



Rearing *Thyridanthrax fenestratus* (Diptera, Bombyliidae) on *Pemphredon fabricii* (Hymenoptera, Crabronidae) prepupae

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Abstract

Thyridanthrax fenestratus (Fallén, 1814) is a bombyliid with poorly understood biology. It was recently shown to locally but frequently parasitize *Pemphredon fabricii* (M. Müller, 1911) (Hymenoptera: Crabronidae), a crabronid wasp that abundantly nests in old *Lipara*-induced galls on the common reed *Phragmites australis* (Cav.) Trin. ex Steud., 1840. The parasitism modes in Bombyliidae and *Thyridanthrax* spp. are not uniform. Here, we report that *Th. fenestratus* switches facultatively between killing the host almost immediately (idiobiont strategy) and killing the host at a later developmental stage (koinobiont strategy). We document the koinobiont parasitoid strategy for a series of *Th. fenestratus* larvae parasitizing *P. fabricii*. We found that a significant portion of *Th. fenestratus* larvae spend winter as young larvae and start feeding on fully developed and defecated prepupae of *P. fabricii* only after the end of cold-induced winter diapause. The time needed for the development of *Th. fenestratus* larvae exceeds several times the time needed for pupation of *P. fabricii* prepupae; the parasitized prepupae, therefore, remain paralyzed until the parasitic larva completes feeding. Fungicides, which alter the pupation of the host larva, seem to have negligible effects on *Th. fenestratus* larvae. The ability to switch between the two parasitism strategies has already been reported for several *Anthrax* spp., though the ability to block the host in the defecated prepupa stage and prevent its pupation following cold-induced diapause is herein reported for the first time.

Keywords

Diptera, eclosion, ectoparasite, idiobiont, koinobiont, parasitoid, pupation

Introduction

Thyridanthrax fenestratus (Fallen, 1814) is a Palearctic species of bee fly (Evenhuis and Greathead 2015). Larval stages are thought to live in the soil (Oldroyd 1969) and be parasitoids of cocoons and predators of eggs of locusts (Acrididae: *Dociostaurus maroccanus* in Spain and Algeria, see Künckel d'Hercule 1893–1905, 1894; Delassus et al. 1929; Del Cañizo 1943; *Arcyptera microptera* in various parts of Russia and at Crimea, see Mokřeckij 1895; Bezrukov 1922; Porčinskij 1894, 1895; *Podisma pedestris* in Russia, see Porčinskij 1894, 1895, and *Schistocerca gregaria* in Algeria, see Sergent 1916) and other grasshoppers (Pamphagidae: *Ocneridia volxemii* in Algeria, see Künckel d'Hercule 1893–1905, 1894). Some butterfly species are probably parasited too (Noctuidae: *Apamea anceps*, see Engel 1932–37; Grigorieva 1958; Zaitsev 1966; Tomaj 1977; Greathead and Evenhuis 1998; Motyčková 2012). Reports from the Netherlands and Britain describe *Th. fenestratus* as parasitizing *Ammophila* spp. larvae (Pontin 1961; Miles and Muggleton 2010; Noordijk et al. 2016; Slikboer et al. 2019). More recently, abundant *Th. fenestratus* larvae in nests of the crabronid wasp *Pemphredon fabricii* were reported (Bogusch et al. 2015, 2018). In contrast, *Th. fenestratus* larvae were rarely reported in nests of the crabronid wasp *Trypoxylon deceptorium* Antropov, 1991 (Bogusch et al. 2015). The larvae are thought to be generalists attacking the nests of various Crabronidae and Sphecidae (Bogusch et al. 2018). For an unclear reason, *Th. fenestratus* host specialization has some local consequences. Although *P. fabricii*, *Tr. deceptorium*, and *Th. fenestratus* distribution ranges overlap in a large part of Europe, the *P. fabricii* and *Tr. deceptorium* nests attacked by *Th. fenestratus* have been reported only in the Pannonian lowland, despite intensive research having been conducted in other regions of occurrence of both these species (e.g., Bohemia, i.e., western part of Czechia) (Bogusch et al. 2018). In Czechia, *Th. fenestratus* adults are active from mid-June until mid-August (Motyčková et al. 2012; Čelechovský et al. 2020). Adults are typically found in sun-exposed forest ecotones, dirt roads (Čelechovský et al. 2020), heathlands (Drake 1991; Lake 2002; Čelechovský et al. 2020), and other dry sandy habitats (Noordijk et al. 2016; Slikboer et al. 2019), where they visit flowers and feed on nectar and pollen.

The bee fly *Th. fenestratus* was abundant in old common reed galls induced by *Lipara lucens* and has been previously reported from the tailing pond of ash and slug from the lignite powerplant near Hodonín (Czechia; 21% of 29 examined *P. fabricii* nests were parasitized) and at a fishpond with sandy bedrock near Sekule (Slovakia; 24% of 89 examined *P. fabricii* nests were parasitized) (Bogusch et al. 2015; Heneberg et al. 2022). They were abundantly present near Hodonín, even though this sampling site was contaminated with arsenic ($40 \mu\text{g g}^{-1}$), copper ($26 \mu\text{g g}^{-1}$), and sulfur ($4616 \mu\text{g g}^{-1}$) (Heneberg et al. 2022). When focusing on *Lipara*-induced common reed galls, *Th. fenestratus* was most abundantly associated with galls 10–14 mm in diameter (also preferred by *P. fabricii*) and present more abundantly in galls on the stems of small or intermediate thickness (also preferred by *P. fabricii*). These stems typically occur in reed beds less stressed by drought or other factors (Astapenková et al. 2017). The larvae of *Th. fenestratus* in galls induced by *L. lucens* frequently suffer from fungal infections (Heneberg et al. 2016).

The mature larva of *Th. fenestratus* was described by Séguy (1932); a drawing of the mature larva was also published by Oldroyd (1969). However, more recently, Bogusch et al. (2015) reported deviations from the original descriptions. Séguy (1932) and Oldroyd (1969) described the larval mandibles of *Th. fenestratus* as bidentate, with lateral hooks, which is in contrast to the findings by Bogusch et al. (2015) and to other larval Bomblyliidae (Oldroyd 1969). Prominent sharp mandibles with three teeth oriented backward were present (Bogusch et al. 2015). Mature larvae reach 4.9 ± 0.8 mm in length. The development and immature larvae of *Th. fenestratus* have been documented by Künckel d'Herculais (1893–1905). There is a report of *Th. fenestratus* rearing from naturally infected *Ammophila pubescens* Curtis, 1836 cocoons (Miles and Muggleton 2010). However, as *A. pubescens* larva spins cocoons, direct observation of their development was impossible. Nevertheless, the above report was the first proof that *Th. fenestratus* allows parasitized larvae to complete their growth, feed on the food supplies provided by the parent wasp, and to spin a cocoon. The cocoon was not intact, and therefore, the authors were uncertain whether the larva was attached to the host larva during the whole time or whether it had eaten some of its host's provisions, bored into the cocoon from below after the cocoon was formed and then only consumed the wasp prepupa or pupa (Miles and Muggleton 2010).

In the present study, we document the development of *Th. fenestratus* on naturally infected *P. fabricii* prepupae under laboratory conditions. The larvae, including host prepupae, were exposed to various field-realistic concentrations of azole fungicides that are commonly used in agriculture (ECDC 2013), namely, difenoconazole, penconazole, and tebuconazole. These azoles have been shown to affect the timing of metamorphosis into adults of the host species (Heneberg and Bogusch 2022), but their effects on *Th. fenestratus* and dipterans, in general, are unknown. Selected other azole fungicides were previously documented to kill dipterans (Kenneke et al. 2009; Saraiva et al. 2018; do Prado et al. 2023). Development was documented, and differences in its timing were tracked.

Materials and methods

To analyze the geographic distribution of the *Th. fenestratus* population parasitizing *P. fabricii*, we considered the data on reed galls induced by *L. lucens*. These consisted of 176 reed beds in Czechia, Poland, Hungary, Slovakia, Austria, Slovenia, and Italy. We included only those reed beds in which the reed galls induced by *L. lucens* were positive for *P. fabricii*. The list of coordinates of examined reed beds that were positive for *P. fabricii* is provided in Suppl. material 1. Numbers of *P. fabricii* obtained by cutting the galls, numbers of *Th. fenestratus* obtained using the same procedure, and the total numbers of galls cut are indicated for sampling sites that were *Th. fenestratus*-positive. We did not show the numbers of *P. fabricii* and *Th. fenestratus* obtained from galls from which the inquilines were allowed to rear but were not cut individually, as *Th. fenestratus* often remain in the galls after rearing in rearing bags, and the detected parasitism intensities would, therefore, be underestimated.

For rearing analysis, we collected prepupae of *P. fabricii* parasitized by *Th. fenestratus* at sampling sites Hajnáčka (48.2126403°N, 19.9546442°E; 20 Jan 2022) and Šíd (48.2660036°N, 19.8795681°E; 21 Jan 2022). Both localities are in the Cerová vrchovina mountains in southern Slovakia. The collected nests were present in ≥ 1 -year-old common reed galls induced by *L. lucens*.

When larvae of *P. fabricii* are 7–20 days old, they stop feeding and line up in the nest one after the other in a row. All of them defecate, shed to become a prepupa, and build a cocoon from their silk on the inner side of the gall, without septa between the larval chambers. Therefore, when collected in January, the reed galls contained defecated fully grown prepupae in cold-induced diapause (Bogusch et al. 2018). The prepupae require only a few days (usually four to six) to pupate and develop synchronously to adults when exposed to the laboratory temperature.

We cut all the collected galls on 22–23 Jan 2022 and kept the excised prepupae at 4 °C until placed in 96-well plates (Brand, Wertheim, Germany) on 25 Feb 2022. We placed the prepupae individually in the wells and allowed them to acclimate to their new environment for 24 h at 23 °C and > 90% humidity. We performed the experiment together with exposure of the nonparasitized prepupae of *P. fabricii* to the same compounds (see the analysis published in Heneberg and Bogusch (2022) for more details). On 26 Feb 2022 (Day 0), we exposed the parasitized prepupae to three triazole formulations, each at a concentration equal to or lower than those recommended by their manufacturers for use in spraying crops to eliminate fungi. The tested compounds were penconazole (Merck, Darmstadt, Germany; batch BCBZ4909, 99.0% purity), difeconazole (Merck; batch BCCD4900, 95.5% purity), and tebuconazole (Merck; batch BCCF9398, 99.1% purity). Before the final dilution, all compounds were first diluted at 50 mg mL⁻¹ in dimethyl sulfoxide (DMSO), and DMSO alone was therefore used as a control. We sprayed the compounds using Potter Precision Laboratory Spray Tower (Burkard Scientific, Uxbridge, UK). The application rate of the applied solutions was constant at 2 $\mu\text{L cm}^{-2}$.

On Days 4 through 48, following the application of triazole fungicides, we checked the treated digger wasps at least every other day for molting, wing development, and mortality (with a break between Days 32 and 42). During the experiment, we kept the digger wasps at 23 °C with >90% humidity. We recorded the time until the full development of wings and mortality.

Results and discussion

We found *Th. fenestratus* to parasitize *P. fabricii* at 15 of the 176 reed beds that were positive for *P. fabricii*. These sites were located mainly in Slovakia (6) and Hungary (4), but several sampling sites were also positive for *Th. fenestratus* in southeastern Czechia (3), eastern Italy (1), and eastern Austria (1). Except for the single sampling site at the border of Italy and Slovenia, all other *Th. fenestratus*-positive *P. fabricii* sampling sites were located in the Pannonian lowland. The restriction of *Th. fenestratus*-positive *P. fabricii* sampling sites to the Pannonian lowland is surprising given that the distribution ranges of

both examined species are much broader and many other sampling sites were located in regions where both species are present (Fig. 1). The *Th. fenestratus* prevalence at *Th. fenestratus*-positive *P. fabricii* sampling sites was $11.6\% \pm 4.1\%$ (mean \pm SE), max 52.9% (reed beds of Neusiedler See near Oggau, Austria), min 0.8% (sampling site Tát, Hungary).

At sampling sites where *P. fabricii* was parasitized by *Th. fenestratus*, two asynchronous populations of this dipteran were present. First, some larvae were overwintering as fully grown; we included one of them in the examined dataset (Fig. 2, P-400-D8). These overwintering larvae were previously found in *P. fabricii* nests at other sampling sites as well (e.g., Hodonín and Sekule mentioned in the Introduction (Bogusch et al. 2015; Heneberg et al. 2022)) and represented the previously described populations parasitizing this host. To date, it is unclear whether *Th. fenestratus* is a true parasitoid or whether it eats part of its host's provisions (Miles and Muggleton 2010). Moreover, as host use in Bombyliidae varies, it was unclear whether these parasitoids act as koinobionts, allowing some further development of infected hosts, or idiobionts, which kill and consume their host in the state in which it is attacked (Yeates and Greathead 2008).

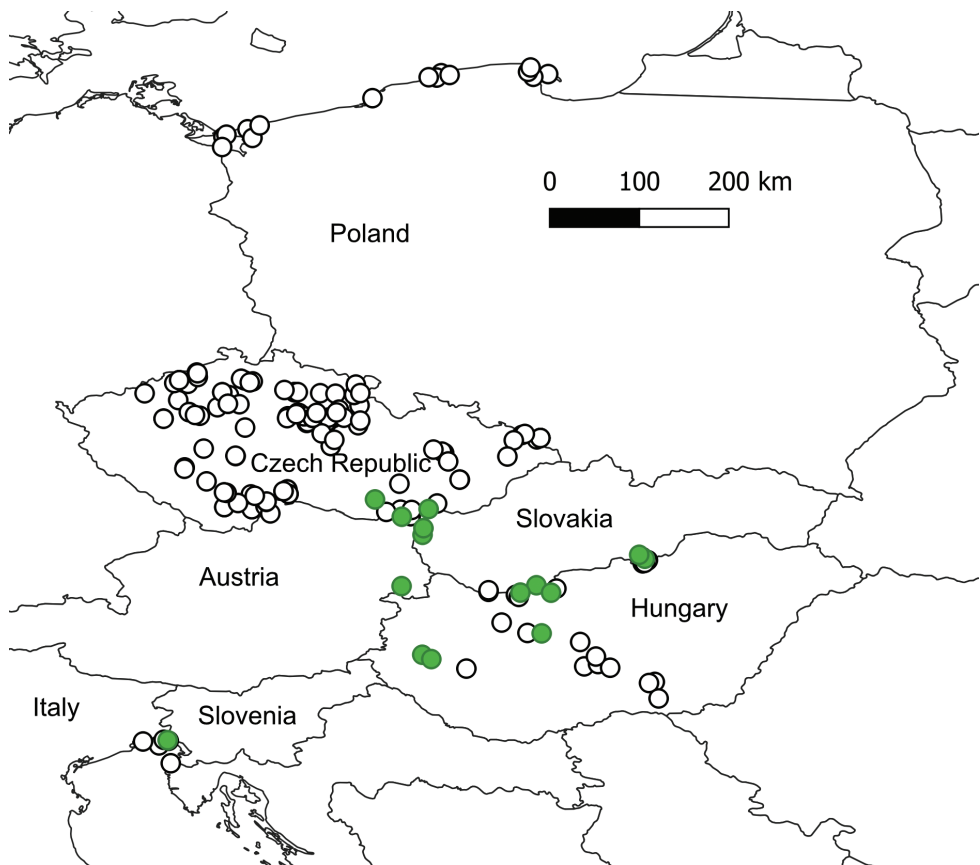


Figure 1. Map of examined sampling sites that were positive for *P. fabricii* (circles). Empty circles indicate sampling sites where only *P. fabricii* was found. Green circles indicate sites where *P. fabricii* was parasitized by *Th. fenestratus*. Source data are available in Suppl. material 1.

A part of the *Th. fenestratus* population, however, adopts a different overwintering strategy. A planidium finds the larva or prepupa of its host and overwinters on the defecated host prepupa. We did not observe the planidium directly, but when we sorted out individual overwintering *P. fabricii* prepupae, tiny attached *Th. fenestratus* larvae were already present on them. The attached larvae induce a block of the development of parasitized prepupae. Under laboratory conditions, the *P. fabricii* prepupae mostly (>80%) developed into pupae four days after removal from cold conditions. Nearly all (>98%) developed into pupae at six days following removal from cold conditions (Heneberg and Bogusch 2022). All the parasitized prepupae remained blocked at this developmental stage – see the illustration figure of one of the analyzed 96-well plates at Day 10 (Fig. 3). The nonparasitized prepupae pupated at the indicated time and metamorphosed to adults at Days 12–14.

We observed the development of 11 individuals spending diapause as first- or second-instar larvae. Seven completed their development successfully, whereas four failed to develop. Two of those that failed to develop had issues with the integrity of their paralyzed prey – the prey changed color and later was overgrown with mold, which also killed the *Th. fenestratus* larvae. In the other two cases, the fully grown larvae failed to pupate, became infected, and died (Fig. 2).

A single host prepupa is sufficient to support the development of a single *Th. fenestratus* larva. The time to pupation was 12 days in the case of the larva that was fully grown at the end of diapause. The seven individuals that grew out only after diapause pupated at Days 18, 20 (3×), 22, 24, and 26 (21.4 ± 1.0 days, median = 20 days) (Fig. 2).

All seven individuals that spent diapause as a first- or second-instar larvae and completed subsequent pupation also succeeded in metamorphosis to adults. Additionally, the single individual observed as overwintering as fully grown succeeded in metamorphosis to adult. It took 20 days for the latter individual to metamorphose from pupa to adult. It took the individuals spending diapause as the first- or second-instar larvae and completed subsequent pupation 20 (2×), 21, 22 (2×), and 24 (2×) days (21.9 ± 0.6 days, median = 22 days) to metamorphose from pupa to adult (Fig. 2). Representative images illustrating critical events in the development of *Th. fenestratus* on overwintering prepupae of *P. fabricii* are shown in Fig. 4.

A long-standing question is whether the larvae of bombyliid flies paralyze and kill the host immediately or at the very late stages of feeding. Adult females lack features that would allow them to paralyze the host. Therefore, it is believed that most bombyliid fly larvae contact the host in the state in which it is consumed (as typical idiobionts) and that the host is not immediately paralyzed or killed (which would be a koinobiont strategy) (Yeates and Greathead 2008). Evidence for other bombyliid species suggests that the host is killed only in the final stages of parasitoid development. However, here, we show clear evidence that parasitic larvae induce a development block of their host prepupae at very early stages, at the latest immediately after diapause. Therefore, while nonparasitized prepupae need only four days to pupate after diapause, the parasitized prepupae never pupate and remain alive and paralyzed for several weeks, which only allows for the complete growth of the parasitic larva until pupation.

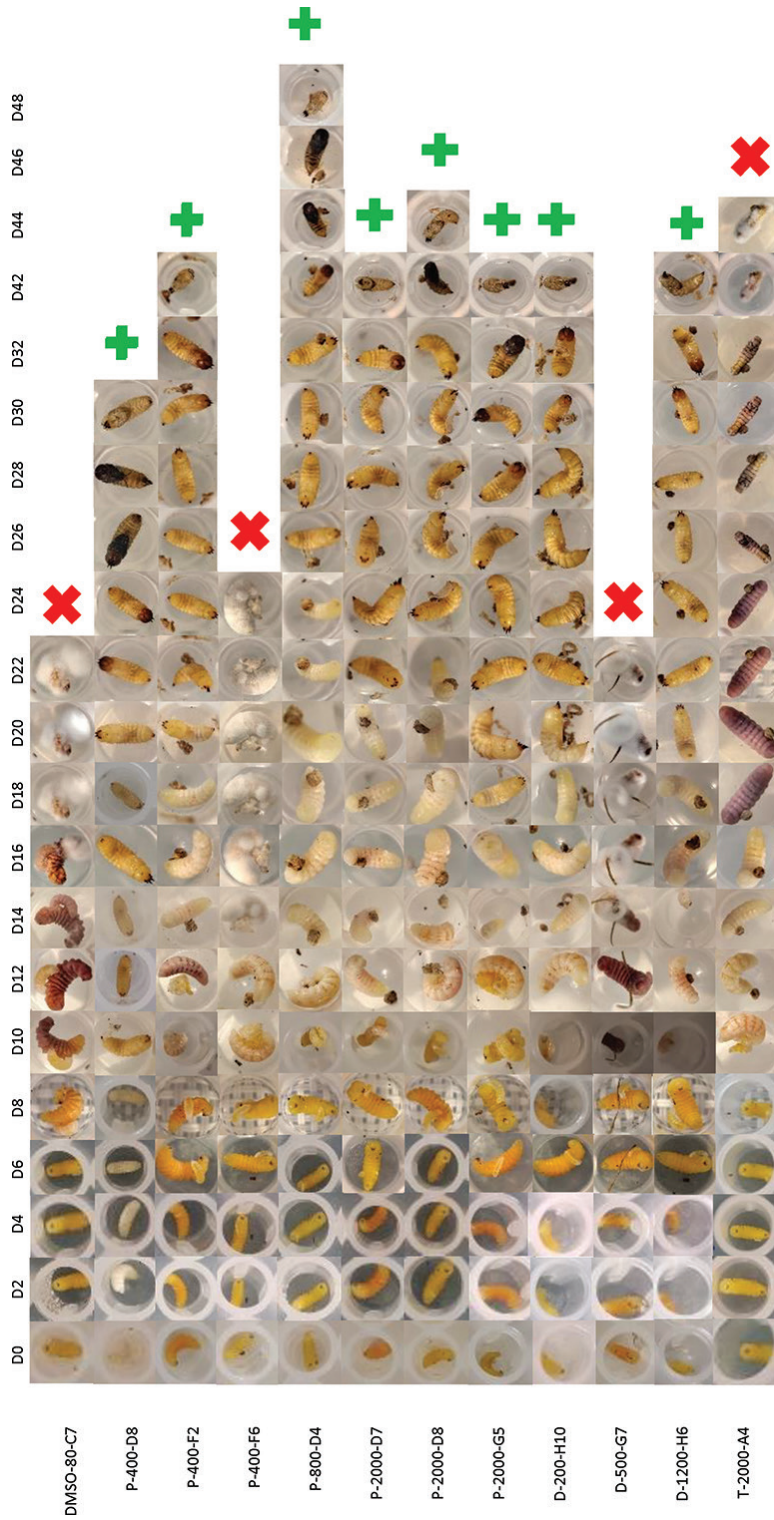


Figure 2. Time-lapse evidence of the development of *Th. fenestratus* under laboratory conditions following cold-induced diapause. The X-axis indicates days elapsed since the termination of cold-induced diapause. Individual lines represent photographs of the same individual. The individual in the second line spent the cold-induced diapause as a fully grown larva; all others spent the winter attached to the defecated prepupa but did not grow before the cold-induced diapause. Letters indicate the treatment (DMSO = control, P = penconazole, D = difeconazole, T = tebuconazole; numbers (200, 400, 500, 800, 1200, 2000) indicate the dilution of the study compounds (for more details, refer to Heneberg and Bogusch 2022)); letters with numbers indicate the positions in 96-well plates.



Figure 3. Representative figure of the 96-well plate at D10 with typically developing *P. fabricii* (already pupated, two to four days ahead of the metamorphosis to the adults) treated with penconazole ($50 \mu\text{g mL}^{-1}$) and with prepupae in wells D7, D8, and G5, which are parasitized by *Th. fenestratus*. The parasitized prepupae did not pupate and remained paralyzed and were killed within the ensuing four days.

Correspondingly, reports of some *Anthrax* spp. claim that their larvae may adopt different strategies when parasitizing tiger beetle (Coleoptera: Cicindelidae) larvae. While they parasitized any tiger beetle larval instar, they remained in a second instar for up to nine months, depending on the host stage and time of year. Once the tiger beetle larva constructs a pupal chamber and becomes a prepupa, the *Anthrax* larva begins rapid growth and reaches full size in one or two weeks (Palmer 1982; Shelford 1913). Thus, the larva appears to behave as a koinobiont or idiobiont based on the time of the year and host developmental stage. Given that two stages of *Th. fenestratus* larvae are found in *Lipara*-induced reed galls, this parasitic species appears to employ a similar strategy. Whether the first-instar larva feeds on the aphid provisions remains unclear, as some other bombyliid species are reported to feed on provisions and develop only on the prepupa or pupa (Bohart 1960; Du Merle 1979). Regarding our experiments with *Th. fenestratus*, we removed all *P. fabricii* prepupae from their cocoons before beginning experiments. Thus, the parasitic larvae must have been attached to them during the cold-induced diapause.

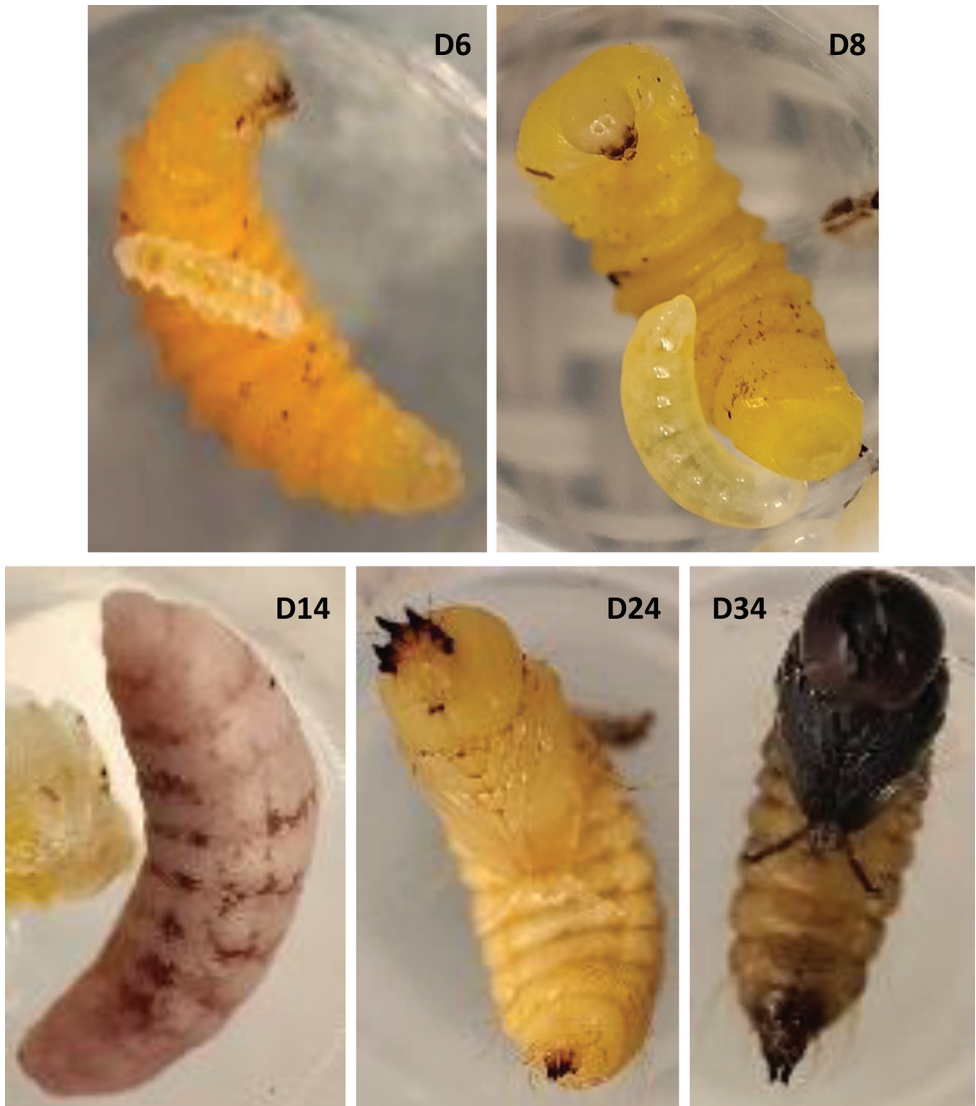


Figure 4. Representative figures illustrating critical events in the development of *Th. fenestratus* on overwintering prepupae of *P. fabricii*. In bombyliid flies, the first stage, triungulin, is active and immediately molts after attaching to its host to the second stage (likely D6), which has already attached itself and is not distinctly curved. This stage can overwinter (as does the pupa). Early third instar (likely D8) and late third instar (likely D14) can be diagnosed by their characteristic curvature. D24 and D34 represent pupae.

We exposed *P. fabricii* prepupae with *Th. fenestratus* larvae to three topically applied fungicides before their growth. However, we did not observe any adverse effects of exposure to the indicated fungicides on the bee flies. The larva treated with DMSO (control) died during development because of fungal infection. There were no concentration-dependent differences in the development and survival of the larvae treated with difeconazole and penconazole, including a lack of effects at the highest

concentrations used (Fig. 2). The only unclear result is with regard to the single tebuconazole-treated larva, which died after it completed its growth, just before pupation. However, more larvae treated with the same compound are needed to provide a definitive answer on tebuconazole's effects on this dipteran.

In conclusion, we found that *Th. fenestratus* facultatively switches between killing the host almost immediately (idiobiont strategy) and killing the host at a later developmental stage (koinobiont strategy). We documented the koinobiont parasitoid strategy in detail using a series of *Th. fenestratus* larvae parasitizing their hymenopteran host *P. fabricii*, which nests in *Lipara*-induced reed galls. We found that a significant portion of *Th. fenestratus* larvae overwinter as young larvae and start feeding on fully developed and defecated prepupae of *P. fabricii* only after the end of cold-induced winter diapause. The time needed for the development of *Th. fenestratus* larvae exceeds several times the time needed for pupation of *P. fabricii* prepupae; the parasitized prepupae, therefore, remain paralyzed until the parasitic larva completes feeding.

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References

- Astapenková A, Heneberg P, Bogusch P (2017) Larvae and nests of aculeate Hymenoptera (Hymenoptera: Aculeata) nesting in reed galls induced by *Lipara* spp. (Diptera: Chloropidae) with a review of species recorded. Part II. PLOS ONE 12: e0169592. <https://doi.org/10.1371/journal.pone.0169592>
- Bezrukov YuG (1922) Kratkoe soobščenie o dejatelnosti Omskoj laboratorii Sibirskogo Entomologičeskogo Bjuro za 1919–1922 g.g. Izvestija Sibirskogo Entomologičeskogo Bjuro 1: 26–20. [in Russian]
- Bogusch P, Astapenková A, Heneberg P (2015) Larvae and nests of six aculeate Hymenoptera (Hymenoptera: Aculeata) nesting in reed galls induced by *Lipara* spp. (Diptera: Chloropidae) with a review of species recorded. PLOS ONE 10: e0130802. <https://doi.org/10.1371/journal.pone.0130802>
- Bogusch P, Havelka J, Astapenková A, Heneberg P (2018) New type of progressive provisioning as a characteristic parental behaviour of the crabronid wasp *Pemphredon fabricii* (Hymenoptera Crabronidae). Ethology, Ecology & Evolution 30: 114–127. <https://doi.org/10.1080/03949370.2017.1323801>

- Bohart GE, Stephen WP, Eppley RK (1960) The biology of *Heterostylum robustum* (Diptera: Bombyliidae), a parasite of the alkali bee. *Annals of the Entomological Society of America* 53: 425–435. <https://doi.org/10.1093/aesa/53.3.425>
- Čelechovský A, Čelechovský D, Čelechovský M (2020) Dlouhososky *Exhyalanthrax afer* (Fabricius, 1794) a *Thyridanthrax fenestratus* (Fallén, 1814) – první nálezy v Národním parku Podyjí (Diptera: Bombyliidae). *Thayensia (Znojmo)* 17: 247–250.
- Del Cañizo J (1943) Parasitos de la langosta en España. I. Dípteros bombilidos. Dirección general de Agricultura, Sección de Plagas del Campo y Fitopatología, Servicio de Lucha contra la Langosta (Madrid).
- Delassus M, Pasquier R, Laffond P, Ruff, Lepigre A (1929) La lute contre les sauterelles en Algérie. Gouvernement general de l'Algérie, Direction de l'Agriculture, du Commerce et de la Colonisation (Alger).
- Do Prado CCA, Queiroz LG, da Silva FT, de Paiva TCB (2023) Toxicological effects caused by environmental relevant concentrations of ketoconazole in *Chironomus sancticaroli* (Diptera, Chironomidae) larvae evaluated by oxidative stress biomarkers. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 264: 109532. <https://doi.org/10.1016/j.cbpc.2022.109532>
- Drake CM (1991) Provisional atlas of the Larger Brachycera (Diptera) of Britain and Ireland. Biological Records Centre, NERC Institute of Terrestrial Ecology, Monks Wood Experimental Station (Huntingdon).
- Du Merle P (1979) Biologie de la larve planidium de *Villa brunnea* Beck., diptère bombyliide parasite de la processionnaire du pin. 1. Recherche et découverte de l'hôte. *Annales de Zoologie Ecologie Animale* 11: 289–304.
- ECDC (2013) Risk assessment on the impact of environmental usage of triazoles on the development and spread of resistance to medical triazoles in *Aspergillus* species. ECDC (Stockholm).
- Engel EO (1932–1937) 25. Bombyliidae. In: Lindner E (Ed.) *Die Fliegen der Paläarktischen Region*. E. Schweizerbart (Stuttgart), 1–619.
- Evenhuis NL, Greathead DJ (2015) World catalog of bee flies (Diptera: Bombyliidae). Revised September 2015. <http://hbs.bishopmuseum.org/bombcat/bombcat-revised2015.pdf> [cited as 22 July 2023]
- Greathead DJ, Evenhuis NL (1998) Family Bombyliidae. In: Papp L, Darvas B (Eds) *Contributions to a Manual of Palaearctic Diptera (with special reference to flies of economic importance)*. Vol. 2. Nematocera and Lower Brachycera. Science Herald (Budapest), 487–512.
- Grigorieva TG (1958) Zernovye sovki i borba s nimi. Gosudarstvennoe Izdatelstvo sel'skhozjajstvennoj Literatry, Moscow & Leningrad. [in Russian]
- Heneberg P, Bizos J, Čmoková A, Kolařík M, Astapenková A, Bogusch P (2016) Assemblage of filamentous fungi associated with aculeate hymenopteran brood in reed galls. *Journal of Invertebrate Pathology* 113: 95–106. <https://doi.org/10.1016/j.jip.2015.12.007>
- Heneberg P, Bogusch P (2022) Commonly used triazole fungicides accelerate the metamorphosis of digger wasps (Hymenoptera: Spheciformes). *Environmental Science and Pollution Research* 29: 67430–67441. <https://doi.org/10.1007/s11356-022-22684-8>

- Heneberg P, Bogusch P, Astapenkova A, Řezáč M (2022) Life in extreme habitats: the number of prepupae per nest of the crabronid wasp *Pemphredon fabricii* is constant even under pressure from high concentrations of toxic elements. *Environmental Science and Pollution Research* 29: 16091–16102. <https://doi.org/10.1007/s11356-021-16881-0>
- Kenneke J, Mazur C, Kellock K, Overmyer J (2009) Mechanistic approach to understanding the toxicity of the azole fungicide triadimefon to a nontarget aquatic insect and implications for exposure assessment. *Environmental Science and Technology* 43: 5507–5513. <https://doi.org/10.1021/es900351w>
- Künckel d'Herculais J (1893–1905) Invasions des acridiens (vulgo sauterelles) en Algérie. Imprimerie administrative et commerciale Girault, A. Franceschi, successeur (Alger).
- Künckel d'Herculais J (1894) Les diptères parasites des acridiens: les Bombyliides. Hypnodie larvaire et metamorphose avec stade d'activité et stade de repos. *Comptes Rendus de l'Académie des Sciences, Paris* 118, 926–929.
- Lake S (2002) The role of livestock grazing in the conservation of lowland heath. PhD Thesis. University of Southampton, Faculty of Science (Southampton).
- Miles S, Muggleton J (2010) The rearing of *Thyridanthrax fenestratus* (Fallen, 1814) (Diptera, Bombyliidae) from a cocoon of *Ammophila pubescens* (Curtis, 1836) (Hymenoptera, Sphecidae). *Dipterists Digest* 17: 45–46.
- Mokržeckij S (1895) O parazitah saranči i kobylok. *Hozjain* 14: 271–272. [in Russian]
- Motyčková V (2012) Dlouhosoky podčeledí Anthracinae a Exoprosopinae v České republice a na Slovensku (Diptera: Bombyliidae). BSc. Thesis. Palacký University in Olomouc, Faculty of Science (Olomouc).
- Noordijk J, Smit JT, Smit J, Vreugdenhil D (2016) De insectengemeenschap van aangelegde steilranden op de heide. *Entomologische Berichten* 76: 48–55.
- Oldroyd H (1969) Diptera Brachycera, Section (a) Tabanoidea and Asiloidea. *Handbooks for the identification of British insects*. Royal Entomological Society (London).
- Palmer MIL (1982) Biology and behavior of two species of *Anthrax* (Diptera: Bombyliidae), parasitoids of the larvae of tiger beetles (Coleoptera: Cicindelidae). *Annals of the Entomological Society of America* 75: 61–70. <https://doi.org/10.1093/aesa/75.1.61>
- Pontin AJ (1961) *Thyridanthrax fenestratus* Fall. (Dipt., Bombyliidae) parasitic on *Ammophila* (Hym., Sphecidae). *Entomologist's Monthly Magazine, London* 96: 26.
- Porčinskij IA (1894) O kobyľkah povreždavših posevy I travy v gubernijah Permskoj, Tobolskoj I Orenburgskoj. Departament Zemledelija (St. Peterburg). [in Russian]
- Porčinskij IA (1895) Les parasites des criquets nuidibles en Russie (Fin). *Eremobia muricata*. Departament Zemledelija (St. Peterburg). [in Russian]
- Saraiva AS, Sarmiento RA, Golovko O, Randak T, Pestana JL, Soares AM (2018) Lethal and sub-lethal effects of cyproconazole on freshwater organisms: a case study with *Chironomus riparius* and *Dugesia tigrina*. *Environmental Science and Pollution Research* 25: 12169–12176. <https://doi.org/10.1007/s11356-017-1180-y>
- Séguy E (1932) Étude sur les diptères parasites ou prédateurs des sauterelles. *Encyclopédie Entomologique, B II, Diptères* 6: 11–40.
- Sergent E (1916) Campagne d'experimentation de la méthode biologique contre les *Schistocerca peregrina* dans la vallée de la Haute Tafna, commune mixte de Sebduou (département

- d'Oran). Existence d'une épizootie autochtone vaccinnante (mai, juin, Juillet 1915). Annales de l'Institut Pasteur, Paris 30: 209–224.
- Shelford VE (1913) The life history of a bee-fly (*Spogostylum anale* Say) parasite of the larva of a tiger beetle (*Cicindela scutellaris* Say var. *lecontei* Hald.). Annals of the Entomological Society of America 6: 213–225. <https://doi.org/10.1093/aesa/6.2.213>
- Shlikboer L, Smit JT, Zeegers T (2019) Honingbijen & wilde bestuivers in defensieterreinen. Deel I: Doornspijkse Hei. EIS Kenniscentrum Insecten (Leiden).
- Tomaj V (1977) Československé druhy čeledi Bombyliidae (Diptera). MSc. Thesis. University of J. E Purkyně in Brno, Faculty of Science (Brno).
- Yeates DK, Greathead D (2008) The evolutionary pattern of host use in the Bombyliidae (Diptera): a diverse family of parasitoid flies. Biological Journal of the Linnean Society 60: 149–185. <https://doi.org/10.1111/j.1095-8312.1997.tb01490.x>
- Zaitsev VF (1966) Paraziticheskie mukhi semeistva Bombyliidae (Diptera) v faune Zakavkaz'ya. Izdatel'stvo 'Nauka', Moscow, Leningrad.

Supplementary material I

Coordinates of sampling sites used to generate Fig. 1

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Data type: xlsx

Explanation note: All the listed sampling sites were positive for *P. fabricii*. Numbers of *P. fabricii* obtained by cutting the galls, numbers of *T. fenestratus* obtained using the same procedure, and total numbers of galls cut are indicated for sampling sites that were *T. fenestratus*-positive.

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