Chemical sabotage in forest health management: Effects of *Allium ursinum* L. based semiochemicals on the catches of bark beetles and their antagonists in stands of European ash (*Fraxinus excelsior* L.)

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Abstract: Chemische Sabotage im Waldschutz: Wirkung bärlauchbasierter Semiochemikalien auf den Fang von Borkenkäfern und deren Antagonisten in Beständen der Gemeinen Esche (*Fraxinus excelsior* L.)

Im Zuge des Klimawandels wird die Bedeutung von Borkenkäfern im Waldschutz zukünftig noch weiter steigen. Die Entwicklung neuer Regulations- und Bekämpfungsverfahren als Ergänzung zu den bisherigen Methoden ist daher notwendig, um Waldbesitzern und -bewirtschaftern eine zielgerichtete Auswahl von Handlungsoptionen im Borkenkäfermanagement zu ermöglichen. Eine Strategie ist dabei der Einsatz von Non-Host-Volatiles zur zur olfaktorischen Abwehr Abwehr von Borkenkäfern. In diesem Zusammenhang wurden in einem Freilandversuch mit Theysohn®-Schlitzfallen in Eschenbeständen in der Nordwestaue Leipzigs verschiedene auf Bärlauch (*Allium ursinum* L) basierende Semiochemikalien getestet, nachdem im Rahmen anderer Versuche vor Ort eine scheinbar repellente Wirkung von Bärlauch auf den Bunten Eschenbastkäfer (*Hylesinus fraxini* (Panzer)) beobachtet wurde. Im Vortrag werden die Ergebnisse des Versuchs kurz vorgestellt und diskutiert.

Keywords: *Allium ursinum*, bark beetles, bark beetle attractants, bark beetle management, *Hylesinus fraxini*, semiochemicals, Theysohn traps

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Introduction

Two decades of ash die back caused by the infection with the fungus *Hymenoscyphus pseudoalbidus* pose a severe threat to European ash resources, a highly valuable tree species from an economic as well as an ecological point of view. Ash dieback, as well as the lowering of groundwater levels and the increasing occurrence of drought periods are predisposing factors for successful attacks by *Hylesinus fraxini*, a secondary pest that requires weakened or stressed host trees and has the potential for mass outbreaks (Schwerdtfeger 1981). Bark beetle attacks on ash do not only accelerate the death of *H. pseudoalbidus* infested trees, they also put trees that only show minor symptoms or are still uninfected, including potentially resistant ash trees, at the risk of dying.

Within the bioProtect project, which aims at developing and implementing biotechnical methods for an insecticide-free management of bark beetles, field experiments were carried out in 2016 and 2017 in ash stands in a floodplain forest near the city of Leipzig. During these experiments it was observed that the presence of wild garlic, *Allium ursinum*, tended to reduce *H. fraxini* colonization on trap logs and trap trees. To comprehensively study the effect of *A. ursinum* and its volatiles on *H. fraxini* as well as other bark beetles and their antagonists, and to assess their potential use in bark beetle management, a trap experiment was set up in 2018 on the same site.

Material and Methods

The floodplain forest northwest of Leipzig is part of the Leipzig floodplain system Flora-Fauna Habitat (FFH) and Special Protected Area (SPA) within Natura 2000. European ash (*Fraxinus excelsior*) is the dominant tree species in the study area, making up 50 % of the tree species mix, along with common oak (*Quercus robur* L., 22 %) and sycamore (*Acer pseudoplatanus* L., 15 %). The ground vegetation is rich in biodiversity, but dominated by *A. ursinum* from March until June. The trap experiment was set up according to Wehnert (2014). Five trap islands consisting of six Theysohn® slot traps, which represent the six test variants, were set up with a minimal distance of 50 m to each other and a distance of about 5 m between traps within an island.

Tested semiochemicals were an *A. ursinum* extract (GSE Vertrieb GmbH) to represent the volatile mixture of the whole plant, as well as two aliphatic sulfides, diallyl disulfide (All) and dipropyl disulfide (Pro), and cis-3-hexenol (Hex), a green leaf volatile. The individual *A. ursinum* components were chosen based on GC-MS analyses of *A. ursinum* leaves carried out by Christine Rachow, a bioProtect project partner at Georg-August-Universität Göttingen, in 2018. For comparison, an ethanol variant was added, which is the standard attractant used to catch *H. fraxini*. Except for the extract, which was applied in 5-ml welted glasses (Carl Roth®), glass capillaries (Hirschmann® ringcaps 100/200 µl) were used as dispensers for the semiochemicals. An ethanol capillary was added to the diallyl disulfide, dipropyl disulfide and cis-3-hexenol variants to be able to prove any potential repellent effect of the substances in the traps. The extract was part of two variants: one with an ethanol capillary (BärEth) and one without (Bär). However, the extract itself contains 20 % of ethanol. Since similar trap experiments had been carried out in the previous two years at the exact same trap island sites, which resulted in sufficient knowledge on the number of *H. fraxini* caught in unbaited traps, we did not add an unbaited variant to the 2018 experiment for capacity reasons. Overall, we tested the following six variants: Bär, BärEth, AllEth, ProEth, HexEth, Eth.

Traps were operated during the main flight period of *H. fraxini* from March 7, 2018 to May 3, 2018 and collected on a weekly basis, except for a two-week break due to a cold spell in the second half of March. In total, traps were collected six times. Statistical analyses were carried out according to the methodology described in Wehnert (2014), which includes a transformation of absolute numbers to percentages, the species-specific selection of the three collection dates with the most individuals, and trap islands plus selected collection dates used as replications, assuming that new individuals emerge every week.

Target taxa for identification included (a) all bark beetle species, (b) bark beetle antagonists (mainly identified to species or genus level) and (c) 'potential' bark beetle antagonists, which were only identified to suborder or family level and therefore can include bark beetle antagonist as well as non-antagonist species.

Results

The total catch of 6.897 individuals of target taxa included eleven bark beetle species, 18 taxa of bark beetle antagonists and five taxa of potential bark beetle antagonists. With 3.099 individuals (44.9 % of total catch) the ash bark beetle *H. fraxini* was the most abundant species, followed by the ambrosia beetle *Xyleborinus saxesenii* (Ratzeburg) with 821 individuals (11.9 % of total catch).

On average, 30.1 % of the total *H. fraxini* catch in a trap island were caught in ethanol baited traps, whereas adding cis-3-hexenol to the same amount of ethanol reduced the catch to 20 % and adding *A. ursinum* extract or dipropyl disulfide reduced the catch to about 18 % (Figure 1). Catches in traps baited with diallyl disulfide (9.3 %) as well as catches in traps baited with the extract alone (4.9 %) were significantly lower than catches in ethanol baited traps.

X. saxesenii reacted differently to the trap variants with most individuals being caught in the two trap variants including the *A. ursinum* extract (26.7 % in traps baited with the extract alone and 29.6 % in traps with extract and ethanol combined) (Figure 1). Catches in AllEth (10.8 %) and ProEth (13.9 %) traps were slightly lower than catches in ethanol baited traps (16.3 %). With an average of only 2.8 % of the total *X. saxesenii* catch in a trap island, traps baited with cis-3-hexenol and ethanol caught significantly fewer individuals than all other trap variants except for AllEth traps. Furthermore, statistical analyses show significant differences between the catches in AllEth and BärEth traps.

The group of antagonists included 190 individuals with *Vinzencellus ruficollis* (54 ind.), *Rhizophagus bipustulatus* (42 ind.) and *R. perforatus* (30 ind.) being the most abundant species. The taxa of this group were often attracted by traps baited with ethanol, but also by traps with the extract alone (*R. bipustulatus*

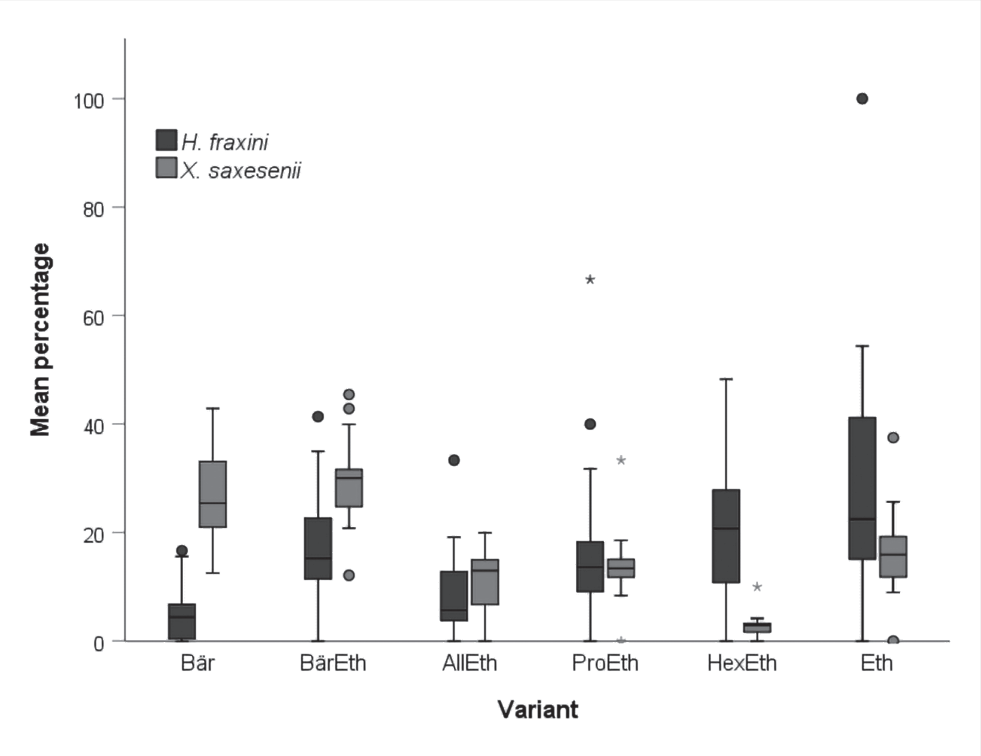

Fig 1: Mean percentage of *Hylesinus fraxini* and *Xyleborinus saxesenii* catches in slot traps baited with different *A. ursinum* based semiochemicals (Bär = *A. ursinum* extract, Eth = ethanol, All = diallyl disulfide, Pro = dipropyl disulfide, Hex = cis-3-hexenol)

and *Uleiota planata*) or traps baited with dipropyl disulfide and ethanol (*Vincenzellus ruficollis*). Despite clear visual trends statistical analyses did not result in significant differences, which is probably due to the overall small number of individuals in the taxa of this group.

The individuals of the families of Elateridae and Staphylinidae as well as the suborders of Apocrita, Brachycera and Heteroptera were defined as potential bark beetle antagonists (see materials and methods section) and made up 17.7 % (1.218 ind.) of the total catch. Data did not show any significant differences among the trap variants, probably due to the mixture of antagonist and non-antagonist species included in the taxa. An identification to genus or species level would be required to gain detailed information on the effects of *A. ursinum* based semiochemicals on the bark beetle antagonists included in these taxa.

Discussion and Conclusion

A. ursinum has a complex volatile profile with high contents of sulfur compounds (Ivanova & al. 2009), such as diallyl disulfide, the most distinct volatile of members of *Allium* spp. (Teuscher 1979) and dipropyl disulfide, which is less dominant in the volatile profile of unwounded and wounded *A. ursinum* leaves (Rachow 2018). Cis-3-hexenol is a common green leaf volatile present in the volatile profile of *A. ursinum* and shows an increased emission rate when leaves are wounded (Fall 1999, Rachow 2018). The reduction of *H. fraxini* catches by *A. ursinum* based semiochemicals as shown in this study may either be the result of a masking of host volatiles or an active repellent effect of these substances that may be a result of the insecticidal quality of *A. ursinum*. Studying the perception of *A. ursinum* based semiochemicals by *H. fraxini* in detail using electroantennography and olfactometer experiments could help answer this question. One of the distinct *A. ursinum* volatiles, diallyl disulfide, which caused a significant reduction of *H. fraxini* catches, is already used as an insecticide in agriculture (Lutomski 1980). Dipropyl disulfide, which had a noticeably lower effect, is said to be more effective in combination with other volatiles (Pierce & al. 1978). Thus, combining it with diallyl disulfide may enhance the repellent effect and simultaneously provide the chance to avoid any ethanol content, which may attract other bark beetle species. In contrast to the above mentioned semiochemicals cis-3-hexenol is not an *A. ursinum* specific volatile but a common volatile of any green leaves and therefore a typical volatile of deciduous forest habitats (Groot & al. 2008). As a volatile that is increasingly released from wounded leaves, for example by feeding of herbivores, it is also a stress marker (Ebel & al. 1995) and therefore a marker of *H. fraxini* suitable host trees.

It cannot be sufficiently explained yet why only about 16 % of *X. saxesenii* individuals were caught in traps baited with ethanol, which is a standard attractant for secondary bark beetle species (Byers 1989) and indicates suitable growth conditions for ambrosia fungi (Klimetzek 1986). It is equally surprising that *X. saxesenii* was instead attracted by both trap variants that included the *A. ursinum* extract, while catches in AllEth traps were reduced, possibly as a result of the fungicidal quality of diallyl disulfide described by Fall & al. (1999). Perhaps the whole *A. ursinum* volatile pattern in combination with ethanol indicates an ideal habitat with stressed deciduous host trees. However, we would have expected that the combination of cis-3-hexenol and ethanol indicates equally optimal conditions, when instead the results suggest a strong repellent effect on *X. saxesenii*.

In conclusion, *A. ursinum* based semiochemicals seem to have the potential to be included as an insecticide-free option in the management of certain bark beetle species, for example as a component of push-pull strategies as described by Cook & al. (2007). However, further research is needed on the effects of different emission rates, the persistency of the substances, the combination of individual semiochemicals and the effects on other bark beetle and forest pest species. Following up on this study, we are currently carrying out an experiment in stands of Norway spruce using the same trap variants.

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