REPRODUCTIVE POTENCIAL OF FEMALE EUROPEAN CORN BORER (Ostrinia nubilalis HBN.) - A USEFUL TOOL FOR BIOLOGICAL CONTROL IN HOPS (Humulus lupulus L.)

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Abstract

The European corn borer (*Ostrinia nubilalis* Hübner), ECB, is polyphagous. In Slovenia it causes the most damage to corn (*Zea mays* L.) and hops (*Humulus lupulus* L.) but it is also often found on vegetables (tomato, pepper) and ornamentals. ECB is a nocturnal moth whose flight can be effectively monitored using a classical light trap. Its population density varies from one microhabitat to another, depending on the presence of host plants. It is often difficult to predict the occurrence of the next generation based on the number of adult ECB caught. One method that can be used is to examine the fullness of the spermatheca – i.e. an ectodermal organ responsible for receiving, maintaining, and releasing sperm for egg fertilization – in female ECB caught in a classical light trap. We analysed 2 years of spermatheca fullness of ECB female and their population dynamic on two locations in Slovenia. Only in high ECB density field were we able to observe a correlation between peak of spermatheca fullness, adult flight peak and oviposition for the first generation of ECB.

Key words: Classical light trap, Degree Day model, *Humulus lupulus*, Lepidoptera, *Ostrinia nubilalis*, spermatheca

REPRODUKCIJSKI POTENCIAL SAMIC KORUZNE VEŠČE (Ostrinia nubilalis HBN.) – KORISTNO ORODJE ZA BIOTIČNO VARSTVO HMELJA (Humulus lupulus L.)

Abstrakt

Koruzna vešča (*Ostrinia nubilalis* Hübner) je polifagna vrsta, ki v Sloveniji povzroča največjo škodo na koruzi (*Zea mays* L.) in hmelju (*Humulus lupulus* L.). Pogosto jo najdemo tudi na vrtninah (paradižniku, papriki) in okrasnih rastlinah. Koruzna vešča je nočni metulj, zato za njeno učinkovito spremljanje uporabljamo klasično svetlobno vabo, ki zahteva vir električne energije. Ima preko 200 gostiteljskih rastlin. Gostota njene populacije se glede na prisotnost gostiteljskih rastlin

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razlikuje na različnih mikrolokacijah. Velikokrat je težava napovedati glede na številčnost ulovljene odrasle populacije koruzne vešče, pojavnost naslednje generacije. Ena izmed metod, ki se je lahko poslužujemo je pregledovanje polnosti spermatek v ulovljenih samičkah, ki jih ulovimo s klasično svetlobno vabo. Spermateka je ektodermalni organ, odgovoren za sprejem, vzdrževanje in sproščanje spermijev za oploditev jajčec. V dvoletnem spremljanju smo analizirali stanje spermatek odraslih osebkov samic koruzne vešče in njihovo populacijsko dinamiko na dveh lokacijah. Pri spremljanju koruzne vešče v Žalcu in na Rojah pri Žalcu, kjer se pojavlja koruzna vešča smo lahko opazili korelacijo med vrhom polnosti spermatek, vrhom leta odraslih osebkov in odlaganjem jajčec prve generacije.

Kjučne besede: *Humulus lupulus*, klasična svetlobna vaba, Lepidoptera, *Ostrinia nubilalis*, spermateka, vsota efektivnih temperatur

1 INTRODUCTION

The European corn borer (ECB), Ostrinia nubilalis (Hübner), is a species that feeds on a variety of agricultural and volunteer plants, which classifies it as a polyphagous pest. In Slovenia, it causes most damage to corn (Zea mays L.) and hops (Humulus lupulus L.), but is also commonly found on vegetables such as tomatoes and bell peppers, and on ornamental plants. The larva is responsible for the boring of the stem of its host, leading to the decay of the plant. Its presence in corn and hops in Slovenia has increased sharply over the last decade, resulting in a noticeable economic damage (Rak Cizej et al., 2010). ECB can harbor two strains (i.e., E and Z) (Anglade et al., 1984), told appart by a polytrophic pheromonal system (Pelozuelo et al., 2004). In central Slovenia, ECB is present with strain E on hops and corn. In addition to the two known strains, there are also three known ecotypes of ECB which differ in voltinism (univoltine, bivoltine and multivoltine ecotypes). In some areas, more than one ecotype and pheromone sex type occur sympatrically (Rak Cizej et al., 2010; Showers et al., 1976). Female fecundity is strongly correlated with species and ecotypes; females that have mated several times are much more fecund than those that mate only once (Fadamiro & Baker, 1999; Jiménez-Pérez et al., 2003). Eggs are usually deposited on the underside of leaves or directly on the fruit. In a healthy population, 95 % of fertile eggs hatch. Sex ratios are 1:1, and some authors have observed slight proterandry (i.e., males emerge a few hours or days before females) (Fadamiro & Baker, 1999).

Researchers also attribute the success of insect evolution to adaptations in the female reproductive organs. Female lepidopteran, ECB included, store the seminal fluid they obtain from the males in an organ called the spermatheca. The spermatheca is an ectodermal organ responsible for receiving, maintaining and releasing sperm to fertilise the egg. After mating, sperm migrate from the proximal part – i.e. the bursa copulatrix – of the female reproductive system, through the spermathecal duct into the spermatheca. Once the eggs are expelled from the ovary, the sperm repeats the journey and fertilises the eggs. The female genital system, including the spermatheca, is involved in the maturation or activation of

the sperm (Pascini & Martins, 2017). Pascini and Martins (2017) state that there is no correlation between the size of the spermatheca and the number of eggs laid by the female. Spermatheca volume was positively correlated with the number of eupyrene and apyrene sperm. However, the number of eggs hatched depended on the male with whom the female mated; fewer eupyrene sperm meant fewer hatched eggs (Muller et al., 2015).

Monitoring of nocturnal insects has been performed by the use of light traps for many years, proving its efficacy in population survey, and ECB in particular (Rak Cizej & Trematerra, 2017). Following of daily catch allow advisers and farmers to adapt their management methods to the current pest pressure of a field. Indeed, management methods such as *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) egg release is dependant of the presence of egg masses on leaves (Hoffmann et al., 2002, 2006; Mertz et al., 1995; Razinger et al., 2016). Eggs are laid by the ECB at ages 3 and 10 days. Researchers have also observed a five-day delay between the emergence of adults and their capture in light traps (Xingquan et al., 2004). Monitoring and surveying of ECB population and life stages can also be modelled thanks to the accumulation of average degree-days (DD) (Calvin & Song Ping Zhong, 1994; Ivezić et al., 2023; Maiorano, 2012).

In order to implement convenient and inexpensive practices in ECB oviposition forecasting, we have introduced spermatheca fullness evaluation in laboratory and population dynamic surveys in corn field in 2023 and 2024. After description of the variation of fullness of spermatheca and population of ECB over the two growing seasons and their offset with simplistic degree-day model (DD model, we aimed at connecting peak of spermatheca fullness, peak of adult flight and oviposition for ECB females.

2 MATERIAL AND METHODS

2.1 Monitoring of the flight of the ECB with classical light trap in 2023 and 2024 at two locations, Roje pri Žalcu and Žalec

In 2023 and 2024, we monitored ECB in two locations in the hop-growing area Roje pri Žalcu (D48/GK; X:5123080, Y: 511464) and Žalec (D48/GK; X:122975, Y: 512459) with a classical light trap using a 160-W mercury (HBO) bulb. The bulb was lit at night from 9 p.m. to 6 a.m. The height of the classical light trap was 1 meter from the ground. The light trap was also equipped with a mechanism that adds 35 to 40 ml of chloroform in the evening (when the light is on) to stun the insects attracted by the light. The contents of the catch at the bottom of the trap were cleaned daily and identified in the laboratory. Chloroform was also added to the trap on a daily basis.

2.2 Weather data

Weather also plays a major role in the successful development of ECB from egg to imago. The combination of favourable temperatures $(8.2 - 41.0 \, ^{\circ}\text{C})$ (Majorano,

2012)), rainfall and, consequently, relative humidity, (70 - 80 %) enables the pest to mate successfully and a high proportion of eggs to develop successfully.

2.2.1 Year 2023

The year 2023 was a rather heavy rainfall year. 826.8 mm of precipitation fell between April and the end of September. The first decade of August is especially notable, with 49.2 mm of rainfall in just one day. A wet and warm weather in the second and third decades of July and early August, when the ECB lays its eggs, was observed.

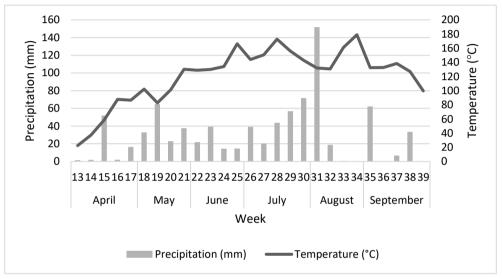


Figure 1: Data for temperature (°C) and precipitation (mm) by week from the beginning of April to the end of September in 2023, agrometeorological station of Žalec (Source: Agrometeorloški portal Slovenije, 2023).

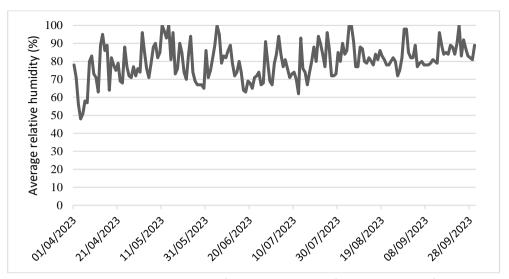


Figure 2: Average relative humidity data from the beginning of April to the end of September 2023, agrometeorological station of Žalec (Source: Agrometeorloški portal Slovenije, 2023).

2.2.2 Year 2024

The year 2024 was quite eventful from the beginning of April to the end of September. Total precipitation during this period was 739.6 mm. Most of the precipitation fell in the second decade of May, the second decade of July and the second decade of September.

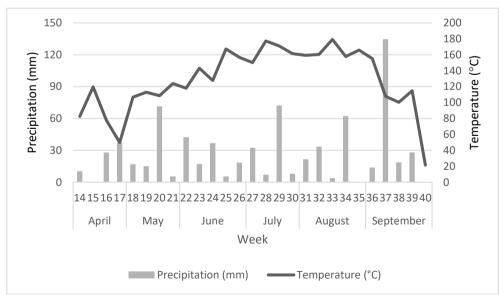


Figure 3: Data for temperature (°C) and precipitation (mm) by week from the beginning of April to the end of September in 2023, agrometeorological station of Žalec (Source: Agrometeorloški portal Slovenije, 2024).

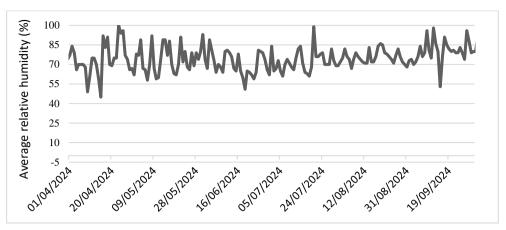


Figure 4: Average relative humidity data from the beginning of April to the end of September 2023, agrometeorological station of Žalec (Source: Agrometeorloški portal Slovenije, 2024).

2.3 Spermatheca examination

The contents of the catch from the light trap were then examined in the laboratory, where the sex of the captured ECB was determined. The females were stored in a prepared 10 % KOH solution. The stored females were then examined under a microscope for the presence of spermatophores in the bursa copulatrix of the female. Spermatheca fullness was determined as Empty when no spermatophore could be found, Half-full when one or more spermatophores were found but feeling only partially (10-70 % approximately). The spermatheca was determined as Full when one or more spermatophore were found feeling the entire spermatheca.

2.4 Degree-Day model

To predict the different life stages of ECB, we used a standard approach of the degree-day model averaging method accumulation (Arnold, 1960), with a base temperature of T_{base} = 10 °C (Got et al., 1991; Ivezić et al., 2023), starting on 1st of January of 2023 and 2024:

$$D(d) = \frac{T_{max}(d) + T_{min}(d)}{2} - T_{base}$$

with

$$T_{min}(d) = T_{base}$$
, if $T_{min}(d) < T_{base}$

$$T_{max}(d) = T_{base}$$
, if $T_{max}(d) < T_{base}$

where D(d) is the degree-day accumulated during the day d, $T_{min}(d)$ is the minimum temperature of the day, and $T_{max}(d)$ is the maximum temperature of the day (Maiorano, 2012). Values of accumulated degree-days (DD) necessary to reach the start of a life stage of ECB were taken from Bessin (2003) and translated from Fahrenheit to Celsius degrees.

3 RESULTS

3.1 Observation of spermatheca fullness

In 2023, the first ECB flight was recorded 9 and 11 days before the DD model estimate in Žalec and Roje pri Žalcu, respectively (Table 1). In 2024, the first ECB flight was this time delayed by 7 days compared with the DD model in Žalec, while in Roje pri Žalcu, the DD model estimated a first flight 2 days later than in the light trap (Table 2). Generations 1 and 2 are separated in 2023 by 45 and 47 days, and in 2024 by 34 and 45 days, at Žalec and Roje pri Žalcu, respectively. First female having full spermatheca is captured on average 7.3 days after first flight capture of ECB (shortest delay is 2 days, obtained in Žalec and Roje pri Žalcu for the 2nd generation in 2023, longest delay is 21 days, in Roje pri Žalcu for the 1st generation in 2023).

Table 1: Description of first occurrence of three life stages for modelled and caught ECB: adults, eggs and larvae of 1st and 2nd generation in 2023 in Roje pri Žalcu, according to date and accumulated degree-days (DD)

	Accumulated degree-days (DD) above 10 °C, 2023	
	Prediction of the occurrence of a stage - temperature threshold reached	Actual catch on classical light trap in Roje pri Žalcu
Start of the adult flight - 1st generation (last year's generation)	2.06.2023 (235 °C)	22. 5. 2023 (140 °C)
Emergence of 1st generation eggs	14.06.2023 (339 °C)	12. 6. 2023 (322 °C) (first female found with full spermatheca)
Emergence of 1st generation larvae	22.06.2023 (431 °C)	15. 6. 2023 (357 °C) (first larval record)
Emergence of 1st generation adults (usually referred to as 2nd generation)	31.07.2023 (906 °C)	8.7. 2023 (613 °C)
Emergence of 2nd generation eggs	7.08.2023 (969 °C)	10. 7. 2023 (641 °C) (first female found with full spermatheca)
Emergence of 2nd generation larvae	15.08.2023 (1043 °C)	21.07.2023 (810 °C)

Table 2: Description of first occurrence of three life stages for modelled and caught ECB: adults, eggs and larvae of 1st and 2nd generation in 2024 in Roje pri Žalcu, according to date and accumulated degree-days (DD)

	Accumulated degree days (DD) above 10 °C, 2024	
	Prediction of the occurrence of a stage - temperature threshold reached	Actual catch on classical light trap in Roje pri Žalcu
Start of the adult flight - 1st generation (last year's generation)	17.05.2024 (236 °C)	15. 5. 2024 (225 °C)
Emergence of 1st generation eggs	31.05.2024 (340 °C)	1. 6. 2024 (346 °C) (first female found with full spermatheca)
Emergence of 1st generation larvae	8.06.2024 (419 °C)	13. 6. 2024 (459 °C) (first larval record)
Emergence of 1st generation adults (usually referred to as 2nd generation)	17.07.2024 (909 °C)	29.6. 2024 (661 °C)
Emergence of 2nd generation eggs	22.07.2024 (976 °C)	6.07.2024 (741 °C) (first female found with full spermatheca)
Emergence of 2nd generation larvae	27.07.2024 (1040 °C)	24.07.2024 (989 °C)

3.2 Population dynamic and its relation to spermatheca fullness

Figure 5 shows the cumulative catch of ECB in two different locations in the years 2023 and 2024. A total of 957 (22 % of females) and 82 (26 % of females) adults, at Roje pri Žalcu and Žalec, respectively, for 2023; and 554 (24 % of females) and 208 (23 % of females), at Roje pri Žalcu and Žalec, respectively, for 2024. The low number of catches in Žalec in 2023 is due to the malfunctioning of the trap after 3rd of August caused by flooding. The first ECB capture in the light trap (adult flight for the 1st generation) is reached at 140 DD and 225 DD at Roje pri Žalcu in 2023 and 2024, respectively (Table 2 and 3), while the adult flight for the 2nd generation is captured at 613 DD and 661 DD, respectively. A one-week delay between 2024 and 2023 can be observed for the first ECB flight, as 2023 experienced colder temperatures and drier conditions in spring (Figure 1-4).

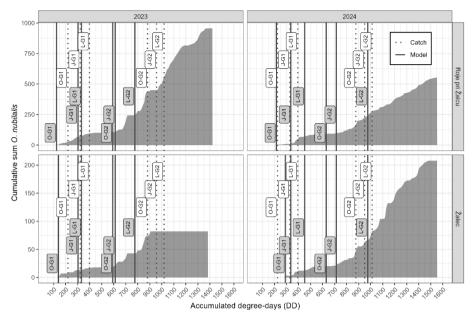


Figure 5: Occurrence of the individual developmental stages of the first and second generation of the ECB for the location Žalec and Roje pri Žalcu in 2023 and 2024 (0 - G1 (1st generation adult), J - G1 (1st generation egg), L - G1 (1st generation larva), and G2 for 2nd generation individuals)

The peak capture of females with full spermathecae corresponds perfectly to the ECB flight peak in 4 of the 7 flight peaks (2 locations * 2 years * 2 generations per year (except one peak impossible to visualize)). The three unpaired flight peaks always occur for the second generation of adults, which also poorly matched the DD model estimates. 51.5 % and 12.7 % of overall captured female ECB had full and half-full spermathecae, respectively (Figure 6).

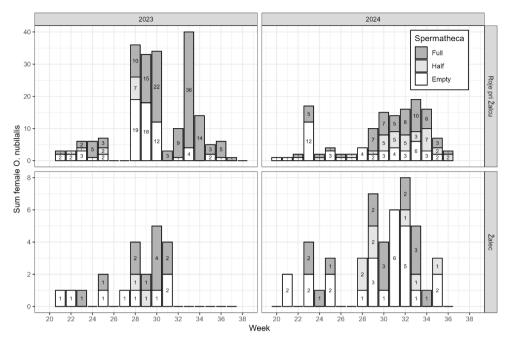


Figure 6: Weekly representation of the ratio of the number of full, half-full and empty female ECB spermathecae at locations Žalec in Roje pri Žalcu, in 2023 and 2024

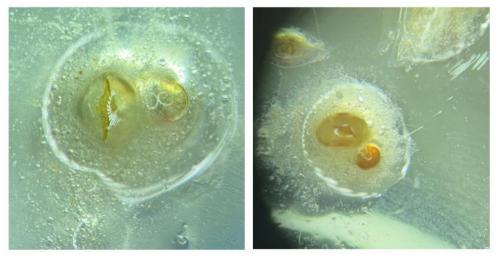


Figure 7: Left picture: Empty bursa copulatrix; right picture: Bursa copulatrix accumulated with spermatophores (Photo: M. Rak Cizej)

4 DISCUSSIONS

Spermatheca fullness status of female ECB during flight of first or second generation was successfully monitored with a daily checking of the content of the spermathecae of female. Location of the trap had a large influence on the number of captured female, however ratio of spermatheca fullness (Full:Empty spermathecae) was equivalent between Roje pri Žalcu and Žalec. Yet, peak of full spermatheca and peak of flight of adults of 1st and 2nd generation was easier to read when density of population was higher (i.e., Roje pri Žalcu). DD model estimates were reasonably matching our ECB catch (±1week) for their first generation. However, large delay was observed between model-estimated oviposition and the actual monitored ECB of the 2nd generation. Indeed, according to DD model, eggs are estimated to be laid 2 weeks earlier than our observation of full spermatheca peak. We can discuss that oviposition peak do not exactly match first laid eggs, however estimated and observed first flight for second generation was as well impacted by a 2-week delay in Roje pri Žalcu. As found by Ivezić et al. (2023), degree-day model does not always give a good estimation of the dynamic of population of ECB, with a high error of the estimated number of adults during the flight of the 2nd generation, due to numerous other factors (e.g., latitude, environment) (Onstad & Brewer, 1996).

In our results, we hypothesize that a full spermatheca found in the female is evidence of an upcoming oviposition period, as found by Showers et al. (1974). In their study, they described the mating status of females with an easy classification into 4 classes using spermatophores found in bursa copulatrix of ECB: Class I - unmated females, ovaries gravid; Class 2 - spermatophore full, ovaries gravid; Class 3 - spermatophore presumed partially sperm-depleted; and Class 4 - spermatophore presumed sperm-depleted, ovaries presumed egg-depleted. The authors found that a peak in egg-laying was observed precisely between the peak capture of class 2 females and class 3 females in light trap. However, for our study too, the peak capture of class 2 females (complete spermatophore) coincided with the peak capture of both male and female ECB, all classes combined, making the description of class or the study of spermatheca contents redundant.

In our study, we did not include the number of spermatophores found in bursa copulatrix, only spermatheca status (Empty, Half-full and Full). Yet, the number of spermatophores can be a valid indicator of mating success, fertility and fecundity rate of ECB. For ECB, Hinton and Andow (2003) found that 7.0 % to 15.1 % of captured females had more than one spermatophore (polyandry). Yet, higher fecundity (i.e., surface of egg mass) and fertility (i.e., number of hatched eggs) are observed in multiple-mated females than in single-mated ones (Fadamiro & Baker, 1999; Jiménez-Pérez et al., 2003). Spermatophore count is not the only relevant factor: five out of seven studies have shown an increase in fertility with increasing spermatophore size (Torres-Vila & Jennions, 2005). The males mating experience also seems to play a role, with female fecundity being higher when mating with virgin males. Indeed, subsequent spermatophores are smaller after the first mating (Jiménez-Pérez et al., 2003; Schlaepfer & McNeil, 2000). However, experienced males (3 matings or more) achieve higher mating success than virgin males,

despite a lower spermatophore volume (Schlaepfer & McNeil, 2000). In fact, the volume of ECB spermatophores decreases over time, resulting in lower fertility over the lifetime of successive partners of a given male (Royer & McNeil, 1993).

Here, for sake of simplicity we did not include the direct environment of the light trap. Yet, food resources are found to be highly relevant in the quality and quantity of spermatophores produced and exchanged. The amount of nitrogen in food, for example, has a positive impact on the lifetime fecundity of male and female ECB (Bonoan et al., 2015), and other lepidopterans (e.g., Heliothis virescens (Fabricius)) (Leahy & Andow, 1994). In organic corn and hop production, Bacillus thuringiensis (Bt) is often used as a method of controlling lepidopteran pests, particularly ECB. Crespo et al. (2010) showed that Bt-resistant adult ECB produced a smaller spermatophore volume and had a lower mating frequency, supporting the use of Bt also to reduce the potential for subsequent generations. We cannot therefore ignore that Bt treatments can have an impact on the study of the mating status of captured ECB. In a broader spectrum, climate change also has a negative impact on spermatophore production of lepidopteran (e.g., climate warming for the grape moth Lobesia botrana (Denis and Schiffermüller) (Iltis et al., 2020)), which supposes a great impact on the mating status of ECB females that we have not taken into account here. However, impact should only be on the density of ECB population, which is not related to the forecast of oviposition period.

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