Project EIVES: Development of innovative methods for the natural regulation of the European spruce bark beetle (*Ips typographus* L.) by antagonists

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Zusammenfassung: In Bezug auf die natürliche Regulation des Großen Buchdruckers (*Ips typographus* L. [Coleoptera: Curculionidae]) nehmen Parasitoide durch die starke Wirtsspezialisierung einen besonderen Stellenwert ein. Vor allem Arten aus der Ordnung der Hautflügler (Hymenoptera) verursachen vielversprechende Parasitierungsraten und zeigen durch ihr aussichtsreiches Potenzial für eine künstliche Massenzucht eine gute Voraussetzung für die biologische Kontrolle von Schädlingen. Das Projekt EIVES verfolgt das perspektivische Ziel, bereits beim ersten Auftreten des Großen Buchdruckers ein hohes Vorkommen dieser Antagonisten auf gefährdeten Flächen zu ermöglichen. Das erste Ziel des Projekts bestand in der Ermittlung einer in der Region des Tharandter Waldes häufig vorkommenden Zielart, welche neben einer nachweisbaren Dichte-Reduzierung von *Ips typographus* auch Chancen auf eine praxistaugliche Vermehrung bietet. Dafür wurde *Coeloides bostrichorum* Gir. (Hymenoptera: Braconidae) gewählt. Für diese Art wird nun im Rahmen von Laborexperimenten eine geeignete Vermehrungstechnik entwickelt, welche die Grundlage für die anschließende künstliche Vermehrung darstellt. Dies umfasst sowohl die Ermittlung von geeigneten Alternativwirten als auch Strategien und Herausforderungen, diese für die Zielart attraktiver zu machen. Als erster Alternativwirt wurde der Tabakkäfer, *Lasioderma serricorne* F. (Coleoptera: Ptinidae) gewählt. Weitere Versuche müssen durchgeführt werden, um eine Parasitierung unter Laborbedingungen herbeizuführen.

Key Words: parasitoid Hymenoptera, *Ips typographus*, *Picea abies*/Norway spruce, *Coeloides bostrichorum*, *Lasioderma serricorne*, alternative hosts, laboratory rearing, biological control

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Introduction

In order to counteract the growing frequency of mass outbreaks of the European spruce bark beetle (*Ips typographus*) in Germany, alternative methods to conventional control strategies with pesticides are increasingly being sought. Numerous parasitoids from the Hymenoptera order cause promising parasitisation rates of potential insect pests in forests as natural antagonists with high regulatory potential (Feicht 2004, Hougardy & Grégoire 2004, Lawson & al. 1996, Weslien 1992). These species are characterised by high mobility, high reproduction rates and promising potential for artificial reproduction (Harris & Okamoto 1991, Hoelscher & Combs 1969, Nierhaus-Wunderwald 1996, Wegensteiner & al. 2015, Whistlecraft & al. 1984) and are particularly interesting for biological control. The regulation of the population density of *Ips typographus* through the release of mass-reared parasitoid Hymenoptera is the basic idea of the EIVES project. The project is funded by the German federal state of Saxony within the framework of the "simul+" - initiative of the Saxon State Ministry for Regional Development and is carried out in close cooperation with the state's public forest company Sachsenforst.

Regarding occurrence and species spectrum in Saxony, only limited information is available on parasitoid Hymenoptera that specifically have the European spruce bark beetle as host. Therefore, the first aim of the project was to identify frequently occurring target species that show a verifiable reduction in the density of *I. typographus* and offer opportunities for an uncomplicated and practical rearing. The second step is to develop a suitable rearing technique for one of the target species, which in the long term will form the basis for the establishment of a functioning laboratory rearing.

Methodical Approaches

In order to determine the species spectrum of parasitoid Hymenoptera in Saxony, various catches were evaluated. First, existing trap catches from the third-party funded project "bioProtect" from 2017, 2018 and 2019 were analysed. In addition, log samples of standing tree infestations were repeatedly generated from several spruce stands in 2020 and 2021. The samples were stored in stem eclectors, so that insects emerging from the logs could be caught in attached jars and preserved in 70 % ethanol solution.

For the laboratory experiments, additional living insects were needed. Depending on the demand, emergence of living insects was made possible in the eclectors by positioning moistened cellulose paper strips in the attached jars instead of the ethanol solution. In 2021, the living parasitoids were stored in a climate chamber at 20 °C and 70 % relative humidity and from 2022 they were stored in the refrigerator at 5 – 7 °C. Honey solution and water were offered continuously as food source on sponges.

In 2021, preliminary tests were carried out for the parasitisation experiments in 2022. Since a functioning laboratory rearing of parasitoid Hymenoptera with their original hosts living in spruce would not be possible using the original host plant, a suitable alternative host and substrate is required. Species were sought that have a low space requirement, a short development period and multiple generations per year. These characteristics are mainly combined in storage pests. Since Auer & al. (2021) had already successfully reared the parasitoid wasp species *Spathius exarator* L. (Hymenoptera: Braconidae) using the tobacco beetle (*Lasioderma serricorne*) as alternative host, this species was first chosen for the experiments. The living *L. serricorne* larvae were exposed in or under different porous materials for bark simulation, e. g. dried bread, crispbread, baking wafers, felt, cardboard and filter paper. The same experimental setup was applied to *I. typographus* larvae, which were only available frozen because of storage reasons.

In 2022, the experiments were further adjusted methodically. The *L. serricorne* larvae were now presented to the parasitoids with frass from *I. typographus* galleries as additional substrate. Vented plastic petri dishes were used as test vessels. The frass was used either pure or mixed with the grain substrate from the tobacco beetle culture. For each petri dish, 10 larvae were placed on individual piles of pure or mixed substrate and covered with moistened filter paper. In addition, there were neutral variants without any substrate. One female of the target species was added to each dish. The experimental setup was maintained for two to three days and the larvae were then examined for possible parasitisation. In addition, photo series were made on some of the test days in order to observe potential parasitisation activities.

Results and Discussion

Coeloides bostrichorum Gir. was selected as target species. The species appears to be suitable for the development of an artificial rearing and for selective use against *I. typographus* because of various characteristics. Due to its lifestyle as an ectoparasitic larval parasitoid, the development of the host is interrupted in the course of parasitisation, allowing the species to achieve parasitisation rates of up to 40 – 50 % (Lozan & Zelený 2002, Nierhaus-Wunderwald 1996, Sachtleben 1952). In contrast to the other species found, *C. bostrichorum* is easily identifiable with the naked eye due to its body size and colouration. In addition, the relatively long ovipositor enables it to penetrate thicker bark layers, meaning that beetle larvae can also be parasitised in lower stem sections (Nierhaus-Wunderwald 1996). *C. bostrichorum* is particularly specialised on *I. typographus* and thus very well adapted to the development of the beetles (Krüger & Mills 1990), so that the species could be used very selectively. The biology with hibernation in the pupal stage (Hougardy & Grégoire 2004, Sachtleben 1952) additionally provides the option of long-term, cooled storage in the context of a laboratory rearing.

No successful parasitisation could be achieved in the 2021 trials. In 2022, egg-like objects were occasionally found on or near deceased larvae. In some cases, these objects were also found within the substrate. In order to identify the supposed eggs, they were sent to AIM – Advanced Identification Methods GmbH for DNA barcoding. Unfortunately no clear result could be obtained because of the insufficient sample quantity. However, after comparison with data and descriptions of Hymenoptera eggs in the literature, it can be assumed that the objects were not eggs of *C. bostrichorum*.

The biggest challenge regarding the parasitisation experiments is the unknown host-finding strategy of *C. bostrichorum*. The available sources make very different and sometimes contradictory assumptions. Richerson & Borden (1972a), for example, have found through several investigations on *Coeloides brunneri* Vier. (Hymenoptera: Braconidae) that there is no evidence of attraction by sound or vibrations produced by the host or by the parasitoid (sonar) as well as magnetism or odor from the host or the host tree itself regarding host search. They also found that artificial „hot spots" were able to trigger parasitoid oviposition (Richerson & Borden 1972b).

In contrast, Mills & al. (1991) studied the species *C. bostrichorum*, *Dendrosoter middendorffii* (Hymenoptera: Braconidae) and *Rhopalicus tutela* (Hymenoptera: Pteromalidae) and conducted experiments with infrared scanner and thermistor probes, whereby no „hot spots" were detected, and concludes that volatiles may be the proximal cues in host foraging for these species Pettersson (2001) concluded from experiments with *R. tutela* that the volatiles used during host search rather emerge from the interaction between introduced microorganisms and the host tree than from bark beetle larvae.

Conclusions and Upcoming Steps

The role of volatiles in parasitoid host search is greater than initially expected. There is much evidence for the importance of volatiles in short-range host localisation (e. g. Mills & al. 1991, Pettersson & al. 2000, Pettersson 2001, Sullivan & al. 2000). The volatiles used for long- and short-range host localisation are also presumably the same (Pettersson 2001). Vinson (1976) notes that indeed a series of physical and chemical cues may be important in host search and that the relevant mechanisms are more sophisticated in more host-specific parasitoid species.

Simulating these natural conditions in the laboratory is challenging in terms of marginal odour differences, odour contamination, artificial bark substrates, etc. Moreover the origin and composition of volatiles used for host localisation is not sufficiently clarified and requires further investigation.

There will be additional experiments with bark from infested logs within the project. Laboratory parasitization experiments will be conducted on bark with the original host (*I. typographus* larvae), bark assembled with the alternative host (*L. serricorne* larvae) and without the host (empty bark with galleries and frass from *I. typographus*). The identification of relevant compounds via gas chromatography is also possible.

Additional experiments with artificial bark substitutes are planned to test the suitability for a laboratory rearing. Another aim is to gain further insights into the behaviour and biology of the target species *C. bostrichorum*, especially under laboratory conditions.

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