Original research

Natural and insecticide-free methods for protecting wood piles from bark beetles - using the example of the ash bark beetle (*Hylesinus fraxini* [Panzer, 1779]) on Common Ash (*Fraxinus excelsior* [L.])

Robert Friedrich[1*], Michael Wehnert-Kohlenbrenner[1], Marco Schneider[1]

Abstract
This paper is investigating the insecticide-free protection of ash wood piles through the use of semiochemical dispensers with allochthonous kairomones to control ash bark beetle populations. For this purpose, borehole counts and brood gallery analyses were carried out on log piles of ash wood stored in the forest. Studies have shown that *Hylesinus fraxini* is repelled by the attractant dispenser with the substance mixture Sexowit®, which was actually developed to catch *Ips sexdentatus*. In addition, the substance mixture has an aggregating effect on various bark beetle antagonists. This causality should be used to find a form of application to protect freshly stacked ash wood from *H. fraxini* infestation. A recommended form of application should be transferable to other bark beetle- and tree species and substances. In adaptation to the so-called Island Method of bark beetle traps, the wood piles of the five test variants were also arranged like islands. Commercially available Sexowit® pheromone dispensers were used, which were attached to the piles. Besides a control without Sexowit® dispenser there were test treatments with one, two, four and nine Sexowit® dispensers. Within the scope of the investigation, effects between the tested variants could be determined and a recommended application method derived. Compared to control most effective protection of ash log piles was reached with variants treated with four or nine lures of Sexowit®. In relation to control infestation was reduced by 78.1% on 4-dispenser variant Sexowit® and 88.9% on 9-dispenser variant Sexowit®.

Keywords
ash bark beetle; bark beetle; *Hylesinus fraxini*; log pile protection

[1] Ostdeutsche Gesellschaft für Forstplanung mbH, Zum Wiesengrund 8, 01723 Wilsdruff, Germany
*Corresponding author: robert.friedrich@ogf.de

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1. Introduction

Bark beetles and other bark breeding insects often occur as secondary damage events, especially after large-scale damage events such as storms, forest fires, or prolonged periods of drought. Depending on the extent of their occurrence, they can threaten all forest functions and forestry objectives in commercial forests. In the interests of clean forest management, potential bark beetle sources, such as windthrows, must be reclaimed and eliminated as quickly as possible. There is no possibility of gaining time to process the quantities of damaged wood before the wood and bark breeders attack it after major damage events such as the storms "Kyrill" (2007) or "Friederike" (2018). The use of insecticides is not permitted in forest stands, meaning directly at the site of damage. In practice, if the processed raw wood lies on the edge of the forest road and cannot be removed immediately, the use of pesticides is often chosen in order to avoid wood devaluation or a swelling effect on the surrounding stands caused by the wood. However, the use of insecticides has one disadvantage: it has an unselective effect. This means that, in addition to the actual target organisms, other non-target species are also killed by the use of pesticides. In addition pesticides are spread further along the food chain.

The main objective of the research work of the Ostdeutsche Gesellschaft für Forstplanung (OGF), the project partners TU Dresden (Institute of Silviculture and Forest Protection, Chair of Forest Protection), and Georg-August-University Göttingen (Department of Forest Zoology and Forest Protection) is to further develop existing methods for natural regulation of bark beetle populations and to test their effectiveness under practical conditions. The bioProtect research project is funded by the Forest Climate Fund.

Initial experience from the development of near-natural regulation methods was limited to the bark beetle trap or the trap-timber-model (Achtnicht, 2010; Friedrich, 2013; Hellmund, 2014; Horn, 2009; Kotte, 2004; Stade, 2008; Wehnert, 2009, 2014; Wehnert and Müller, 2012). In order to transfer these procedures into practice, the effectiveness of the near-natural regulation principles is tested on the model of test log piles. Based on the active principle of allochthonous kairomones according to Müller and Zülke (2007), a fundamental application experiment was carried out with the semiochemical Sexowit®, which is allochthonous to the ash habitat. It is known from literature that the substance, which was actually developed as an attractant for the pine bark beetle (*Ips sexdentatus*), has both a repellent effect on the ash bark beetle (*Hylesinus fraxini*) and an aggregating effect on the generalist bark beetle predator *Thanasimus formicarius* (Wehnert and Müller, 2012). *Thanasimus formicarius* is typically a predator of pine forests, but studies have shown that the beetles colonise other forest habitats with increasing age and feed on other bark beetle species, including the ash bark beetle (Warzée and Grégoire, 2003).

The aim of the work presented here was to find a suitable form of application to protect ash log piles from infestation by *H. fraxini* using commercially available Sexowit® dispensers. At the same time it was aspired to accumulate a high population of *T. formicarius* around the log pile in order to increase the pressure from a predator in addition to the repellent effects of the substance on *H. fraxini*. In a next step the transfer of results of log pile examinations to an even larger scale (e.g. forest stands) is conceivable. In ash forest stands, *H. fraxini* is a common pest that has long been classified as harmless. In the course of the spread of ash dieback, a fungal disease caused by the pathogen *Chalara fraxinea*, the importance of the beetle as a secondary forest pest has strongly increased (Pfister, 2012). Methods for protecting ash log piles from bark beetle infestation might be transferable to ash forest stands.

Since the Sexowit® dispensers are relatively price-intensive to purchase, and economic efficiency plays a decisive role in practice, special attention was paid to these factors. The derived application form should also serve as a basis for further substance experiments in other habitats with other target organisms.

2. Materials and methods

2.1 Experimental setup

In order to work as practically as possible, the test log piles were designed to create conditions inside and on the surface that are comparable to those of larger scaled log piles commonly used in forestry. For this purpose, 15 ash logs each with central diameters of about 10 cm and a length of 2 m were stacked pyramid-shaped onto a base. To avoid major machine-related damage to the logs, for example through the use of harvesters, the wood used in the examination was processed by hand. Only fresh ash wood without dead wood components was used.

One experiment consisted of five variants to be tested, ordered in a circular arrangement with a distance of 30 m to

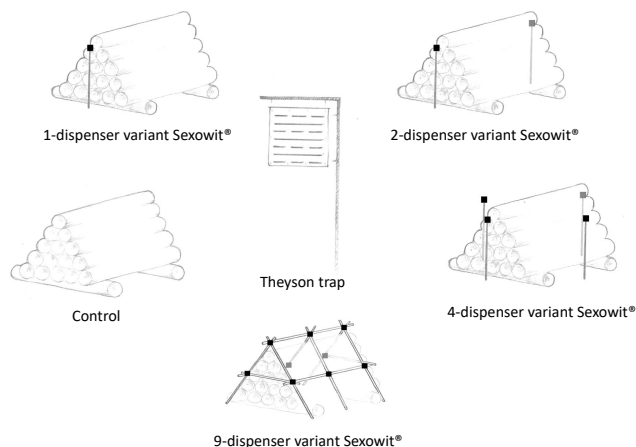each other and was referred to as a so-called log pile island (Figure 1).

Figure 1. Structure of a log pile island with the different application forms

A Theysohn® slot trap modified with ethanol, in accordance with Müller et al. (2008) was placed in the middle of a log pile island for the detection of the target species (Figure 1). This was intended to create a standard for the abundance of *H. fraxini* and to allow further conclusions on the colonisation of the log piles. Table 1 provides an overview of the semiochemicals and substance mixtures used.

In addition to the control (a log pile without a Sexowit® dispenser) as a control, four other variants were equipped with the substance Sexowit®, which differed in the number of dispensers attached. With the 1-dispenser variant Sexowit®, this was inserted into the floor at the end of a metal rod close to the log pile, so that the dispenser hung just above the upper edge of the stack. The 2-dispenser variant Sexowit® was arranged similar to the first one, but with dispensers on both front sides. The third variant was the 4-dispenser variant Sexowit® whereby, besides the two front sides, the wood stack dispensers were also attached to the left and right. In order to simulate a net-like dispenser arrangement and to ensure a gapless application of the substance, the film dispensers of the 9-dispenser variant Sexowit® were evenly distributed on a frame of bamboo rods spanning the log pile. All variants were arranged randomly within a log pile island. A total of four log pile islands were built. This means three repetitions of the actual experiment were carried out.

2.2 Test areas

Of the four log pile islands, two were set up in the Leipzig alluvial forest south of the city of Schkeuditz. In the following, these are referred to as SCHK 1 and SCHK 2. The two other log pile islands were built in the district of Saxon Switzerland-East Ore Mountains, at the dam of Gottleuba (LSH 1) on the one hand, and at the Mordgrund near the village Oelsen (LSH 2) on the other. When selecting the experimental areas,

attention was paid to the fact that they are pure stands or mixed stands of ash, which could be assumed to have a confirmed occurrence of *H. fraxini* in the year of investigation on the basis of findings of old feeding patterns. The location of the individual test sites in the Free State of Saxony is shown in Figure 2.

Table 2 gives an overview of the characteristics of the individual test areas.

The two log pile islands LSH 1 and LSH 2 were set up on 23.02.2016 and equipped with the Sexowit® dispensers on 24.03.2016. The log pile islands SCHK1 and SCHK2 were built on 03.03.2016 and also equipped on 24.03.2016. Attaching the lures before 24.03.2016 was not necessary since climatic conditions were not suitable for swarming of bark beetles. Before installing the dispensers, the logs were checked for existing boreholes (=BH) to ensure equal initial conditions.

2.3 Data collection borehole test

After activation, the colonisation activity of *H. fraxini* was examined five times within seven weeks depending on prevailing climatic conditions and expected flight activity. The last check was carried out after a period of about two weeks due to a decline in flight activity. The exact control dates and intervals are listed in Table 3.

At each examination, the colonisation intensity of *H. fraxini* on the individual stem was examined and differentiated according to the position of the trunk in the wood pile by means of counting the number of boreholes. This was carried out on eight so-called test logs of the 15 logs of each log pile. Colonisation examination was not carried out on the entire trunk but in three test windows, each 30 cm long and reaching around the whole trunk, as shown in Figure 3. The test windows were placed at the thicker end of the trunk starting as follows:

- Test window A: end face (0.0 to 0.3 m)
- Test window B: trunk centre (0.85 to 1.15 m)
- Test window C: middle of the second trunk half (1.4 to 1.7 m)

Both the positioning and the orientation of the logs in the stack was random during the first assembly, but was subsequently consistently maintained during each dismantling and reassembly of the stack. A centre diameter was measured for each test window and the lateral surface was calculated. The position of the test windows on the trunk and the boreholes detected during the examinations were permanently marked with a weatherproof marker (waxy forest chalk) to rule out the possibility of shifting the test windows on the trunk and a multiple acquisition of boreholes.

In addition to recording the boreholes of *H. fraxini*, the slit traps on the log pile island were emptied on each examination date and the catches were taken along in the conservation solution benzoic acid to determine the species. The catches were determined with the aid of a stereo microscope and suitable literature (Grüne, 1979).

Table 1. Semiochemicals and substance mixtures used

Substance	Origin of substance	Substance class	CAS	Purity level [%]	Manu-facturer	Discharge rate [Δul/24h]	Dispenser type
Ethanol	VOC	Alcohol	64-17-5	96	VWR	13.361	Reservoir
Sexowit®	*Ips sexden-tatus*	Mixture of substances	–	–	WITASEK	17.120	Film dispenser
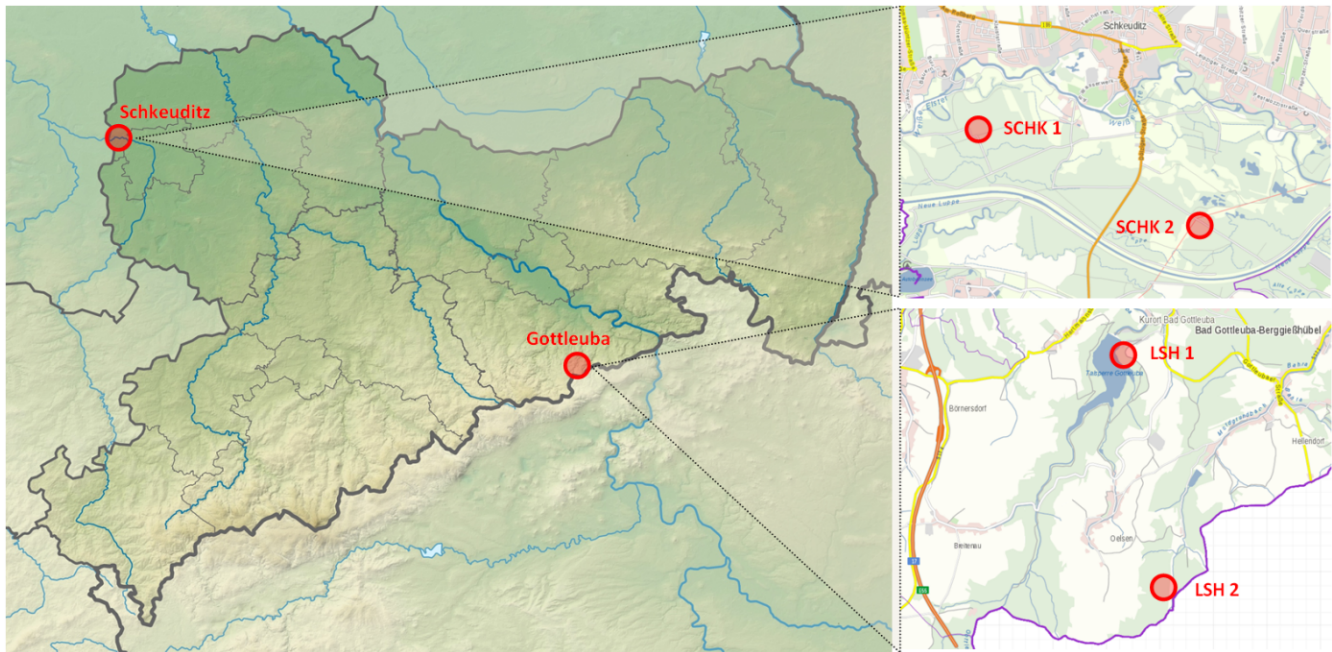
Figure 2. Location of the test areas in the Free State of Saxony (Germany)
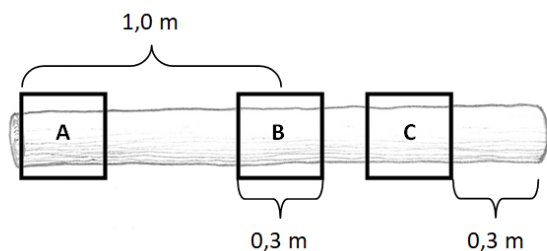
Figure 3. Position of the test windows on the trunk

The appearance of bark beetle antagonists at the log piles was recorded if there were individuals located on the stems during a borehole examination.

2.4 Data collection brood gallery analysis

After completion of the borehole test, the log piles were left unchanged until mid-August so that the eggs and larvae of *H. fraxini* could evolve and the brood gallery could be developed with egg niches, larval ducts and pupa cradles. In addition,

active antagonists also had the possibility to influence the breeding of the bark beetles under the bark. Subsequently, from 15.08. to 18.08.2016 on the areas LSH1 and LSH2 and from 19.08. to 24.08.2016 on the areas SCHK1 and SCHK2, the test windows of the test trunks were peeled.

The exposed galleries were measured according to Wehnert (2014) (Figure 4). From the quotient of the actually populated area and the area that was potentially available, settlement quotients were determined for the individual variants. By counting egg niches, larval ducts and pupa cradles, conclusions could be drawn about *H. fraxini* settlement success.

Analysis of the galleries of *H. fraxini* revealed the following parameters:

- number of brood galleries per m^2
- size of the brood pictures [cm^2]
- length of the maternal ducts MG1 and MG 2 [cm]
- length of the larval ducts [cm].

For some of the brood galleries, the number of egg niches, larval ducts and pupa cradles was recorded over a length of 1 cm of the mother duct (windows a1 and a2).

Table 2. Overview of the stands of the test areas; B°: Stand density index, CA: Common ash, SM: Sycamore maple, CB: Common beech, UC: Undercrop, FM: Field maple

Area	Coordinates (Gauss–Krüger)	Age [a]	Tree species on the area	B°	Height above sea level	Forest site mapping data
SCHK 1	51°23′7.64″ N 12°12′2.29″ E	58	CA, Valuable broadleaved trees (SM), UC: FM	0.9	115m	Utt / ÜR2
SCHK 2	51°22′37.79″ N 12°14′2.22″ E	42	CA, Valuable broadleaved trees UC: FM	0.9	120m	Utt / ÜR2
LSH 1	50°50′5.34″ N 13°56′0.04″ E	64	Horst CA, adjoining spruce and larch	0.8	465m	Mf / SM3
LSH 2	50°47′52.69″ N 13°56′45.99″ E	96	CA mixed tree by tree into CB stock	1.1	520m	Mf / M1

Table 3. Timeline and examination intervals of the ash logs

	SCHK 1		SCHK 2		LSH 1		LSH 2	
	Date	Interval	Date	Interval	Date	Interval	Date	Interval
superstructure	03.03.16		03.03.16		23.02.16		23.02.16	
activation	24.03.16		24.03.16		24.03.16		24.03.16	
1. examination	31.03.16	7	31.03.16	7	01.04.16	8	01.04.16	8
2. examination	07.04.16	7	08.04.16	8	11.04.16	10	11.4.16	10
3. examination	13.04.16	6	13.04.16	5	18.04.16	7	18.04.16	7
4. examination	20.04.16	7	20.04.16	7	25.04.16	7	25.04.16	7
5. examination	04.05.16	14	04.05.16	14	13.05.16	18	13.05.16	18
total of days		**41**		**41**		**50**		**50**

2.5 Statistical evaluation of the borehole test

For the statistical evaluation, only the sum of the recorded boreholes during the catching period from the activation of the variants to the end of the main swarming phase of *H. fraxini* was considered. This applies to the first two examination dates on all areas. Since only very few boreholes were detected during the first examination, which were almost equally distributed among the variants, it can be assumed that the experiments were not overly influenced by pheromone production of the beetles at this time. The strains were free of infestation at the beginning of the trial period.

With increased boring activity and the associated pheromone production of the ash bark beetle in the wood, the smell emitted by the trunk changes continuously. The original smell of uninhabited ash wood is increasingly overlaid by beetle-produced odours. Therefore, the individual examination dates with the boreholes recorded in the catch interval cannot be regarded as real repetitions of the experiment. The assumption formulated by Müller et al. (2017), that a new beetle population was available in each examination period and on each experimental site (log pile island), can still be assumed. Thus, the same test setups on different surfaces are real repetitions. However, not all areas were included in the statistical evaluation. On the area SCHK 2, hardly any settlement took place on the log piles. A lush, strong-smelling carpet of *Allium ursinum* (wild garlic) formed on this area during the growing season. Since other accompanied experiments led to the suspicion that the intensive smell of *Allium ursinum* had a negative influence on the substance test (or the activity of the ash bark beetles in general), the SCHK2 area was not included in the statistical evaluation to test the effectiveness of the Sexowit® dispensers. This means that there were two repetitions of the test for the statistical evaluation.

For each repetition, the boreholes drilled up to the reporting date were set in relation to the potentially colonized stem surface. The average boreholes per m^2 of the individual variants determined in this way were then transformed as a percentage (p values) of the sum of all boreholes per m^2 of the repetition (log pile island). The p-values of the repetitions are normally distributed, but variance homogeneity could not be confirmed. Therefore, a nonparametric test was performed for two independent (non-linked) samples (Mann-
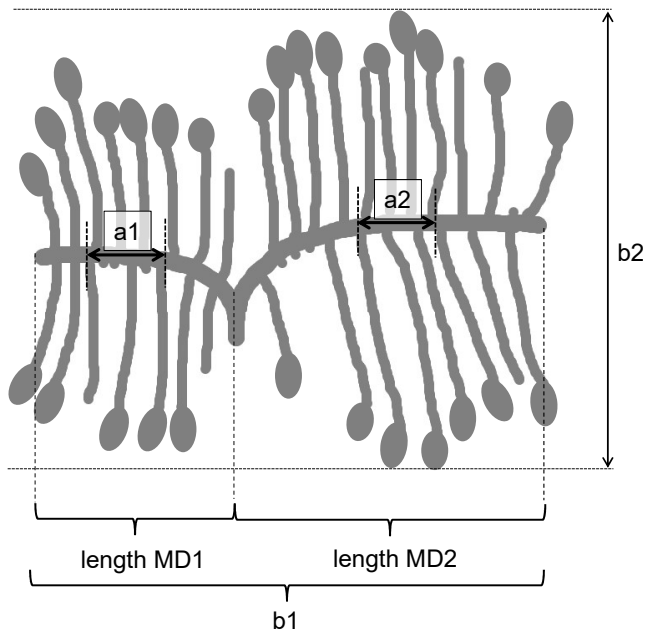
Figure 4. Recording parameters during colonization analysis for *H. fraxini*, a1/a2: 1 cm length of each of the maternal ducts, b1: added length of both maternal ducts, b2: length of the larval ducts, MD: maternal duct

Whitney-U-Test). This was done with the SPSS 15.0 program for Windows.

3. Results

3.1 Borehole test

A total of 6,066 boreholes (BH) on the areas SCHK 1, LSH 1, and LSH 2 were recorded in the test windows of the test logs during the entire investigation period from 24 March to 13 May 2016. The area SCHK 2, with only 149 recorded wells during the investigation period, is not shown in the following results. Based on observations on the log piles and the determination of specimen copies, all boreholes were assigned to the species *H. fraxini*. Since the trunks of the test piles grew naturally and were therefore not standardized, the potentially colonized stem area at each log pile was different. Therefore, the recorded boreholes were put in relation to the summed shell surface of the log piles. On average, 181 drilled holes per m^2 of the potentially populated area were recorded for all variants.

The most significant results are considered to be the results obtained from the beginning of the drilling activity until the decay of the main swarming phase (first and second examination of the log piles); this is because the influence of ash bark beetles already present in the wood on their conspecifics which have not yet drilled in is less than with advanced colonisation. As colonisation progresses, more and more beetles drill into the wood pile and produce pheromones. Thus, the experiment

is increasingly manipulated by beetle-produced odours and the effect of the Sexowit$^®$ dispensers is influenced.

During the period from activation of the tests to the end of the main swarming period, an average of 200 BH/m^2 was recorded at the control (Figure 5). On the 2-dispenser version Sexowit$^®$ the penetration rate was 231 BH/m^2, an increase of 15.5% compared to the control. The 1-dispenser variant Sexowit$^®$ with 153 BH/m^2 was 23.5% less populated than the control. For the 4- and 9-dispenser variants Sexowit$^®$, the reduction in infestation was 83.5% each, which corresponds to 33 BH/m^2.
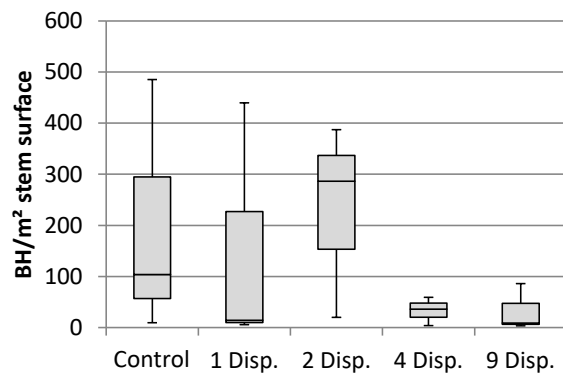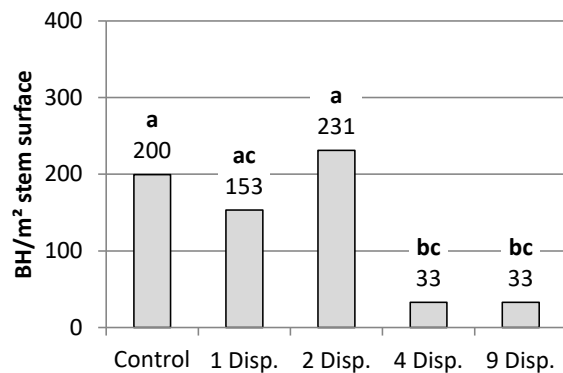

Figure 5. Main results of the borehole (BH) measurements in BH/m^2 stem surface (test areas SCHK 1, LSH 1 and LSH 2); only period from activation until end of main swamp phase (1st/2nd examination)

The highest density of *H. fraxini* boreholes over the entire study period with all five examinations was found on the control, with an average of 342 BH/m^2 (Figure 6). 18.4% fewer drill holes (279 BH/m^2) were recorded on the 2-dispenser variant. The 1-dispenser variant had an average of 170 BH/m^2, 50.3% fewer boreholes than the control. The 4-dispenser variant showed an average of 75 holes per m^2 and the 9-dispenser variant only 38 holes per m^2, a reduction of 78.1 and 88.9%
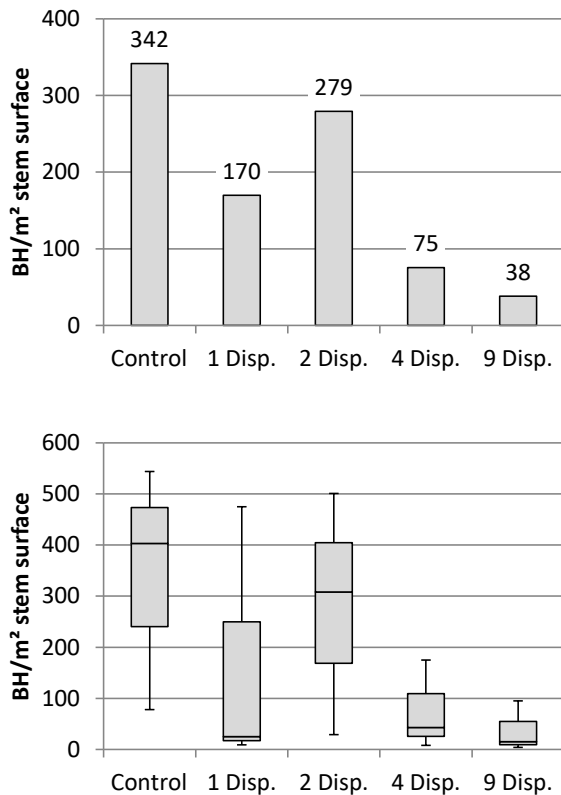
respectively compared to the control.

Figure 6. Main results of the borehole (BH) measurements in BH/m^2 stem surface (test areas SCHK 1, LSH 1 and LSH 2); total examination period (5 examinations)

Figure 7. Average borehole density (BH/m^2) compared to the quotient of dispensers per m^2 pile surface

The statistical evaluation (Table 4) of the main swarming phase of the borehole test (holes/m^2 transformed into percentages) showed that the 4- and 9-dispenser variants Sexowit$^\circledR$ differ significantly (p = $a \leq$ b 0.05) from the control and the 2-dispenser variant Sexowit$^\circledR$. The 4-dispenser variant Sexowit$^\circledR$ and the 9-dispenser variant Sexowit$^\circledR$ are not significantly different from each other.

The average population density (BH/m^2) of the variants during the main swarming phase and the dispenser density per m^2 total surface area to be colonized is shown in Figure 7. The linear formula of the compensation function (coefficient of determination 0.599) was used to determine the theoretical amount of Sexowit$^\circledR$ dispenser necessary to provide total protective effect. The results showed that theoretically 2.1 Sexowit$^\circledR$ dispensers were needed to achieve a number of 0 boreholes per m^2 of the log pile's surface. In relation to the average pile (4.90 m^2 surface), this means that theoretically 10.22 dispensers would be necessary to ensure 100 percent protection.

Looking at the distribution of the boreholes on the position of the logs in the stack, it was found that the logs in the
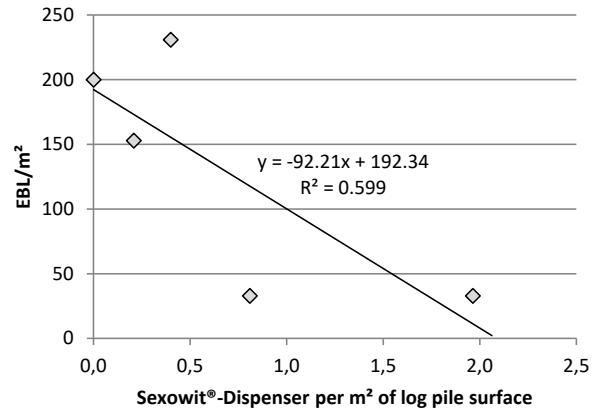
lower outer area of the stack (log numbers 11 and 15) were most heavily populated. Trunk number 13, which was also to be found in the lowest layer of the log pile, was settled somewhat less intensively than the outer trunks of the lower layer. The least settlement was recorded at trunks 1 and 4. In summary, it can be seen that two logs (11 and 15) were particularly noticeable with regard to the number of boreholes per m^2. The differences between the other test strains of the log piles were not so clear. When evaluating the position of the test windows, it was found that all three test windows were equally intensively accepted by the beetles. No preference or avoidance of a particular test window was registered.

The course of flight and breeding activity of *H. fraxini* during the investigation period is shown in Figure 8. The results described in the following refer to the sums of the boreholes at all log piles and the individuals of *H. fraxini* recorded in the slot traps. These sums are shown separately for boreholes at the stack and individuals in the slit traps as a percentage of the examination dates. It can be seen that in the period from the application of the dispensers (24.03.2016) to the first examination (31.03./01.04.2016), the lowest flight activity (0.7% total catch share) was observed within the five examination periods. In the subsequent period between the first and second examination, the majority of the total recorded ash bark beetles were caught both at the log piles and in the slot traps. During these 7 and 10 days (SCHK and LSH, respectively) the highest swarming activity could be observed with about 70.5% of all recorded *H. fraxini*. Thus, the maximum swarming activity was detected in the phase from the beginning to mid-April during the study period 2016. Flight activity was reduced in the period between the second and third examinations, as well as between the third and fourth examinations. In these periods, 5.9 and 5.5% of the total number of ash bark beetles recorded were registered. In the fifth and last examination period, flight activity increased again. 17.4% of the total data were collected during this

Table 4. Mann-Whitney-U-test (non-parametric) for two independent (non-affiliated) samples; asymptotic significance (2-sided), significance level 5%

	Control	1-Dispenser Sexowit®	2-Dispenser Sexowit®	4-Dispenser Sexowit®	9-Dispenser Sexowit®
Control		0.827	0.127	**0.050**	**0.050**
1-Dispenser	0.827		0.275	0.513	0.513
2-Dispenser	0.127	0.275		**0.050**	**0.050**
4-Dispenser	**0.050**	0.513	**0.050**		0.275
9-Dispenser	**0.050**	0.513	**0.050**	0.275	

period. However, it should be noted that the last period of 14 and 18 days was about twice as long as the four before.

A comparison of the two study sites SCHK (SCHK 1 only) and LSH (LSH 1 and 2) shows that the swarm intensity was even higher in Schkeuditz at the time of the highest swarming activity than in the East Ore Mountains (LSH). Furthermore, it is noticeable that flight activity in the East Ore Mountains increased again towards the end of the trial period (beginning to mid-May). Whether this also applied to Schkeuditz can only be conjectured, since the investigations there were finished nine days earlier.

3.2 Brood gallery analysis

The results of the brood galleries (BB) of *H. fraxini* exposed and recorded during peeling of the trunks are shown in Figure 9 as average values of the three test areas SCHK 1, LSH 1 and LSH 2. It is clear that the distribution of the brood galleries per m^2 on the individual variants (Figure 9) strongly resembles the distribution of the bore holes per m^2 over the entire examination period (Figure 6). The highest brood gallery density per m^2 (281 BB/m^2) was observed for the control, which accounted for a total share of 37.9%. The second most intensive populated variant was the 2-dispenser variant Sexowit® with 219 BB/m^2. The 1-dispenser variant Sexowit® was slightly less populated with 141 BB/m^2. The greatest successes in avoiding colonization compared to the control were achieved with the 4-dispenser variant Sexowit® (71 BB/m^2) and the 9-dispenser variant Sexowit® (28 BB/m^2) with 74.7 and 90.0% infestation reduction.

For all variants, the number of actual brood galleries was lower than the previously recorded boreholes. The biggest percentage difference occurred with the 9- and 2-dispenser variant Sexowit®. Here, 26.3% and 21.5% fewer brood galleries were recorded than boreholes. For the control and the variant with one Sexowit® dispenser, the reduction is 17.8% and 17.1%. The smallest discrepancy was observed with the 4-dispenser variant Sexowit® (5.3%).

The population ratios of all *H. fraxini* test windows averaged over all three test areas also tended to agree with the results of the brood galleries and drill holes per m^2. The only significant difference is that here not the control but the 2-dispenser variant Sexowit® is the one with which the potentially available breeding material was most intensively accepted (Figure 10). However, the difference to the control is very small. The least breeding material was accepted for the 4- and 9-dispenser variants Sexowit®. The 1-dispenser variant Sexowit® lies between the control and the 2-dispenser variant Sexowit®.

Figure 11 shows the average brood gallery size per variant in cm^2 (green illustration). This is contrasted with the average number of brood galleries per m^2 of the lateral surface (red illustration). By equipping the log piles with Sexowit® dispensers, it was possible to reduce the average density of population per m^2 of lateral surface. This effect tended to increase as more Sexowit® dispensers were used. Nevertheless, the figure shows that a decreasing population density tends to cause an enlargement of the brood galleries. However, as shown in Figure 10, the settlement percentage of the variants with 4 or 9 Sexowit® dispensers is distinctly lower than with the control. The reduction in population density is therefore not compensated by the increasing size of the brood galleries.

In order to assess the success of the development of the brood of *H. fraxini* from egg to larva to pupation, these stages of development were documented as described in chapter 2. From this, the effectiveness of the natural antagonists of the ash bark beetle or the influence of intraspecies competition in the breeding galleries can also be derived.

On average, 9.77 egg niches (=100%) were applied per cm of mother gallery length over all variants. Approximately 8.33 larvae hatched (85.3%), of which 6.10 pupated (62.4%) (Figure 12). Looking at the individual variants separately, it can be seen that the lowest development successes were achieved with the control and the 1-dispenser variant Sexowit®. Most of the niches were created in the 2-dispenser variant Sexowit®, but fewer larvae hatched and fewer pupa cradles created than in the 9-dispenser variant Sexowit®. The highest development successes of the ash bark beetles were recorded for the 4- and 9-dispenser variants Sexowit®. In the 4-dispenser version Sexowit®, 65.5% of the niches also developed into a chrysalis cradle. The figure for the 9-dispenser variant Sexowit® was 66.2%.
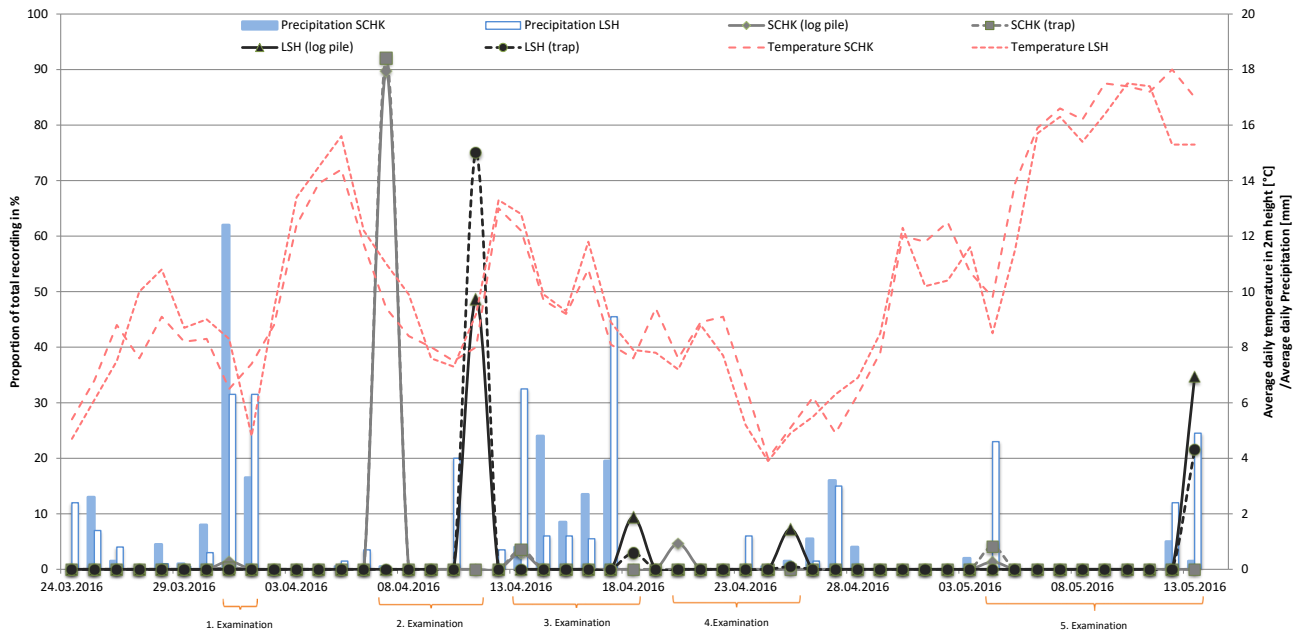
Figure 8. Percentage share of individual examination dates per area in the total survey; recorded boreholes and individuals caught in the slit traps

3.3 Observations of natural antagonists of H. frax-ini

Figure 13 shows an overview of the natural antagonists of *H. fraxini* perceived as visual observations on the bark surface. Since the detection of the antagonists was not methodical, the explanations in this respect refer to random observations. The largest part of the antagonists is the European red-bellied clerid (*Thanasimus formicarius*). Of a total of 37 recorded antagonists, 21 were *T. formicarius*. In addition, 13 beetles of the genus *Rhizophagus* and 3 individuals of *Vincenzellus ruficollis* were recorded. With 2 specimens, the fewest antagonists were registered at the control. In contrast, 10 recorded bark beetle predators, 6 of them *T. formicarius*, were detected at the 4-dispenser variant Sexowit®.

4. Discussion

Concerning the number of boreholes per m², as well as the number of brood galleries per m², the controls were the ones with the most intensive population of the ash bark beetle over the entire experimental period. Each variant treated with Sexowit® was less well accepted. With 88.9% and 78.1% compared to the control, the best infestation reductions were achieved with the 9- and 4-dispenser variant Sexowit®. When considering the period from activation of the variants to the end of the main swarming phase (i.e. the initial phase with a minor influence of superimposed, beetle-produced odours), the reduction in infestation for the 4- and 9-dispenser variants Sexowit® is 83.5% compared to the control and can be proven to be significant. This efficiency corresponds in magnitude to the results achieved by Wehnert (2014) on catch log

piles. The latter found a reduction of 80.2% in the colonisation activity of catch log piles compared to the control. The efficiencies of protection mechanisms based on spray insecticide use are 97% (Fastac® Forst liquid) or 100% when using an insecticide-treated net (Storanet®, BASF manufacturer's data on application efficiency). To Hellmund (2014); Müller and Hellmund (2009); Müller et al. (2008); Wehnert (2009) or Wehnert (2014), a higher Sexowit® concentration seems analogous to lead to an increased incidence of *T. formicarius* but also of antagonists in general, which the visual observations tended to show. The reduction of the infestation at the log piles equipped with Sexowit® can probably be attributed to two effects. On the one hand, it seems to be due to a repellent effect on the ash bark beetle of the standard dispenser Sexowit®, originally developed to catch *Ips sexdentatus*, as described by Wehnert (2014). On the other hand, Sexowit® leads to the aggregation of predators of the ash bark beetle (e.g. *T. formicarius*). The statement formulated by Wehnert (2014), regarding the mode of action of Sexowit® as an allochthonous kairomone in the ash habitat, could thus also be confirmed on the ash wood piles.

In further investigations it should be clarified to what extent the spatial arrangement of the 4 dispensers on the object to be protected has a positive or negative influence on the prevention of infestation, and whether the achieved efficiencies can be increased even further. In particular, it has to be checked whether the increased discharge rate of the four dispensers or the selected spatial distribution have a greater influence. If the increased concentration of the active ingredient is more influential than the spatial arrangement, the use of a dispenser
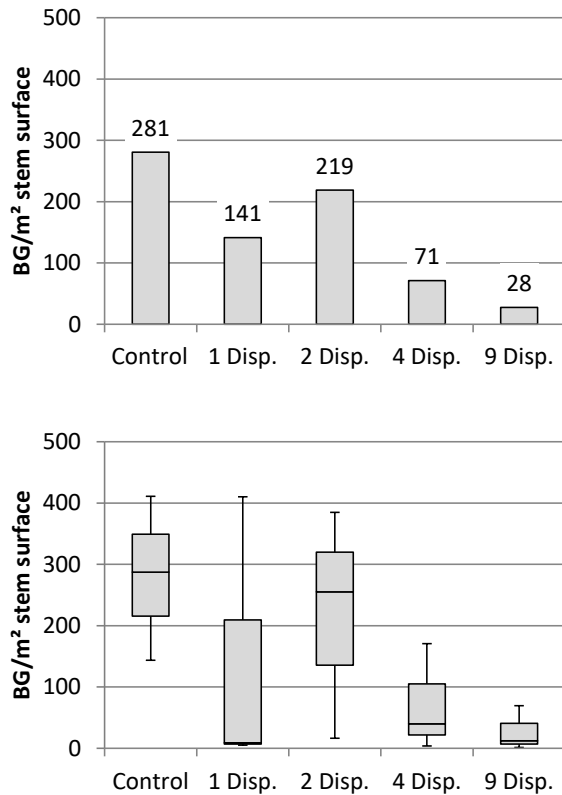
Figure 9. Average number of brood galleries (BG) per m² stem surface; complete experimental period (test areas SCHK 1, LSH 1 and LSH 2)

Figure 10. Quotient of actually populated area and potentially populated area per variant over all test areas (test areas SCHK 1, LSH 1 and LSH 2)

with a higher emission rate is recommended.

It could not be conclusively clarified why the use of one dispenser Sexowit® seemed to lead to a reduction in infestation while two dispensers Sexowit® did not. This outstanding effect could only be observed on test area LSH 1. Here the 1-dispenser variant Sexowit® was the least populated variant in this log pile island. At the same time the 2-dispenser variant Sexowit® was the most intensively populated log pile in the whole examination including all test areas. The reasons for this distribution of infestation cannot be safely identified. It would be conceivable that special trees used for the set-up, or small-scale conditions on the experimental area could be named as reasons.

The distribution of infestation at the examination log pile showed increased penetration activity in the trunks in the lower outer pile area. However, this probably has less to do with the position of the trunks in the log piles and more with their texture. For reasons of stability, the heavy lower parts of the trunk base were often placed in the log positions mentioned above. These were mostly characterized by a rough, strongly structured bark surface, which *H. fraxini* seems to infest preferentially. Pfister (2012) also describes
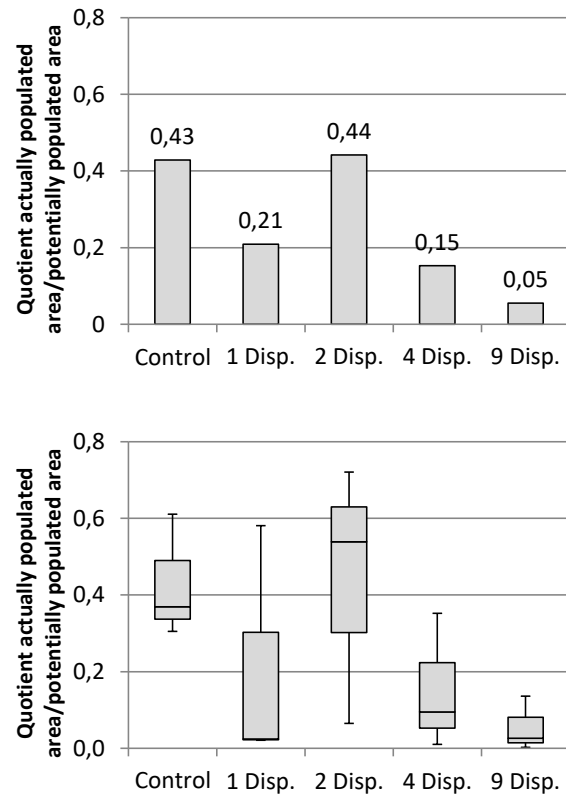
such a preference of the beetles for branch collars and bark excrescences over the smooth bark. Contrary to (Nakládal and Turčáni, 2008), the mostly weaker, thin-barked sections from the uppermost trunk area were less intensively populated. The position of the test window on the trunk had no discernible influence on the colonisation activity of ash bark beetles. All three test windows were accepted with almost the same intensity. Any drying effects at the front of the trunks therefore do not seem to have any influence on breeding activity. The orientation of the holes on the trunk was not recorded during the investigations.

The first two weeks of April were identified as the main swarming phase. The classification formulated by Pedrosa-Macedo (1979) as early swarmers with a swarming period between March and May can thus be confirmed and concretized for the year of investigation, whereby the minimum temperature of 16 °C formulated for the beetle flight appears excessive. It is difficult to explain the increase of the recorded boreholes and of the individuals of *H. fraxini* caught in the slot traps on the test areas LSH 1 and LSH2 in the period from the beginning to mid-May. According to Pedrosa-Macedo (1979), only one generation occurs per year, whereby the developed beetles fly out of the brood galleries from July to start
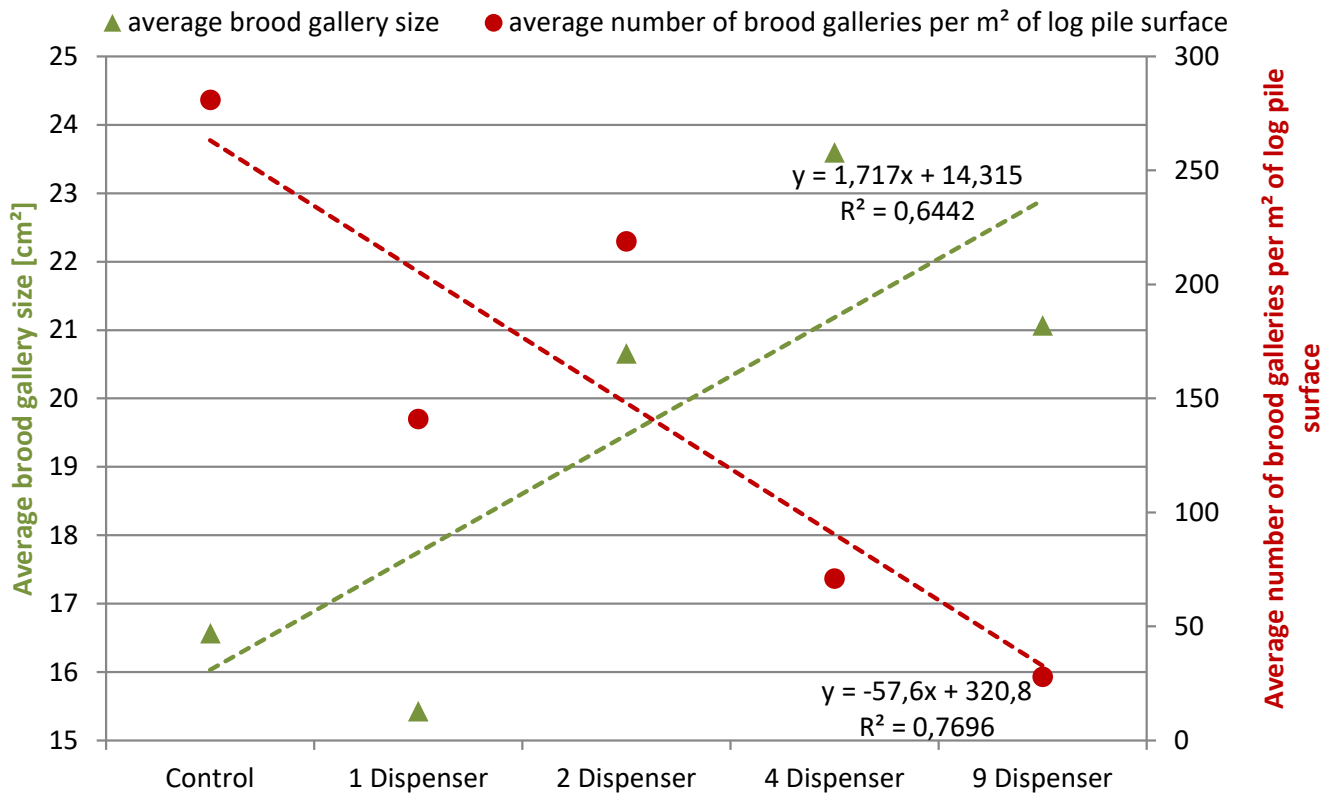
Figure 11. Comparison of the average brood gallery size and the average number of galleries per m^2 of the available lateral area

their maturation feeding. This may still be the main swarming phase with a renewed increase after unfavourable flight conditions. Especially in the low mountain ranges (LSH 1 and LSH 2), night frosts can increasingly occur in early spring, which may additionally slow down the activity of the beetles.

The results of the brood gallery analysis are in line with the results of the borehole counts. Between 0.1 and 26.3% less brood galleries per m^2 were recorded per variant than boreholes per m^2. This means that not every borehole that has been started has led to breeding success. Possible reasons for this effect could be aborted drilling attempts, suboptimal breeding conditions, too high breeding space competition, or the influence of predators. According to (Nierhaus-Wunderwald, 1996), some ground beetle species outside the hatcheries are active bark beetle predators in addition to the *Thanasimus* species. Birds are also believed to be able to regulate bark beetle populations outside their breeding systems.

The distribution of the population percentages of the test windows among the variants also tends to correspond to the results of the borehole counts and the number of recorded brood galleries.

Comparing the average brood gallery size and the average number of brood galleries per m^2 it can be generalized that the trend is recognizable that with increasing dispenser density of Sexowit® the number of brood galleries decreases while the area of the individual gallery increases. This proportionality

is reversed.

In contrast to Wehnert (2014), it could be documented that the success of the development from the created niche to the pupated caterpillar increases with a higher density of the Sexowit® dispensers. This is presumably due to the fact that the higher the number of dispensers, the lower the competition for breeding space. The brood galleries created by the adult beetles are larger on average, but the egg niches are still created at a close distance. The hatched larvae can create longer feeding passages without reaching the border to the next brood gallery. A suspected stronger hunting of the brood of *H. fraxini* at the variants with high dispenser density by *Thanasimus* larvae does not seem to have taken place or could not be documented.

In conclusion, it can be stated that it is currently possible to achieve a reduction in infestation of approximately 70 - 85% with justifiable effort by means of near-natural procedures in ash. In practice, however, the devaluation of forest storing ash wood by the ash bark beetle is meaningless, since the insect only eats superficially on the outermost layer of the sapwood. For the marketing of the trunks, only insect feeding that reaches deeper into the wood is relevant. The protection of forest stands could be more important than the protection of wood poles. The ash bark beetle acts as a vector for the fungus responsible for ash dieback. It should be investigated if the protective effect achieved at the log pile can also be
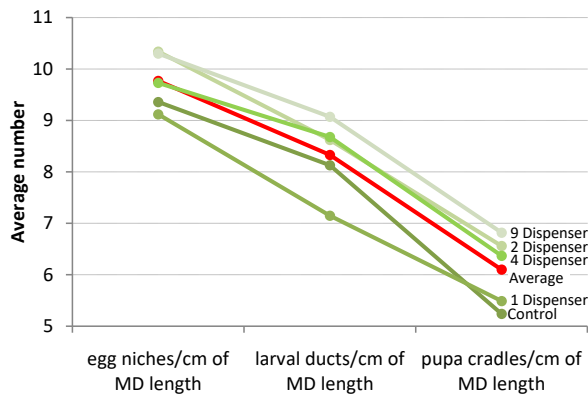
Figure 12. Developmental successes of the brood of *H. fraxini* from the egg niche to the pupa cradle, MD: maternal duct
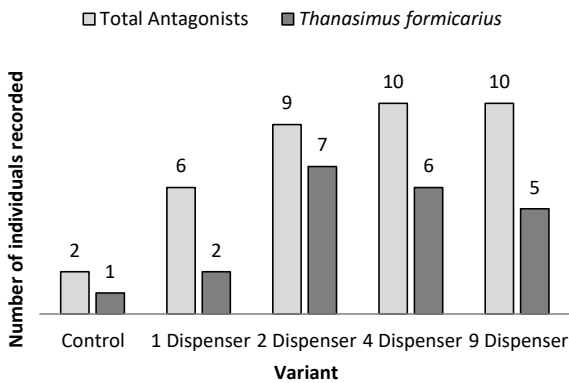

Figure 13. Overview of the natural opponents of *H. fraxini* recorded at the log piles

produced in forest stands. Here the most complete possible prevention of infestation should be achieved, as very few bark beetles are sufficient to transmit the fungus.

5. Conclusions

It could be significantly proven that ash log piles treated with four or nine Sexowit® dispensers are less intensively populated by *H. fraxini* than untreated variants. The results of the experiments confirmed that the near-natural regulatory principles can be practicable to achieve a reduction in infestation of approximately 75-90% on log piles at reasonable expense. For reasons of economy, however, it is not recommended to equip the small quantities of wood described at the beginning with 9 dispensers. The price for one dispenser of Sexowit® used in the experiment was 12,75 €. This is to be considered cost intensive regarding the small amount of wood that is

being protected from it. According to linear regression, 11 Sexowit® dispensers would theoretically be necessary to provide 100% protection to a log pile with a total surface of 4.9 m^2. In addition, the reduction in infestation of the 4-dispenser variant Sexowit® during the main swarming phase was identical to that of the 9-dispenser variant Sexowit®. The reduction in infestation of both variants was significant at the same level compared to the control ($p = 0.05$) and they did not differ significantly from each other. The version with 4 dispensers Sexowit® seems to be sufficient to protect ash wood piles in a practical way.

The transfer of the methodology used here to other tree species, insect pests, and semiochemicals in the right combination should also be considered in the future. The devaluation of ash timber as well as deciduous timber in general has not been regarded as problematic so far.

Analyses of the individual strains of the examination log piles have shown that *H. fraxini* prefers structural trunk areas such as the trunk base or branch collars. Furthermore, the position of the trunk in the log pile and thus also the climatic conditions inside the pile seems to play a subordinate role. There was no preference for certain areas of the individual trunk in the log pile.

H. fraxini is an early swarming bark beetle species which, depending on the weather, occurs early in the year and can then very quickly reach high population densities. Measures to protect ash wood from colonisation with *H. fraxini* must therefore be taken early in spring. In addition, the climatic conditions that influence the beetles' flight should be carefully observed.

The results of borehole count and brood gallery analysis tend to agree well. Therefore, this procedure also appears suitable for further investigations. The oversight error in borehole counting is within a tolerably low range. Analysis of population percentages of individual variants shows that the more potentially populated an available area is, the larger the brood galleries will be. Especially for the 4- and 9-dispenser variants Sexowit®, the percentage of population was higher than the number of boreholes or brood galleries per m^2.

Also, the account of the average brood gallery sizes of the individual variants showed an increased area expansion with a lower population density. The documentation of the development of the individual stages in the brood galleries does show that an increased density of Sexowit® dispensers has increased the brood success within a brood gallery. The expected result of reduced breeding success with a high dispenser density due to the action of *Thanasimus* species did not occur.

Nevertheless, it was observed that an increase in Sexowit® concentration also led to increased sightings of potential antagonists.

Acknowledgments

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