Both BTO1 and BTO2 were tested. The snails were collected close to the Departmental building in the city of Lahti, Southern Finland. Adult snails were allowed to reproduce in 1.7 l glass jars (Ø 10 cm, height 19 cm) containing a soil monolith (4 cm thick), and covered with gauze. Fresh food consisting of carrot, lettuce and earthworms was given to the snails twice a week. Maturity of the adult snails was determined by the thickened outer lip framing the aperture of the shell (Baur and Raboud 1988, Raboud 1986, Terhivuo 1978). The jars were kept outdoors under natural light and temperature conditions. The eggs laid by the snails were removed daily, placed into glass jars with moist soils and kept in the fridge (+5 °C) before the start of the experiment. After two months there were sufficient numbers of eggs to conduct the experiment.

Toxicity bioassay

The experiment was performed in 1.7 l glass jars with a soil monolith (3 cm thick) growing *Trifolium repens*, *Festuca pratensis* and *Taraxacum officinale*. Four mature snails and about 55–65 eggs were placed on the soil in each jar. Three treatments, each with 5 replicates, were established: 1) jars sprayed once with BTO1 (0.5 dl m⁻²; the amount equalling 500 l ha⁻¹), 2) jars sprayed with BTO2 (0.5 dl m⁻²) and 3) jars sprayed with equal amount of water (control). The containers were covered with gauze and kept at 20 °C under a 16:8 h light:dark cycle. During the experiment, the snails were fed twice a week with fresh carrots, lettuce and leaves of *T. officinale* and *T. repens*. Fresh water was sprayed weekly in the jars to maintain favourable moisture conditions for the snails. Hatching of the eggs and movement of the adult snails were observed weekly. After three months the snails were removed to clean jars with fresh plant material to activate and check the survival of the snails. The following day the number of surviving snails was recorded.

Experiment 2: Repellent effect of BTO2 on *A. arbustorum* - Perspex® fence barrier

Experiment 2.1 – Snails inside the fences

Transparent Perspex® fences (height 40 cm, area 0.74 m²), partly buried in the soil (3 cm deep), were constructed in mid June 2005. The fences were established in five home yards containing grassy vegetation, in the city of Lahti. The fences received four treatments, each with three to five replicates, fenced areas: 1) without Vaseline® or BTO2 (control; n=4); 2) receiving Vaseline® only (n=3); 3) with BTO2 only (n=5); and 4) with a mixture of Vaseline® and BTO2 (n=5). The Vaseline®, the BTO2, and the mixture of BTO2+Vaseline® (40/60, v/v.), was spread using a brush on the outer upper side of the walls to form a 10 cm wide barrier. The upper 5 cm of the fence was bent so as to form “a rain shadow” for the area at which the repellent was applied. Spreading of the smears was done only once at the start of the study. The next day, 50 snails were placed into each fenced area. The study lasted for 38 days and within this time, the number of snails in the fenced areas were monitored at 1, 4, 6, 8, 11, 14, 18, 21, 28, 36 and 38 days after initiation of the study.

Experiment 2.2 – Snails outside the fences

The set up of experiment was identical to the Experiment 2.1 described above except that no snails were added to the fenced areas. The experiment was conducted in the city of Lahti in a fertile fallow meadow growing tall herbs, grasses and some deciduous trees. The study was conducted from late July to early September in 2005. The *A. arbustorum* population in the area was >10 adults m⁻² (visual assessment). Naturally occurring snails were removed from the fenced areas at the start of the study. Three treatments, each with 2 to 4 replicates, were established: 1) fenced areas without Vaseline® or BTO2 (control; n=2); 2) Vaseline® only (n=2); and 3) a mixture of Vaseline®+BTO2 (n=4). Spreading of the smears on the outer upper side of the walls was carried out only once at the onset of the study. After removing all the snails, pieces of carrot were placed inside the fence to attract snails

into the fenced area. The study lasted for 42 days within which time the entrance of snails into the fenced area was monitored at 3, 6, 9, 18 and 42 days after initiation of the study. During the first month the snails were removed from the systems at every inspection, after which, the snails that entered the systems were left untouched. One control and one BTO2+Vaseline® treated fence were left in the meadow over winter. In the following summer, the entrance of snails into the fenced area was monitored five times.

The weather during Experiments 2.1 and 2.2 (mid June to early September) was variable with heavy rains (collective rainfall during the study 271 mm) to periods of dry and warm weather (average temperature 15 °C; Finnish Meteorological Institute).

Experiment 3: The repelling effect of a BTOm barrier against *A. lusitanicus*

Experimental set up

The experiment was established at MTT Agrifood Research Finland, Jokioinen, in 2005. The field area (4.5 × 42 m) was surrounded by glasshouses on both long sides. One of the short ends was bounded by the storehouse and the other end was separated from the main field by a 50 cm high tin plate treated with BTO to prevent slugs from escaping. The field vegetation consisted mainly of *Elymus repens*, *Trifolium repens* and *Taraxacum officinale*. The vegetation was left to grow wild except for a mowed strip (1.20 m wide) in the middle of the area to facilitate moving without crushing the slugs (Fig. 1).

Slug population

A. lusitanicus were originally collected in 2001 from Åland and transported to Jokioinen for the purpose of testing pesticides. The slugs were reared and contained outdoors in a secured grassy field area. Within four years the population had developed from a few individuals in the area into approximately 20–100 individuals m⁻², the density varying in different parts of the field according to the weather and vegetation. Some naturally occurring



Fig. 1. *Experiment 3*. The experimental area was situated in the middle of a small field with slugs and surrounded by glasshouses.

A. fasciatus (Nilsson) slugs were also found in the experimental field and included in the experiment.

Test plants

Chinese cabbage seedlings, *Brassica pekinensis* var. Yamiko, were produced in the glasshouse and grown in 1.5 l black plastic pots containing commercially available horticultural peat (Kekkilä: N-P-K 14-4-20). At the end of June the plants were placed outdoors in the experimental field. Strips of irrigation-mat (40 × 40 cm) were placed on the mowed strip of grass and the pots were centred on the mats at a distance of one meter from each other. Automatic drip irrigation was used to keep the irrigation mat constantly wet.

The experiment was designed on information and experiences from a preliminary field trial arranged in the same way. The experiment was completely randomized with four replicates. The use

of irrigation-mats prevented grass from reaching the pots and creating passage ways for the slugs to cross the BTO barrier. Splashing of rainwater with soil on the treated surface of the pots was also reduced, which minimized any further possibility of access to the potted plants.

Treatments

The experiment consisted of 24 pots, half of which were equipped with a plastic collar, 3 cm in breadth, fastened around the rim of the pots to prevent the washing effect by raindrops. A mixture (BTOM) of BTO1 and BTO2 (30/70, v/v) was painted evenly on the whole outer surface of the pots. The pots received six treatments (completely randomized design), each with four replicates: 1) pots without collar and repellent (control), 2) pots without collar but with repellent (BTOM × 2) applied twice (fortnightly), 3) pots without collar and with repellent (BTOM × 4) applied four times (weekly), 4) pots with collar but no repellent, 5) pots with collar and with repellent (collar + BTOM × 2) applied twice (fortnightly), 6) pots with collar and with repellent (collar + BTOM × 4) applied four times (weekly). The weekly and fortnightly treatments with BTOM started on the first day of the experiment, which extended from 23 of June to 8 of August. A bird-net was put up over the entire field to prevent thrushes from eating the slugs.

Data collection

The plants were checked in the morning on a daily basis for the duration of the study. The number of slugs entering the pots and accessing the plants were counted and then placed back into the field outside the grass strip of the experiment. At the same time, all old cabbage leaves hanging over the pot rims were removed. The damage to the plants caused by the slugs was estimated by visual assessment as a percentage of the damaged leaf area (Fig. 2). Observations from the first 35 days covered the period of BTOM treatments. The second part of the study, the follow-up period, monitored the duration of the repellent effect of BTOM on the slugs after day 35. Observations were made until it could be verified that slugs had entered all treatments.

Climate conditions during the study



Fig. 2. *Experiment 3*. The number of *Arion lusitanicus* slugs in pots were counted and the leaf area damage was estimated. Treatment 4) pot with collar but no birch tar oil mixture (BTOM).

The experimental area was surrounded by greenhouse walls on two sides making the area less exposed to normal weather conditions than in an open field. Subsequently, the area received more rainfall due to runoff from the greenhouse roofs. Compared to data from the local weather station (Fig. 3) the total rainfall in the experimental area was approximately three times higher than in the open field. Temperature changes in the pots during the experiment were equivalent with those in an overturned black plastic pot on the ground where the temperature was monitored by a data-logger (CelciPick CK-39/+75 °C).

Statistical analysis of all experiments

Statistical analyses were performed using the SPSS 10.0 statistical software (SPSS 1999). To examine the differences in the amount of adult and newly hatched young snails between the treatments in Experiment 1, analysis of variance (one-way ANOVA) was conducted. In Experiment 2.1 and Experiment 3, ANOVA for repeated measurements was used. Data from Experiment 2.2 was analyzed by non-parametric 2-way Kruskal-Wallis analysis (Ranta et al. 1989) with time and distillate treatment as factors. In Experiment 3, only data from observations in the period of BTO treatments were statistically analysed.

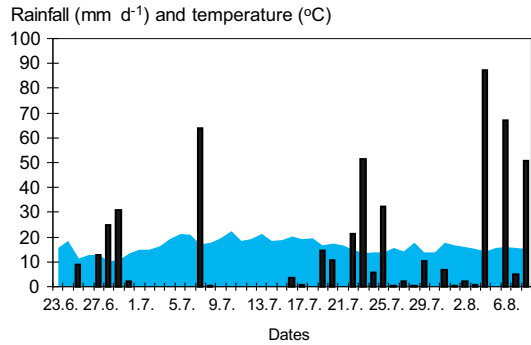


Fig. 3. *Experiment 3*. Rainfall (mm d⁻¹) and temperature (°C) in the enclosed experimental area based on information from the local weather station at Jokioinen. Bars indicate the rainfall and the shaded area indicates the temperature. The excess water runoff from greenhouse roofs tripled the amount of water received in the experimental area.

Results

Toxic effect of BTOs on *A. arbustorum* - direct spray application

The results of the laboratory study (Experiment 1) proved BTOs to be ineffective in eliminating snails; neither BTO1 nor BTO2 had a statistically significant effect on the survival of adult snails (ANOVA, F=1.68, p=0.226) (Fig. 4). The snails excreted extensive amounts of slime directly after spraying of BTOs and aimed at escaping from the jars. On day 2, all adult snails in the BTO1 and BTO2 treated jars were inactive and a slime plug excretion was observed in the front aperture of the shell. The eggs started to hatch one week after initiation (and spraying) of the study in all treatments with BTOs having no clear effects on the number of hatched eggs, the activity, or survival of the young snails. During the 3-month study period, most adult snails in the BTO1 and BTO2 treated systems remained passive while those in the control treatments were active. After being removed to jars with fresh food at the end of the study, almost all adults, irrespective of the treatment, were still alive. The data was not always normally distributed and the variances were sometimes heterogenous, even after data transformation.

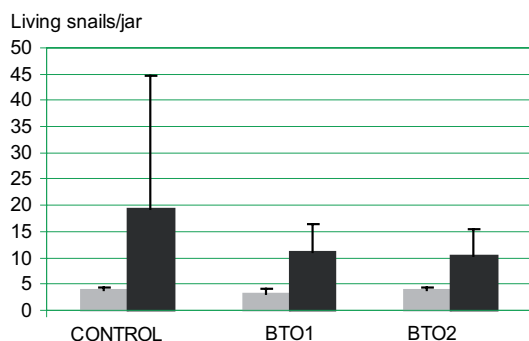


Fig. 4. Experiment 1. The number of living adult and young *Arianta arbustorum* in the control and treated jars at the end of the study. Soluble (1) and viscous (2) forms of birch tar oil (BTO) were used. Black bars = adult snails, grey bars = young snails (<3mm). Mean values + SD, n=5.

Repellent effect of BTO2 on *A. arbustorum* - Perspex® fence barrier

In the field enclosure study (Experiment 2.1), BTO2 displayed a clear repelling influence on the *A. arbustorum*. The day after placing the snails in the fenced systems, it was found that only 20 % remained in the control systems, while all individuals were still present in the systems painted with the Vaseline®+BTO2 - mixture. Both BTO2 and Vaseline® alone repelled the snails to some extent (repeated measures ANOVA, $p=0.000$), but these effects were short-term and less effective when compared to the results produced by the Vaseline®+BTO2 - mixture (repeated measures ANOVA, $p=0.000$) (Fig. 5). Data from Experiment 2.1 was not always normally distributed and the variances were sometimes heterogeneous, even after data transformation. The data was log10-transformed due to the high variation of snail densities in the controls and in the treatment with Vaseline® only. Although this did not normalize the data completely, ANOVA for repeated measurements using log10-transformed values was applied. We were interested in the BTO induced temporal patterns in the field studies. Therefore a repeated-measures (not available in non-parametric test packages) model was considered to be the most appropriate form of analysis. The fact that data

transformation did not improve the data, warrants caution when interpreting the data.

The results from Experiment 2.2 support those obtained from Experiment 2.1, i.e. that the Vaseline®+BTO2 - mixture was the most effective snail repellent (2-way Kruskal-Wallis, $p<0.05$) (Fig. 6). This mixture formed an effective barrier preventing all snails from entering the fenced systems up to seven weeks after initiating the study, whereas in the control fences, between two and six snails were found at every inspection. As was seen in Experiment 2.1, Vaseline® alone also seemed to repel the snails in Experiment 2.2, although the effect was not statistically significant (2-way Kruskal-Wallis, $p>0.05$). Unlike in the control and in the Vaseline® treated systems, the carrots remained intact and untouched in the Vaseline®+BTO2 treated systems. Data from Experiment 2.2 was analyzed by a non-parametric 2-way Kruskal-Wallis test (Ranta et al. 1989) with time and distillate treatment as factors. The data was not always normally distributed and the variances were sometimes heterogeneous. Transformations had no effect on data.

The monitoring results (Experiment 2.2) from summer 2006 (one year after the initiation of study), showed that the repelling effect of the BTO2+Vaseline® -mixture remained over winter.

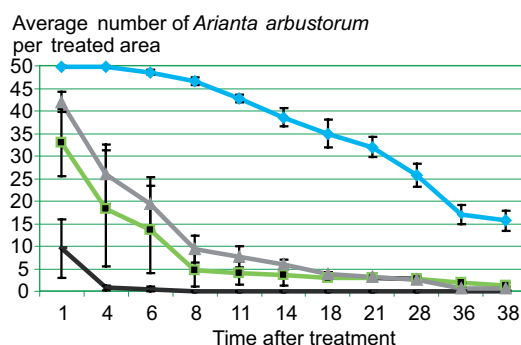


Fig. 5. Experiment 2.1. The number of *Arianta arbustorum* (mean values + SD) found inside the differently treated fences at different times. At day zero, 50 adult snails were placed inside the fenced areas. Control (black line); Vaseline® (green line with squares); viscous birch tar oil (BTO2) (grey line with triangles); BTO2+Vaseline® (blue line with diamonds).

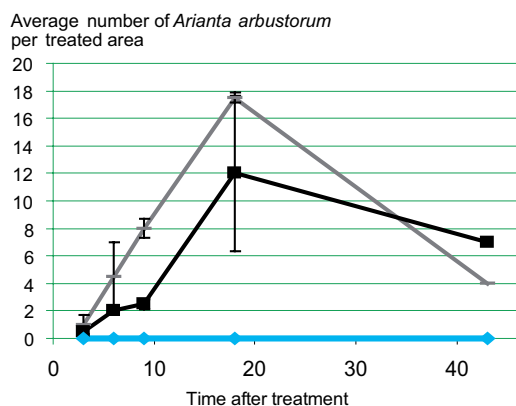


Fig. 6. Experiment 2.2. The number of *Arianta arbustorum* (mean values \pm SD) found inside the differently treated fences at different times. Control (grey line); Vaseline® (black lines with squares); viscous birch tar oil (BTOM)+Vaseline® (blue lines with diamonds).

At all five monitoring times during the year 2006, we found >3 snails inside the control fence but no snails from inside the BTOM+Vaseline® treated systems.

The repelling effect of a BTOM barrier against *A. lusitanicus*

It was found that BTOM effectively repelled *A. lusitanicus* from potted cabbage plants when applied as a protective barrier around plastic pots (Experiment 3). Upon reaching the BTOM barrier on the plastic pots, the slugs shrank away immediately before coming in contact with the smear. The slugs were strongly attracted to the cabbages and tried repeatedly, but unsuccessfully, to find a way to cross the BTOM barrier.

The first part of the study clearly showed that treatment of pots with BTOM significantly reduced the amount of damage on the cabbage plants (repeated measures ANOVA, $p=0.000$), which were almost left untouched (Fig. 7). The plants in the control pots were completely consumed 18 days after start of the experiment (Fig. 8). Figure 8 also shows that the collar alone did not have any hin-

dering effect on leaf damage caused to the plants (repeated measures ANOVA, $p=0.527$). This was further highlighted by the lack of interaction between the collar and the BTOM treatments. Interestingly, there was no difference in the repelling effect between the weekly and fortnightly applications. The data was normally distributed and the variances were homogenous.

The follow-up period revealed that 21 days after the final BTOM treatment (36 days after starting), 12.5% of the leaf area of the plants in the pots treated with BTOM was damaged compared to the total damage in the control pots. As expected, the effect of BTOM in the fortnightly treated pots began to decrease sooner than in the weekly treated pots (Fig. 9). At the end of the experiment, only 7.8% of the leaf area of the plants was damaged in the weekly treated pots with and without collar, compared to 30.1% in the fortnightly treatments. The species *A. fasciatus* was not found in the treat-



Fig. 7. Experiment 3. Birch tar oil mixture (BTOM) efficiently prevents *Arion lusitanicus* from reaching the plants when applied as a protective barrier outside the pots (July 25). Treatment 2) no collar + BTOM fortnightly application. The photo was taken 19 days after painting with the BTOM.

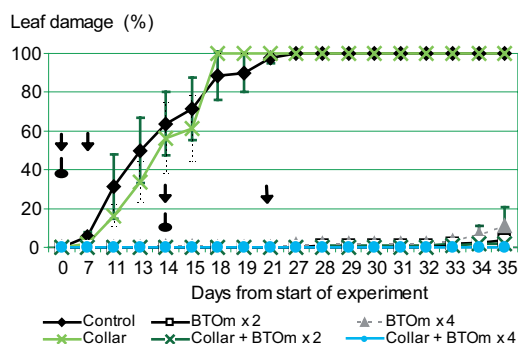


Fig. 8. Experiment 3. Percent leaf damage (mean values \pm SD) of potted *Brassica pekinensis* by *Arion lusitanicus* in the control and birch tar oil mixture (BTOm) treatments during the first part of the study. Arrows with a triangle indicate the weekly treatments, and arrows with a circle, fortnightly treatments.

ed pots and thus the leaf damage was solely caused by *A. lusitanicus*.

Discussion

Toxic effect of BTOs on *A. arbustorum*

Our preliminary studies (unpublished data) have clearly shown the negative influence of BTOs on the two slug species *Deroceras agreste* and *A. lusitanicus*: BTO1 sprayed over land areas growing grasses and herbs resulted in the death of these molluscs soon after spraying. However, as was clearly shown in the current study, the mortality effect of BTOs against snails was low. Furthermore, the eggs and the newly hatched juveniles with a fragile shell also survived the spraying. The results suggest that shells of the snails, irrespective of their age, provide these organisms an efficient shelter against substances that are seemingly toxic to other molluscs. The slime plug excreted by the snails in the frontal aperture further enhances their survival under unfavourable, even hostile conditions. That the adult snails became temporarily inactive for a period of three months after the BTO treatments

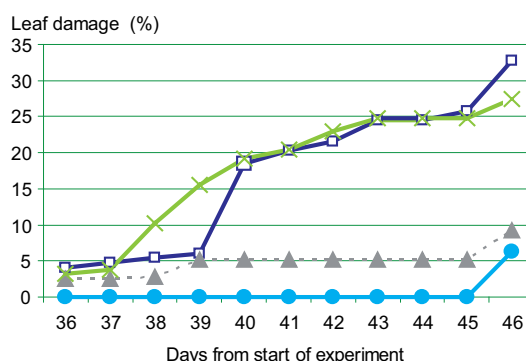


Fig. 9. Experiment 3. Percent leaf damage (mean values) of potted *Brassica pekinensis* by *Arion lusitanicus* in birch tar oil mixture (BTOm) treatments during the follow-up phase. Treatments: weekly in pots without collar (triangles) and with collar (circles), fortnightly in pots without collar (squares) and with collar (crossed squares). Untreated control pots were not included in the graph because the plants were completely eaten and the leaf damage could not be estimated.

indicates that the food source, also receiving BTO spray, of the snails remained repellent for a long time. It should be noted that the inactivating effect of BTOs on snails in the field would be shorter as the effect of BTOs is likely to be reduced by rain and UV. However, BTO could still be useful in IPM strategies, where the aim is not always to kill insect pests, but rather to prevent yield losses. Yield losses may be possible to be reduced by the inactivating effect of BTO on snails. Furthermore, a relatively long time of inactivity is bound to affect the fecundity and fertility of *A. arbustorum* which is likely to have a negative impact on the population densities of the snails.

Repellent effect of BTO against slugs and snails

The results clearly showed that BTO exhibited a repellent effect against *A. arbustorum* and *A. lusitanicus* in confined areas heavily infested with these molluscs. BTO2 indisputably showed high potential as a mollusc repellent against *A. arbustorum* given

that none of the snails crossed the BTO2+Vaseline® barrier of the fenced systems during the 43-day experiment. However, repeated applications of the cabbage pots over a period of several weeks were required to maintain the repellent mode of action against *A. lusitanicus*. In doing so, the concentration of the active constituents was maintained at a level high enough to prevent slugs from crossing the BTOm barrier. Weekly treatments with BTOm provided the best protection against slugs, as it took them more than three weeks after the last treatment to enter the pots. Moreover, the interval between the treatments should preferably not exceed two weeks, which seem to be the critical point for the BTOm barrier to start breaking down. Unexpectedly, the pot collar, intended to protect the BTOm barrier from rain and sunshine, did not improve the repelling effect. Rather, it was more relevant to keep the outside of the pots free from dirt and grass as the slugs avoided the BTOm barrier provided it was clean. The large individuals of *A. lusitanicus*, which are the most active and able to travel long distances (Grimm and Schaumberger 2002), were also found in the pots with collars.

According to the results in Experiment 2 with *A. arbustorum*, it can be assumed that BTO mixed with a greasy substrate such as Vaseline® could extend the repelling effect against *A. lusitanicus* as well. Although the mechanism is not yet known, we suggest that Vaseline® prevents BTO2 from drying, thereby retaining the repelling volatiles in the mixture. Vaseline® can also prevent the water-soluble compounds from dissolving and leaching out under heavy rain. It seems that Vaseline® alone works as a repellent against snails, although the effect is short-term and far less intense as in BTOs. Whether the repellency of Vaseline®, as a mineral oil based grease, bases on volatiles or its unpleasant physical properties for the snails, remains open.

Interestingly, there appears to be a specific, hitherto unknown, compound or group of compounds in the BTO that acts as an efficient repellent to both slugs (unpublished data) and snails. These molluscs appear to be able to detect the repellent compounds in BTO by olfaction only at a short distance. When confronted with BTO, the molluscs stop at a distance of approximately 1 cm from the

substrate, and turn around to escape from the obvious unpleasant odour. It is noteworthy to mention that common pine tar (manufacturing process having similarities to that of BTO) has a similar physical structure and odour to that of BTO, but is far less effective at repelling molluscs when compared to BTO. Where pine tar is concerned, the snails stop by the substrate for a while but then glide over the sticky substrate with slightly increased mucus production (Hagner 2005).

Locomotion of slugs via olfaction cues is a well known phenomenon (Gelperin 1974). Some plant extracts, such as extracts of *Saponaria officinalis* and *Valerianella locusta*, are known to have a similar effect on the behaviour of *A. lusitanicus* (Barone and Frank 1999). The repelling or attracting properties of the specific active compounds in a substance is also connected to the level of concentration in the substance (Clark et al. 1997). Further studies are needed to find out how many treatments, or which concentrations give the best result for protection against molluscs.

Our results indicate that BTO smeared on plastic pots, Perspex® fences (or other non-absorbing material), has a definite repellent effect against *A. lusitanicus* and *A. arbustorum*. Although shelled molluscs do not appear to be killed by BTOs when directly sprayed, the fact that these distillates reduced the active period of the snails is likely to reduce the damages brought about this snail in northern latitudes with already a short growing season. To fully understand the effect of BTO on slugs and snails, a thorough investigation of the chemical composition of BTO is required. This information will enable the better selection of the most effective type of BTO for control of molluscs. Furthermore, although BTO is included in the worldwide substance database, knowledge of its chemical composition is also essential for EU registration and future use as a biological plant protection product.

To conclude, our studies give strong evidence on the potential of BTOs to be applied as an effective, non-costly, easy-to-use, and an environmentally friendly (Hagner et al. 2010a, 2010b) method against molluscs. As biological plant protection methods are needed to compensate for the potentially harmful and decreasing range of chemical

molluscicides, this method could be a useful contribution as an alternative pest management strategy not only in home gardens, but also to some extent in organic farming practices and IPM strategies.

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