

## **BIOLOGICAL CONTROL OF WOOD DESTROYING BEETLES WITH *SPATHIUS EXARATOR* (HYMENOPTERA: BRACONIDAE)**

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**Abstract** For nearly a decade, the wood pest *Anobium punctatum* has been effectively controlled in historic monuments with its natural antagonist *Spathius exarator*. This braconid wasp parasitizes its host species by piercing its ovipositor directly through the wood surface followed by oviposition onto the beetle larva. After development, it hatches as an adult wasp through a hole it gnaws itself. This tiny exit hole, 0.5 mm wide, can be easily distinguished from the 2 mm wide exit hole of *A. punctatum*, allowing us to monitor parasitism success. Between 2012 and 2021, these parasitic wasps were successfully released into more than 200 different *A. punctatum* infested objects across Europe. Between 400 and 2000 wasps were released per treatment, depending on severity of infestation and quantity of infested objects within the building. A monitoring program was established in parallel, and parasitism rates were calculated as the proportion of parasitized *A. punctatum*. We started in 2012 with a release protocol that included 5-8 individual treatments per year. In subsequent years, the protocol was slightly modified and reduced to up to 4 treatments per year to allow for a longer treatment period. Here we present parasitism rates of *A. punctatum* infested objects (n=42), treated and monitored up to three years in relation to the number of treatments per year. Parasitism rates increased significantly after one year of treatment (Wilcoxon rank-sum-test, p=0.0008; n=42) and continued to increase in the second and third year. Corresponding annual parasitism rates, based solely on new exit holes of *A. punctatum* and *S. exarator* per year, ranged from 0.66 to 0.74. There were no significant differences in the parasitism rates between the group of churches with 5-8 and 1-4 annual treatments during all three years of treatment. Long-term monitoring data, as shown for church Pa. indicate the need to continue biological control with one or two individual releases per year to maintain infestations at the low levels achieved. These data prove this biological method of pest control as an efficient, sustainable and non-toxic long-term option to manage the common furniture beetle.

**Key words** *Anobium punctatum*, wood preservation, parasitic wasps, antagonist, historic monuments

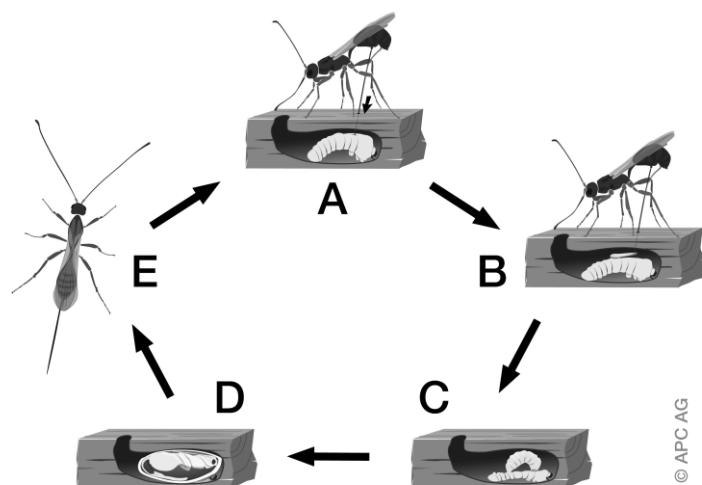
### **INTRODUCTION**

The common furniture beetle, *A. punctatum* De Geer (Coleoptera, Anobiidae), is one of the most frequent and destructive anobiid beetle (Child and Pinniger, 2014) and now, although originating from Europe, spread worldwide (Pinniger and Child, 1996). *A. punctatum* can infest the sapwood of almost all conifer and deciduous wood species as well as several tropical wood species (Paul et al., 2008) and thus, cause huge damage by degrading wooden attics, monuments and antique inventories as well as building fabric in historical buildings. Furniture beetles might decompose the wood up to a third of the original structure, as they are highly philopatric and lay their eggs in or onto the same objects for many generations (Becker, 1942). Adult beetles hatch between May and September and might live 20 to 30 days for mating (Child and Pinniger, 2014). Female beetles lay up to 50 eggs in rough wood, cracks and crevices or old *A. punctatum* exit holes (Child and Pinniger, 2014; Cymorek, 1975). After hatching, larvae feed within the wood for two to five years (Pinniger and Child, 1996). Right before pupation, adult larvae feed towards the surface, and pupate within an enlarged pupal chamber for a period of two to three weeks. Adult beetles gnaw through the remaining wood and eclose through a characteristic 1–2 mm wide exit hole (Pinniger and Child, 1996).

In the laboratory of the pest control company APC AG, anobiid infested wood was examined for hatching insects for a time span of four years. Although several insect species were hatching, the braconid wasp *S. exarator* was by far the most frequent antagonist of *A. punctatum* with 3530 hatched individuals (own unpublished data). That confirms the examination by Becker (1954) who classified *S. exarator* as the most important and most efficient antagonist of *A. punctatum*.

*S. exarator* is a hymenopteran wasp with a body size of up to 9 mm. After locating the host larvae within the wood, the female wasps pierce the wood with their ovipositor and lay one single egg on the woodworm larva. After

hatching, the parasitoid larva feed on their host, thereby killing it. Afterwards, the parasitoid larva pupates, and 28 to 30 days after oviposition an adult *S. exarator* ecloses through its own 0.5 mm wide eclosion hole (Figure 1; see also APC AG et al., 2017). Thus, a newly hatched *S. exarator* wasp corresponds to one killed *A. punctatum* beetle. As eclosion holes of *S. exarator* and *A. punctatum* easily can be distinguished by their size and shape, we are able to estimate the relative frequency of parasitized beetles by counting the wasp and beetle eclosion holes and calculating their proportion (see also Lyngnes, 1956). Thus, the aim of our work was to establish a biological method to control the common furniture beetle and to examine biology and population dynamics of *S. exarator* within infested buildings.



**Figure 1** Life cycle of *S. exarator*: the braconid wasp localizes its host within the wood, paralyzes it (A), followed by oviposition onto the *A. punctatum* larva (B). The wasp larva feeds from the beetle larva (C). The anobiid larva dies and *S. exarator* larva pupates (D) and hatches about 28–30 days after oviposition (E).

## MATERIAL AND METHODS

### Treatment and monitoring procedure

From summer 2012 until fall 2020 infested objects in Germany and Switzerland (n=42; 40 churches, 1 museum, 1 historical building) were treated at a room temperature of  $>15^{\circ}\text{C}$ . Generally, a minimum of twelve treatments over a period of three years was conducted for each object. Depending on the remaining infestation, single annual treatments followed. Per treatment, wasps were released directly in infested areas indicated by beetle exit holes, the presence of frass or the appearance of *A. punctatum* beetles. The number of released wasps per object varied from four to thirteen treatment units, depending on the number of infested areas and intensity of infestation. Each “treatment unit” contained 100 *S. exarator* wasps with a proportion of 80% females to 20% males to ensure mating.

The objects were continuously monitored to evaluate the success of treatment. Three to five active areas with easy-to-count *A. punctatum* exit holes were selected. Each area was about  $0.1\text{--}0.3\text{ m}^2$  in size and had at least 30 to max. 150 existing *A. punctatum* exit holes. In these areas, exit holes of *A. punctatum* and *S. exarator* were counted separately before the first treatment started. Holes  $> 0.8\text{ mm}$  were classified as *A. punctatum*, and holes  $< 0.8\text{ mm}$  as *S. exarator* exit holes. Beetle and wasp exit holes of the monitored areas were summed up separately and the “basic” parasitism rate was calculated as the relative frequency of parasitized *A. punctatum* (Lyngnes, 1956). At the end of each full hatching period of *A. punctatum*, the total number of exit holes of both species was recounted and the “treatment” parasitism rate after x treatment years was calculated.

Unlike the basic and treatment parasitism rates, which include the number of exit holes resulting from beetle and wasp activity during several decades or centuries, the “annual” parasitism rate was calculated by counting only one years’ new exit holes. The number of new holes per year results from the difference between the total number of holes of the current and the previous year, thus representing the annual relative frequency of parasitized *A. punctatum*. For more details see Auer et al. (2021).

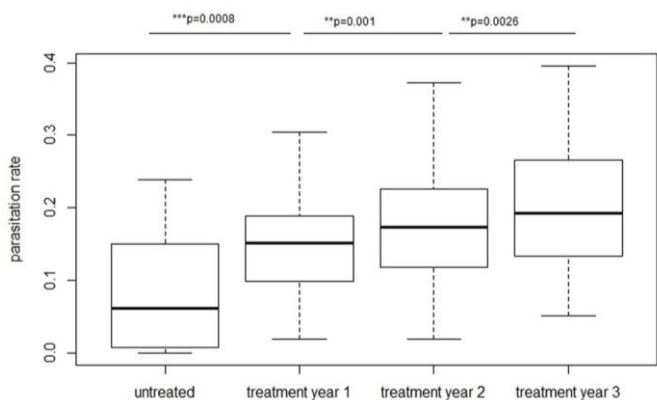
### Data analysis

Statistical analyses and figures were conducted using R (R Core Team, 2019) and Excel. Mean values of parasitism rates were evaluated whether they meet the assumptions of normality and homogeneity of variance. Differences between mean parasitism rates were determined using the Wilcoxon rank-sum-test.

## RESULTS

### Parasitism rates after a three-year treatment period

Parasitism rates in 42 *A. punctatum* infested buildings significantly increased after one year of treatment with *S. exarator* (Wilcoxon rank-sum-test,  $p=0.0008$ ;  $n=42$ ; Figure 2). Likewise, the increase between the first and the second (Wilcoxon rank-sum-test,  $p=0.001$ ) and the second and third year of treatment was significant (Wilcoxon rank-sum-test,  $p=0.0026$ ). “Basic” parasitism rates before treatment varied from 0 to 0.24 ( $0.08 \pm 0.07$ ; mean  $\pm$  SD), while “treatment” parasitism rates after one year ranged from 0.02 to 0.31 ( $0.14 \pm 0.07$ ; mean  $\pm$  SD), after two years from 0.02 to 0.37 ( $0.17 \pm 0.08$ ; mean  $\pm$  SD) and after three years from 0.05 to 0.40 ( $0.20 \pm 0.08$ ; mean  $\pm$  SD). The corresponding “annual” parasitism rates of all infested objects, including only new exit holes of *A. punctatum* and *S. exarator* per year, steadily increased over the treatment period from  $0.66 \pm 0.27$  up to  $0.74 \pm 0.26$  (mean  $\pm$  SD;  $n=42$ ) (see Table 1; column 1-8 treatments).



**Figure 2** Parasitism rates, based on monitoring data of *A. punctatum* infested buildings ( $n=42$ ; 40 churches, 1 museum, 1 historical building), found before first treatment (“basic” parasitism rate; untreated), after one year of treatment (“treatment” parasitism rate; number of treatments:  $4.74 \pm 1.40$ ; mean  $\pm$  SD;  $n=42$ ), after two years of treatment (number of treatments:  $4.19 \pm 1.58$ ; mean  $\pm$  SD;  $n=42$ ) and after three years of treatment (number of treatments:  $3.05 \pm 1.70$ ; mean  $\pm$  SD;  $n=42$ ). Asterisks indicates significant differences among the parasitism rates ( $*p \leq 0.05$ ;  $**p \leq 0.01$ ;  $***p \leq 0.001$ ; Wilcoxon rank-sum-test). Central rectangle: first quartile to third quartile of the values. Line inside rectangle: median ( $n=42$ ); whiskers above and below the rectangle: minimum and maximum of the values. Data were collected between 2012 and 2020.

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### Impact of the number of annual treatments on parasitism rates

To determine if there is a difference in parasitism efficiency between the number of annual treatments, the objects were divided into groups with 1-4 treatments and 5-8 treatments per year (Table 1). In the objects with 1-4 treatments per year, the mean annual parasitism rate increased steadily from the first to the third year of treatment. Contrary, in the objects that were treated 5-8 times per year, the highest parasitism rate could be achieved after the first year of treatment and remained at a similar level until the third year of treatment. Although in the group with 1-4 treatments, the mean annual parasitism rate in the first year was slightly lower compared to that with 5-8 treatments, this difference was not significant (Wilcoxon rank-sum-test,  $p=0.249$ ;  $n=21$ ).

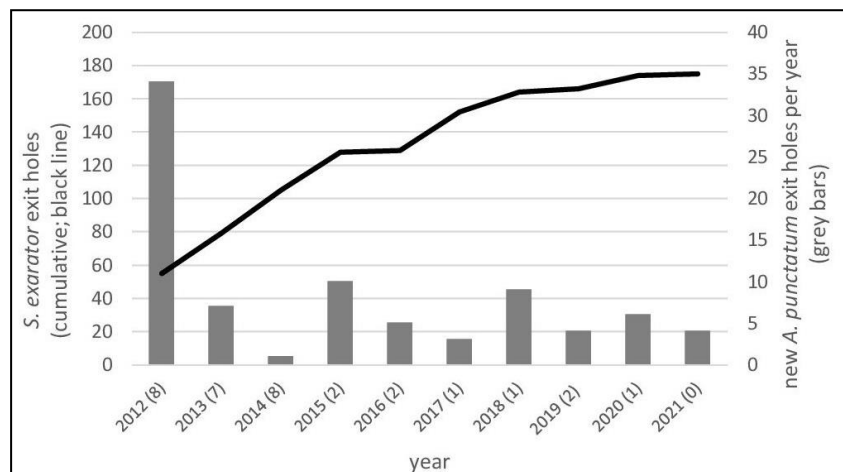
Treatment year	1-4 Treatments	5-8 Treatments	1-8 Treatments
1	$0.61 \pm 0.28$ ( $n=21$ )	$0.70 \pm 0.26$ ( $n=21$ )	$0.66 \pm 0.27$ ( $n=42$ )
2	$0.69 \pm 0.25$ ( $n=28$ )	$0.69 \pm 0.24$ ( $n=14$ )	$0.69 \pm 0.25$ ( $n=42$ )
3	$0.75 \pm 0.26$ ( $n=36$ )	$0.67 \pm 0.29$ ( $n=6$ )	$0.74 \pm 0.26$ ( $n=42$ )

**Table 1** Annual parasitism rates of objects ( $n=42$ ; 40 churches, 1 museum, 1 historical building) treated and monitored over a period of three years. The objects were divided into two groups in terms of the number of treatments: 1-4 treatments and 5-8 treatments per year.

73

### Long-term treatment of church P.

As an example for population dynamics of *A. punctatum* and *S. exarator* over a treatment period of ten years, Figure 3 shows the number of newly hatched beetles per year and the cumulative number of hatched wasps, as indicated by the number of their eclosion holes. After ten years of treatment with different number of releases per year, a cumulative amount of 175 *S. exarator* exit holes was counted, representing 175 killed *A. punctatum* larvae on the monitored area. According to the increase in parasitation, the maximum reduction in furniture beetle hatching was observed after three years of treatment with 97% and has remained at this level to date.



**Figure 3** Cumulative number of *S. exarator* eclosion holes per year (black) and number of new *A. punctatum* eclosion holes per year (grey) in the pewage of church “Pa.” after each treatment year (2012-2021). Number within brackets indicate number of annual treatments.

## DISCUSSION

In the historical buildings from this study, an average of 8% of anobiid beetle larvae was parasitized and killed by *S. exarator* before treatments started. Our method for evaluating this natural parasitism rate is based on the ratio of *S. exarator* and *A. punctatum* eclosion holes and consequently includes all eclosion holes that have been originated from beetle and parasitoid activity since the construction of the respective buildings. This natural parasitism rate of *S. exarator* is similar to the natural parasitism rate of *Habrobracon hebetor* on the millet head miner *Heliocheilus albipunctella*, which was between 8 -12% (Amadou et al., 2019).

The low natural parasitism rates on anobiid beetle larvae within historical buildings indicate the need for augmentative biological control to reduce pest population rapidly. Indeed, repeated releases of *S. exarator* in infested buildings led to a remarkable increase of the parasitism rate after three years. As shown in table 1, the anobiid beetle activity could be reduced by up to 74% after three years of treatment. Interestingly, the objects treated 1-4 times and those treated 5-8 times per year showed no significant difference in the resulting parasitism rates after three years of treatment. These data demonstrated a quick and efficient colonization of wasps in infested objects, regardless of the frequency of treatments per year. However, the slight but steady increase in the number of new *S. exarator* exit holes as well as new hatching beetles even after 10 years of treatment in church Pa., as shown in Figure 3, indicates that there might be still a small number of *A. punctatum* larvae inside the wood after the period of intensive treatments.

To ensure the greatest possible success, the best time to release the parasitoids must be found, especially with few treatments per year, as these must coincide with the accessibility of the susceptible host stages. Studies with the closely related parasitoid species *Spathius agrili* and *Spathius galinae* showed that all 5 larval stages of the Emerald Ash Borer (*Agrilus planipennis*) are parasitized with L4 being preferred (Belokobylskii et al., 2012; Yang et al., 2005; Yang et al., 2012). However, the prepupae and pupae were not parasitized. As *S. exarator* shows a similar parasitization preference (own observations) and due to the multi-year development period of the host larvae (Pinniger and Child, 1996), multiple annual releases provide the parasitoids with sufficient susceptible host stages to parasitize. In addition, the activity phases of the host larvae and the parasitoids must be taken into account when planning treatments. Although the key stimuli in the host search of *S. exarator* are not known, it seems very likely that *Spathius* depends on the feeding activity of host larvae to locate them by vibrations, feeding sounds, chemical cues or color, as has been demonstrated for other parasitoids (Roese et al., 1997; Steidle and Schöller, 1997; Steidle and Ruther, 2000; Turlings et al. 1990). According to that, the release of parasitoids from temperatures greater than 15°C in spring before pupation of the host-beetles and in late summer after hatching of the host-larvae from the egg might be most crucial and efficient.

Given that, it can be concluded that for the successful treatment of an *A. punctatum* infested object, it is not decisive to carry out as many releases of parasitoids as possible within the shortest time. Rather, it is important to set up a control program for the longer term to control *A. punctatum* efficiently and economically, and, in this respect, a number of 4 treatments between May and September in each of the first three years is sufficient. Additionally, if treatments were abruptly stopped after this period of intensive treatment, the pests might multiply again uncontrolled within a few years. Thus, single annual treatments should follow to maintain the low level of anobiid activity achieved.

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