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## Thermal disinfection of stored grains by solar energy

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### Abstract

Chemical control especially fumigants is the most commonly used method to control stored-grain pests. A safer alternative for disinfection is by heating up grains to a temperature of 50-60 °C. However, this alternative consumes high thermal energy due to the relatively high temperature required to achieve the required goal. Using solar energy as heat source for low temperature applications has become a viable mean for heating applications. Heating of grains using solar energy requires special design of grain storage system as well as development of efficient heat transfer mechanism to increase grain temperature over a limited period of time. The main objective of the current study is to use thermal disinfection as a non-chemical, safe control method for grain management. A heating system based on solar energy has been developed as heat generator to control stored-grain insects. The target temperature range is 50-60 °C, which is enough to kill most of stored-grain insects. The grain hopper heating system relies on hot water supplied from a solar collector. The temperature of grains can be controlled based on the amount of grains in the hopper and the amount of energy transmitted to grains inside the hopper. The effectiveness of the system will be measured by reaching the best temperature and time combination for each insect species without affecting the seeds quality. The best temperature and time combination for cowpea beetles will be discussed in more details.

## Retrospect, insights and foresights:

### Biological control of *Anobium punctatum* with *Spathius exarator*

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### Abstract

Biological control using beneficial organisms is getting more and more important in Integrated Pest Management. An effective strategy in the fight against the most common timber pest species, the furniture beetle *Anobium punctatum*, is based on the parasitoid wasp species *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 feeding on the larva and pupation, the adult wasp emerges through a tiny 0.5 mm wide wood hole, which can be clearly distinguished from the 2 mm wide hole of *A. punctatum*. This enables us to observe easily the treatment success as each new *S. exarator* exit hole is equivalent to one killed beetle larva.

Between 2012 and 2017, the braconid wasps were introduced into about 80 *A. punctatum* infested buildings. At least twelve treatments over a period of up to three years were performed. On exactly defined areas, the newly emerged exit holes of *A. punctatum* and *S. exarator* were counted and the parasitisation rate was calculated. Here we present pooled data of 29 *A. punctatum* infested churches, successfully treated and monitored over a period of one to five years. Furthermore, as a representative sample, we show the results of one church over a period of six years.

We demonstrate the biological control of the common furniture beetle with this braconid wasp as an efficient, sustainable alternative to conventional residual methods. However, after a period of up to three years intensive treatment, a continuous monitoring-program with necessary additional single treatments should follow.

**Key words:** biological control, wood pest, cultural heritage, common furniture beetle, parasitic wasps

## 1. Introduction

Many chemical products for wood preservation are currently in a review process and possibly will be restricted from the European Biocidal Product Regulation. Thus, the expansion of alternative methods for pest control will be required like physical treatments or biological control. Physical methods like heat treatment or anoxia for controlling wood boring insects are well established and have a long tradition in practical experience. Biological control using natural enemies, on the other hand, had not been applied so far, although many antagonists against common wood boring species are known (Haustein, 2010; Schmidt 1952) and some reports of laboratory research or practical experience had been published (Lygnes, 1956; Haustein, 2010). Advantages in using parasitoids for biological control are the exclusive feeding on their host individual and the pinpointing of their hosts in hidden places, even at low densities (Schöller & Prozell, 2011). Described enemies of the common furniture beetle *Anobium punctatum* are amongst others the checkered beetles (Cleridae) *Opilo domesticus* and *Korynetes caeruleus* and the parasitoids *Spathius exarator*, *Sclerodermus domesticus* (Schmidt, 1952) and *Cerocephala cornigera* (Becker, 1942). So far, laboratory breeding experiments with predators in the family Cleridae revealed less success in mass rearing, thus making them currently unsuitable candidates for biological control (Haustein, 2010). Further practical monitoring (Ott, 2005; Paul et al., 2008; Schöller et al., 2008) confessed, that the braconid wasp *S. exarator* is one of the most common natural enemies of the furniture beetle in historic buildings in Germany. With the scientific knowledge of Becker (1942) and Lygnes (1956), the innovative pest control company APC AG from Nuremberg bred *S. exarator* as a commercial biological control method against *A. punctatum* (Auer and Kassel, 2014). After successful mass rearing, first results of laboratory as well as several praxis tests were published (Kassel and Auer, 2015; Biebl and Auer, 2017).

This publication shows the practical use of the parasitoid *S. exarator*, presenting pooled data from 29 infested objects, as well as one representative church, selected from currently more than 80 *S. exarator* treated objects.

## 2. Material and Methods

### Biology of *Spathius exarator*

The hymenopteran wasp *S. exarator* has a body size of up to 9 mm, with females possessing an ovipositor about their body length. A female wasp localizes its host feeding within timber by its movements and gnawing. After drilling the ovipositor through the wood, it paralyzes the larva and lays a single flexible egg onto it. At a temperature of 20°C and humidity of 60%, the *S. exarator* larva hatches after 3 to 5 days and feeds from its host larva. After pupation, the adult wasp hatches 28 to 30 days after oviposition from the wood through a self-gnawed exit hole (diameter up to 0.5 mm), which can easily be distinguished from the exit holes of *A. punctatum* (diameter 1-2 mm).

### General treatment and monitoring procedure

Depending on intensity of infestation, at least twelve treatments over a period of up to three years at a building temperature of >15°C were performed by the company APC AG. About 100 bred *S. exarator* were assembled for each defined infested area inside the building. Usually, a total of 500-800 wasps were released per object and treatment. After a period of up to three years of intensive treatment, monitoring was continued and, if necessary, further single treatments were conducted.

On exactly defined areas, the new exit holes of wasps and furniture beetles were counted and documented after each treatment. From these data, the reduction of newly hatched *A. punctatum* beetles per year was calculated. As data of hatching beetles in the year before the treatment are lacking, a real blank value is missing. Thus, additionally the parasitisation rate using the following formula was calculated:

$$\frac{\text{no. of } S. \text{ exarator exit holes}}{(\text{no. of } S. \text{ exarator exit holes}) + (\text{no. of } A. \text{ punctatum exit holes})}$$

## Pooled Data

Presented data compare the basic parasitisation rate at the day of the first treatment with the parasitisation rate after the last monitoring of 29 buildings treated with *S. exarator* up to five years, using Mann-Whitney U-test. Furthermore, we show the data of the decline in newly appeared *A. punctatum* exit holes and the parallel mean cumulative increase of *S. exarator* exit holes in these objects. From 53 treated objects up to 2016, 24 could not be included in the analyses since monitoring modalities have changed (n=5), basic parasitisation rates could not be calculated because of missing data (n=6) or monitoring data were not collected (n=13).

## Chapel P. (Bavaria)

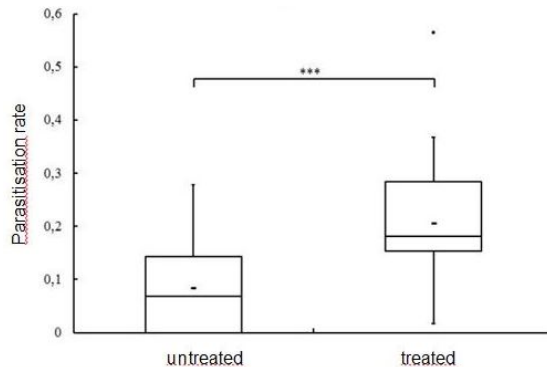
In the Chapel P. in Bavaria, eight, six and eight treatments were performed in the years 2012 to 2014. In 2015 and 2016 two treatments per year and in 2017, one treatment was done. At each treatment, about 500 braconid wasps were released at infested foot stools and the altars of the chapel.

## Statistics

Statistical analyses were conducted using the software PAST: Paleontological Statistics software package for education and data analysis (version 2.12; Hammer et al., 2001). Figures were made with Microsoft Excel or the Microsoft Excel add in SSC-Stat (Statistical service center, University of Reading, UK).

## 3. Results

Results from five years of practical application in 29 *S. exarator* treated objects showed promising results. The mean number of treatments per year were 5.8, 5.4, 3.7, 0.7 and 1.7 for treatment years one to five, respectively. Parasitisation rates in the monitored areas increased after treatment with *S. exarator*: Before the onset of applications, parasitisation rates ranged from 0 to 0.276 (0.085±0.088; mean ± standard deviation; n=29). Parasitisation rates in objects treated for one to five years were significantly higher, ranging from 0.017 to 0.565 (0.206±0.114, mean ± standard deviation; n=29; Mann-Whitney U-test, p<0,001; Fig. 1).



**Figure 1.** Mean parasitisation rates ± SD of 29 churches. Untreated: before first treatment; treated: during last monitoring.

Asterisks indicate significant differences (Mann-Whitney U-test, p≤0.001). Rhombus: outlier.

In table 1, an overview is given on the number of treated objects, the mean number of treatments per year as well as the mean number of newly hatching adult furniture beetles and wasps over treatment years one to five.

Number of newly hatched adults of *A. punctatum* continuously decreased over the first three years of treatment, as indicated by the declining number of newly appeared exit holes of *A. punctatum*. In the second year, an overall reduction by 61.22% was reached, and in the third year, a significant

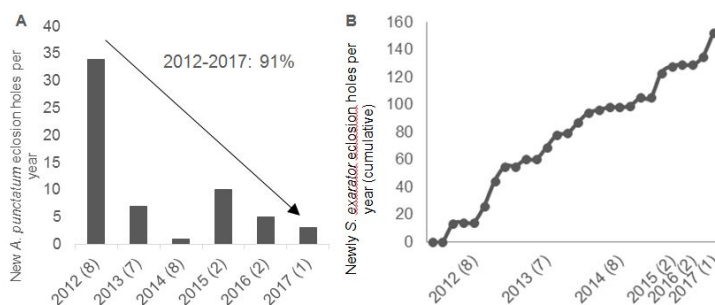
reduction of even 92.61% was achieved (Mann-Whitney U-test,  $p=0.016$ ). After three years of treatment, the number of adult *A. punctatum* slightly increased. Compared to the first year, the overall decline of newly hatched *A. punctatum* still was 74.14% in the fourth and 67.68% in the fifth year. However, only few objects were treated over a period longer than three years ( $n=3$ , year four and five, respectively) and the number of applications (0-2) in these objects was rather small.

Simultaneously to the decreasing number of newly appeared *A. punctatum* exit holes, the number of *S. exarator* exit holes continuously increased over the treatment years (table 1).

**Table 1.** Number of annually new eclosion holes of *A. punctatum* and the cumulative number of eclosion holes of *S. exarator* in treatment years 1 to 5; SD: standard deviation

Year of treatment (n=number of objects)	Mean number of treatments per year	Mean number of <i>A. punctatum</i> eclosion holes ( $\pm$ SD)	Mean number of <i>S. exarator</i> eclosion holes (cumulative) ( $\pm$ SD)
1 (n=29)	5.8	15.47 ( $\pm$ 17.74)	37.10 ( $\pm$ 30.35)
2 (n=17)	5.4	6.00 ( $\pm$ 7.13)	59.12 ( $\pm$ 51.53)
3 (n=7)	3.7	1.44 ( $\pm$ 0.83)	61.43 ( $\pm$ 47.69)
4 (n=3)	0.7	4.00 ( $\pm$ 4.33)	116.33 ( $\pm$ 34.08)
5 (n=3)	1.7	5.00 ( $\pm$ 4.08)	123.67 ( $\pm$ 40.27)

In Figure 2A, the number of newly hatched furniture beetles in the Chapel P. in Bavaria is shown, as indicated by the number of their eclosion holes. From 2012 to 2014, after repeated treatments each year, a strong decline was measured (34, 7 and 1 new exit holes, respectively). However, a slight increase after the fourth treatment year was found (10 new exit holes). In the following treatment years, a decline in newly hatched furniture beetles could be observed to an overall reduction of 91% compared to the first year (2016: 5 and 2017: 3 new exit holes).



**Figures 2 A–B:** Eclosion holes by (A) *A. punctatum* and (B) *S. exarator* in foot stoles in Chapel P.

A. Number of newly found *A. punctatum* eclosion holes per year; B. Cumulative number of newly found *S. exarator* eclosion holes; Numbers in brackets indicate numbers of treatments per year

Figure 2B shows the amount of newly hatching *S. exarator*, represented by the number of their eclosion holes. In 2012 before first treatment, no *S. exarator* exit holes were found. At the end of 2012, after eight treatments, we found 55 new exit holes. This amount continuously increased over the treatment years up to a cumulative amount of 152 newly occurring adult wasps until now.

#### 4. Discussion

The monitoring of the treated objects, presented in this publication, showed promising results. In all treated churches, the decline in newly hatched adult furniture beetles can be attributed to parasitisation by the released wasp *S. exarator*, as indicated by the increased number of their

eclosion holes. Thus, *S. exarator* appears to be an efficient and sustainable biological control method against the common furniture beetle. Furthermore, the success of a treatment can be estimated easily, as the eclosion holes of *S. exarator* and *A. punctatum* can be distinguished just by their size.

Larvae of the parasitic wasp *S. exarator* need larvae of *A. punctatum* for their development and, in consequence, each exit hole of *S. exarator* represents a parasitized and killed larva of *A. punctatum*. Over a period of five years of treatment, a mean number of 123.67 new *S. exarator* wasps hatched in the narrow-monitored areas of each treated church, representing as much killed furniture beetle larvae in this area. Corresponding to that, the number of annually newly appeared *A. punctatum* exit holes continually decreased with an overall reduction of 92.61% between the first and the third treatment year. However, in the fourth and fifth year of treatment, the number of newly eclosed furniture beetles slightly increased. However, only few objects were treated over a period longer than three years ( $n=3$ , year four and five, respectively) and additionally, the number of application (0–2) in these objects was rather small. Thus, monitoring objects over a longer period will reveal further insights in the population dynamics of *A. punctatum* and *S. exarator*.

It has to be noted that the amount of new *A. punctatum* exit holes in the first treatment year is already a reduced value. We started the first treatment about two months before the yearly one-time eclosion of *A. punctatum*. Thus, the wasps had been able to parasitize the beetle larvae for about two months until we documented the number of hatching *A. punctatum* beetles for the first treatment year. For a better estimation of the infestation level, it might be useful to monitor the eclosion of *A. punctatum* for one year before the onset of the treatment period.

As shown in literature (Ott, 2005; Paul et al., 2008; Schöller et al., 2008; Hausteine, 2010), *S. exarator* appears to be a naturally occurring parasitoid of *A. punctatum*. Our own observations confirm that, since we found eclosion holes of *S. exarator* in two third of the 29 *S. exarator* treated churches before first treatment. After collecting data of exit holes of *A. punctatum* and *S. exarator* in untreated objects for two years, Hausteine (2010) calculated a prey-predator relationship of 26.5 yearly eclosed *A. punctatum* per *S. exarator* (1:26.5). Even despite the knowledge of different data sets, our data show clearly reduced relationships of 1:1.6 after one year of treatment and 1:0.2 after the second treatment year (Auer and Kassel, 2017). Due to natural predator-prey relationships, the use of a natural antagonist in controlling pest organisms will not result in a 100% elimination of the pest (Graf, 1992; Querner, 2017) but in a decline of their population under a predefined minimum infestation level (Hausteine, 2010). This is approved by our data, as the number of newly found eclosion holes of *A. punctatum* in treated objects slightly increased after a short period with a reduced number of treatments or without releasing *S. exarator*. Consequently, a continuous monitoring program with a well-adjusted treatment protocol is strongly recommended. This is even more important in the light of the relatively long development period of *A. punctatum* which can take up to 5 years (Pinniger, 1996).

Additionally, the parasitisation success of *S. exarator* also depends on various factors like paintings on infested objects (Biebl and Auer, 2017), previous insecticide treatments, type of wood and infestation level and should be investigated in further laboratory experiments as well as practical experience. By revealing the effects of these parameters, an elaborated application program adapted to the respective conditions in the treated objects can be developed and enhances the efficiency of the biological control of *A. punctatum*.

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## Prospects of Entomopathogens in Post-Harvest Integrated Pest Management

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### Abstract

In these exploratory experiments, entomopathogenic nematodes and fungi were investigated for the management of the populations of postharvest insect pests. Nematodes were screened for pathogenicity to *Plodia interpunctella* (Hübner), while nematodes and fungi were investigated for virulence to the maize weevil, *Sitophilus zeamais* (Motschulsky). Adults and larvae of *P. interpunctella* were screened for susceptibility to the following six nematodes: *Heterorhabditis bacteriophora* Poinar (HP88, Lewiston and Oswego strains); *H. indica* Poinar, Karunakar and David (Homl strain); *H. marelatus* Liu and Berry (Point Reyes strain); *H. megidis* Poinar, Jackson, and Klein (UK211 strain); and *H. zealandica* Poinar (NZH3 strain). The nematodes that had the highest virulence to larvae and adults of *P. interpunctella* were *H. indica*, *H. megidis*, and *H. marelatus*. Six strains of nematodes were studied, namely *H. bacteriophora*, *H. indica*, *H. georgiana* (K22), *Steinernema feltiae* SN and *S. carpocapsae*. All strains of fungi, *Beauveria bassiana* (GHA) and *Metarhizium brunneum* (F52) were evaluated for infectivity to adults of *S. zeamais*. The two strains of Steinernematidae nematodes and a strain of fungus, *B. bassiana* were found to cause significant mortality of the weevils compared to the rest of the entomopathogens and the control. To demonstrate the practical application of entomopathogens, wettable dust of *B. bassiana* were dispensed on jute bags after which weevils were exposed to the treated surfaces for 30 min. The exposed weevils recorded between 90 to 100% mortality 14-d after exposure. Additional study demonstrated that the parasitoid, *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae) could be integrated with entomopathogenic nematodes. These experiments demonstrate the potential usefulness of entomopathogens in the management of stored product Lepidopteran and Coleopteran pests.

**Keywords:** entomopathogens, nematodes, fungi, parasitoid, virulence.