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Lifecycle insights as a prerequisite for targeted management of *Cydalima perspectalis*

Poznavanje životnog ciklusa preduvjet je za uspješno suzbijanje šimširovog moljca *Cydalima perspectalis*

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ABSTRACT

The box tree moth (*Cydalima perspectalis* (Walker, 1859)), originally from Asia, has rapidly expanded its range in Europe, first detected in Germany and the Netherlands in 2007, likely via infested box tree (*Buxus* spp.) seedlings. In Croatia, the species was first recorded in 2012 in the Istria region. The larvae feed extensively on box trees, leading to defoliation and, in severe cases, plant death. This study, conducted on the Croatian mainland, focused on the development of first-generation larvae (n = 292) collected in April from the Garešnica region, just as they emerged from winter diapause. The pest development was monitored over six months, revealing that larvae resume feeding in mid-April and continue until late August before re-entering diapause. Degree day (DD) accumulation was tracked, showing that the first generation required approximately 560–640 DD to complete its development, while the second generation required 530–730 DD. Environmental factors, such as temperature, humidity and photoperiod, influenced larval development. Key periods for pest control were identified between late April to early May (when accumulated 53 DD) and late June to early July (when accumulated 478 DD), when larvae are in the early, more susceptible stages. Targeting larvae during these windows significantly reduced the second-generation population and minimized the number of overwintering larvae, which contributes to damage in the following spring. Understanding the lifecycle and environmental influences on *C. perspectalis* through degree day models is essential for optimizing management strategies and improving the timing and effectiveness of interventions to protect box trees from long-term damage.

Keywords: biology, degree days, defoliation, diapause, invasive species management, lifecycle

SAŽETAK

Šimširov moljac (*Cydalima perspectalis* (Walker, 1859)), porijeklom iz Azije, brzo je proširio svoj areal u Europi, a prvi put je otkriven u Njemačkoj i Nizozemskoj 2007. godine, vjerojatno putem zaraženih sadnica šimšira (*Buxus* spp.). U Hrvatskoj je ova vrsta prvi put zabilježena 2012. godine na području Istre. Gusjenice se intenzivno hrane šimširom, uzrokujući defolijaciju, a u težim slučajevima i potpuno sušenje biljaka. Ovo istraživanje provedeno je u središnjoj Hrvatskoj i započelo je razvojem gusjenica prve generacije (n = 292) prikupljenih u travnju na području Garešnice, netom nakon izlaska iz zimske dijapauze. Razvoj štetnika praćen je šest mjeseci, pri čemu je utvrđeno da gusjenice počinju hranjenje listom šimšira sredinom travnja i nastavljaju do kraja kolovoza, nakon čega ponovno ulaze u dijapauzu. Akumulacija efektivnih temperatura (DD) pokazala je da je prvoj generaciji bilo potrebno približno 560–640 DD za dovršetak razvoja, dok je drugoj generaciji bilo potrebno 530–730 DD. Na razvoj gusjenica utjecali su okolišni čimbenici poput temperature, vlage i fotoperioda. Ključni period za suzbijanje štetnika je kraj travnja do početka svibnja (kada se akumulira 53 DD) i krajem lipnja do početka srpnja (kada se akumulira 478 DD). Gusjenice su tada u ranijim, osjetljivijim stadijima razvoja. Provođenje suzbijanja u tim vremenskim okvirima značajno smanjuje populaciju druge generacije i minimizira broj prezimljujućih gusjenica koje uzrokuju štetu sljedećeg proljeća. Razumijevanje životnog ciklusa i utjecaja okolišnih čimbenika na vrstu *C. perspectalis* te korištenje efektivnih temperatura ključno je za optimizaciju strategija suzbijanja i poboljšanje učinkovitosti tretmana u suzbijanju s ciljem dugoročne zaštite šimšira.

Ključne riječi: biologija, stupnjevi dana, defolijacija, dijapauza, upravljanje invazivnim vrstama, životni ciklus

INTRODUCTION

Invasive species are non-native organisms that are introduced into ecosystems either intentionally or unintentionally. Once established, they can pose a significant threat to local biodiversity (Lemić, 2011). According to Mc Neeley's (2001), invasive alien species are organisms that have negative ecological, economic or health impacts. In recent decades, numerous cases have been discovered in which invasive species have been accidentally introduced into new areas where they do not naturally occur, leading to a variety of negative consequences (Canelles et al., 2021).

One of these species is *Cydalima perspectalis* (Walker, 1859) (Lepidoptera: Crambidae), known as the box tree moth or box tree pyralid - box tree caterpillar. Due to its uncontrolled introduction and rapid spread, this invasive species has become a major problem for plants of the genus *Buxus*. Through their feeding activity, they cause damage to plant leaves. Their feeding patterns cause distinctive damage, while the formation of cocoons and silk threads makes their presence on the plants easily identifiable (Ostojić et al., 2015). Additionally, they may feed on tree bark (Bunescu and Florian, 2016), which can lead to the death of branches or even the entire plant (Leuthardt and Baur, 2013). If populations left unchecked, they could cause heavy defoliation of boxwood (Burjanadze et al., 2019) which can kill the plant (Kawazu et al., 2007; Van der Straten and Muus, 2010; Kenis et al., 2013; Leuthardt and Baur, 2013; Nacambo et al., 2014.). This pest not only reduces the plant's market value but also diminishes its aesthetic appeal. For example, in a cemetery in Basel, Switzerland, plants affected by *C. perspectalis*, estimated at $2.7 million, would require $1.4 million for replacement (Leuthardt, 2013). In 2010, a massive infestation of box tree caterpillars in combination with the fungus *Cylindrocladium buxicola* Henricot led to the loss of almost 100 hectares of boxwood forests in southwestern Germany and northwestern Switzerland (Kenis et al., 2013; Wan et al., 2013). This species is responsible for significant economic losses and has demonstrated strong adaptive capabilities, facilitating its rapid and widespread distribution globally.

Originally from Asia, the box tree moth was first detected in Europe in Germany and the Netherlands (Krüger, 2008). Since then, it has spread to France, Austria, Switzerland, Belgium, the Czech Republic, Hungary, England, Slovenia, Italy, Greece, Turkey, Liechtenstein, Serbia and Croatia. In Croatia, it was introduced in the Istria region in 2012. It is assumed that the box tree moth came to Europe through the import of box tree plants and plant material (Hizal et al., 2012; Koren and Črne, 2012; Wan et al., 2013; Fora et al., 2016; Plant et al., 2019; Bird et al., 2020).

In its native habitats, *C. perspectalis* can be found on four identified plant species within the genus *Buxus*, including both wild and cultivated varieties (Leuthardt and Baur, 2013) as well as other non-*Buxus* plant hosts in its native habitat including *Euonymus alatus* Siebold (burningbush), *E. japonicus* Thunberg (Japanese spindletree), *Ilex chinensis* Sims (purple holly) and *Murraya paniculata* Jack (orange jessamine) (Wang et al., 2013; Wan et al., 2014). Given that species of the genus *Buxus* are prevalent in urban gardens, parks, private and historic gardens, and nurseries, the proliferation of the *C. perspectalis* in these environments represents a significant threat (Strachinis et al., 2015). In Europe, the pest has also been documented on alternative hosts such as *Rubus plicatus* Weihe & Nees (plaited-leaved bramble), *Ruscus aculeatus* Linné (butcher's-broom), and *Ruscus colchicus* Yeo (Matsiakh et al., 2018).

Cydalima perspectalis undergoes four distinct developmental stages: egg, larva (caterpillar), pupa, and adult. Young larvae are yellow-green with a black head, while mature caterpillars are green with thick black and thin white stripes, along with black dorsal spots (Matošević, 2013). Fully grown larvae reach approximately 4 cm in length before pupation (Korycinska and Eyre, 2010). Early instars feed collectively on leaves, whereas later instars disperse and consume entire leaves individually (Göttig and Herz, 2017). Frass and leaf fragments are readily observable on the ground around the base of the plant (Gutue et al., 2014). Adults *C. perspectalis* have a wingspan of 4 to 4.5 cm (Gutue et al., 2014). They are nocturnal and rest on leaf undersides during the day (Brua, 2013). This species has two color morphs: a common white form with dark brown borders and a melanic form with solid brown wings and a white streak or spot (Korycinska and Eyre, 2010; Hizal et al., 2012.).

The pest can produce 2 to 5 generations annually, depending on climatic conditions (Maruyama and Shinkaji, 1991). The number of generations varies depending on the temperature and the accumulation of developmental degree days (DD) (Nacambo et al., 2014) and the timing of adult emergence varies depending on the location. According to Matošević (2013) larvae undergo six developmental instars while Leuthardt and Baur (2013) observed five to seven instars for the summer generations, and three to seven instars for the overwintering generation. The average development time for larvae, from egg hatch to pupation, at temperatures ranging from 22 to 24 °C varied between 16 and 24 days (Leuthardt and Baur, 2013). Species larvae undergo obligatory diapause and overwinter within silk shelters constructed between two or more leaves (Nacambo et al., 2014). Diapause can commence between the second and fifth instars, with the majority overwintering as fourth instars in Japan (Maruyama and Shinkaji, 1991) and as third instars in Europe (Nacambo et al., 2014).

Therefore, we hypothesied that the lifecycle of *C. perspectalis* on the Croatian mainland differs from currently established knowledge. Our aim was to establish the degree day (DD) requirements for the first and subsequent generations of *C. perspectalis* under the climatic conditions of the Croatian mainland.

Control of *C. perspectalis* is essential to mitigate damage and prevent its further spread. The flight activity of this pest, along with other Lepidoptera species, is typically monitored using pheromone traps (Barić and Pajač Živković, 2020). Management strategies include both mechanical and chemical approaches, with mechanical control being the most effective and environmentally sustainable. A critical component of mechanical control is the removal of overwintering sites where the caterpillars enter diapause, helping to prevent the emergence of new generations in early spring (Ostojić et al., 2015).

Accurately determining optimal treatment parameters for *C. perspectalis* requires a thorough understanding of its biology in relation to specific environmental conditions. The purpose of this study was to examine the development and lifecycle of *C. perspectalis* under the climatic conditions of Croatia, with a focus on how temperature, humidity, and degree day (DD) accumulation influence each developmental stage. By analyzing the timing and duration of the larvae, pupae, and adult stages in this localized context, the goal is to create targeted pest control strategies that are finely tuned to regional climate dynamics. This approach not only improves the precision of interventions but also maximizes their effectiveness by aligning treatment applications with the most vulnerable periods in the pest's lifecycle. Through this detailed biological insight, pest management efforts can be better timed to reduce population levels, limit damage to box trees, and prevent overwintering larvae from causing early spring infestations.

MATERIAL AND METHODS

For this research, *Cydalima perspectalis* caterpillar samples were collected from infested boxwood plants from a garden. The sampling site was in Garešnica (45°32'18.1"N, 16°50'23.3"E), where significant box tree moth infestations were found, making it an ideal location for studying the pest's development and behavior under local environmental conditions.

The caterpillars were collected in mid-April 2021, at the time when the larvae emerged from diapause. They were then carefully transported in ventilated containers under controlled temperature and humidity conditions to the research site at the University of Zagreb Faculty of Agriculture laboratory. Upon arrival, the larvae were sorted into groups based on size and stage to standardize the population, ensuring uniformity for subsequent measurements and developmental observations. Growth and development were monitored weekly, with fresh boxwood leaves provided every two days as a food source. After each feeding, the caterpillars were measured, and the number of larvae at each developmental stage was recorded to ensure uniformity before pupation.

Mortality rates and sex ratios were assessed throughout the study to provide insights into population dynamics. Mortality was recorded at each developmental stage (larva, pupa, and adult), allowing for the calculation of stage-specific and overall mortality rates. The males of *C. perspectalis* have a more extensive brown coloration and tufts at the end of the abdomen than the females (Brua, 2013), making them easy to distinguish. These measurements helped establish the overall survival rates and the sex ratio of the first-generation adults.

After pupation, cages were set up with the pupae. After hatching, pairs of moths were formed, each consisting of one male and one female. The males of *C. perspectalis* have a more extensive brown coloration and tufts at the end of the abdomen than the females (Brua, 2013), making them easy to distinguish. A total of 10 male-female pairs being placed into individual cages, with each cage containing one pair of caterpillars for controlled observation or experimentation. Monitoring encompassed the observation of oviposition and the subsequent hatching of the caterpillars. The moths were fed a diet consisting of honey diluted in water and a slightly saturated saline solution, which provided the sodium necessary for optimal muscle and nerve development (Chapman, 1998).

The study lasted until September 1, 2021, with temperature and humidity conditions carefully maintained to replicate local environmental conditions of central Croatia. Developmental stages were recorded daily, allowing for comprehensive descriptive analysis. The data used in this study (i.e. maximum and minimum air temperature, mean daily relative humidity) were obtained from the Croatian Meteorological and Hydrological Service for the year of sampling. The maximum distance between the meteorological stations and the collecting location was 15 km.

The degree-day model (DD) was used for determining the beginning and end of each life stage of *C. perspectalis*. This model is based on the standard and widely used mean value method (Arnold, 1960) with a lower temperature threshold (namely the base temperature, T base):

$$D^{\circ}(d) = \frac{T\,max\;(d) + T\,min\;(d)}{2} - T\,base$$

where *D°(d)* being the degree-days accumulated during day *d*, *T min (d)* the minimum temperature of the day and *T max (d)* the maximum temperature of the day. This is a linear method because it assumes that the development rate is a straight line directly related to temperature (Wilson and Barnett, 1983). This model, used with a T-base of 10 °C, is most used to estimate insect development times (Trnka et al., 2007).

RESULTS

During the trial period from April to August, the weather conditions were characterized by a gradual increase in temperature and relatively stable humidity (Figure 1). In April, the mean air temperature was 10.1 °C, with a relative humidity of 68.5%. By May, the temperature increased to 15.1 °C, with slightly higher humidity at 71.5%. The highest temperatures were recorded in June (23.0 °C) and July (23.8 °C), with humidity levels at 64.9% and 70.5%, respectively. In August, the temperature slightly decreased to 21.6 °C, while humidity remained steady at 70.5%. These warm temperatures and moderate humidity levels provided favorable conditions for the development and activity of *C. perspectalis*, supporting rapid larval and pupal progression throughout both generations.

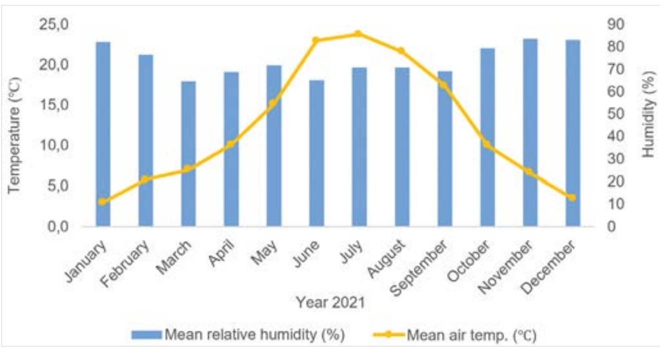

Figure 1. Weather conditions in central Croatia in 2021

Caterpillar size was utilized as a primary indicator for determining developmental stages. Although literature indicates that *C. perspectalis* undergoes six developmental stages in Croatia (Matošević, 2013), specific size-to-stage correlations were not observed. As a result, larval stages were determined independently based on our measurements. It was observed that the caterpillars did not exceed 30 mm in length, despite literature suggesting a potential maximum of 40 mm (Korycinska and Eyre, 2010). The criteria for categorizing the developmental stages are detailed in Table 1.

Table 1. Developmental stages and sizes of caterpillars

Developmental stage	Caterpillar size / mm
L1	0 – 5
L2	5 – 10
L3	10 – 15
L4	15 – 20
L5	20 – 25
L6	25 – 30

Table 2 records observations of *C. perspectalis* larvae, pupae, and adults at specific intervals after the collection of larvae that had emerged from winter diapause. The observations track developmental progress from larval to adult stages, as well as egg-laying activities, over the course of the study. For better visualization of developmental progress across two generations see Figure 2.

Figure 2 shows the distribution of *C. perspectalis* individuals across different developmental stages (L1–L6, pupae, and adults) from April to September 2021. Initially, on 23 April, a large population of larvae is observed, primarily in the L2 stage, with some in L3 and L4 stages. As time progresses, larvae transition to later stages (L4–L6), and by 19 May, adults begin to emerge, marking the start of the adult phase for the first generation. The first-generation development concludes in late May, with most larvae having pupated and emerged as adults.

Table 2. Developmental stages, adult emergence, and reproductive output of *Cydalima perspectalis* across two generations (April–September 2021)

Readings in 2021	L1	L2	L3	L4	L5	L6	Pupae	Adults	Male/ female	Eggs
23.04.	-	179	70	40	2	1	-	-	-	-
28.04.	-	30	33	28	12	3	-	-	-	-
05.05.	-	30	31	24	1	1	2	-	-	-
12.05.	-	5	21	27	17	5	5	-	-	-
19.05.	-	4	12	21	12	6	12	3	1/2	-
26.05.	-	3	10	15	10	6	21	7	3/4	-
01.06.	-	1	3	7	8	8	16	4	3/1	110
09.06.	-	-	1	2	5	4	18	5	2/3	80
14.06.	-	-	1	1	-	-	22	2	3/3	64
19.06.	-	-	-	-	-	-	-	11	7/4	71
21.06.	225	-	-	-	-	-	-	-	-	-
28.06.	100	87	12	-	-	-	-	-	-	-
07.07.	88	42	10	8	5	1	-	-	-	-
21.07.	34	76	12	3	3	1	2	-	-	-
27.07.	23	60	10	3	2	2	1	1	1/0	-
31.07.	16	50	15	8	3	6	2	1	0/1	-
05.08.	15	30	5	8	3	6	8	2	1/1	-
13.08.	14	28	11	2	3	2	8	8	5/3	44
19.08.	13	20	10	1	1	1	1	-	-	34
25.08.	11	17	7	1	1	-	2	-	-	-
01.09.	-	5	12	3	4	-	-	-	-	-

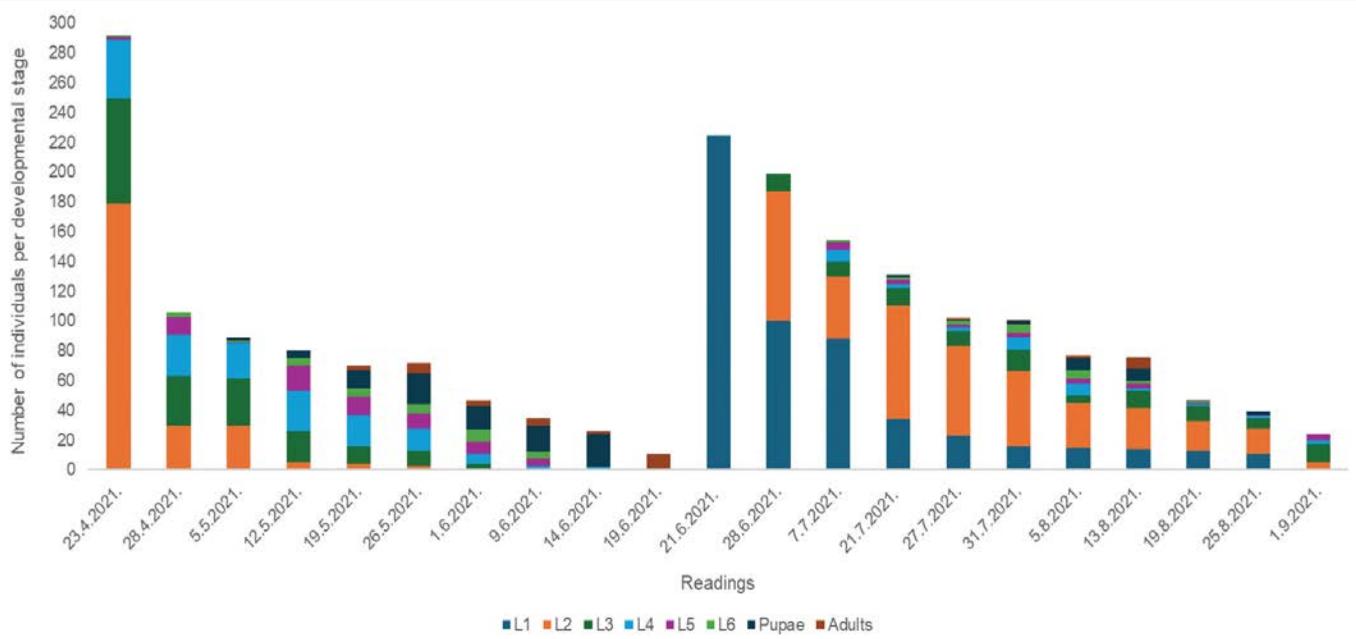

Figure 2. Developmental Progress of *Cydalima perspectalis* Across Two Generations

Starting on 21 June, the second generation emerges, with larvae predominantly in the L1 and L2 stages, like the first generation. A noticeable overlap between developmental stages is observed, particularly as the first-generation larvae are pupating and emerging as adults, while the second-generation larvae are hatching and developing. This overlapping of stages is important as it reflects the continuous lifecycle of the moth, requiring careful timing of control measures. By late August, the population across all stages declined, reflecting the maturation of the second generation.

Post-diapause larval development

On April 23, 2021, the first observation is made after field collection of larvae that emerged from diapause. At this point, most larvae are still in the early developmental stages, with 179 in L2 and 70 in L3. Only a small number of larvae have progressed to later stages, such as L4 (40 larvae), L5 (2 larvae), and L6 (1 larva). As the larvae develop, the subsequent observations on April 28, May 5, and May 12 show a gradual progression through the developmental stages. By May 12, there is a significant shift, with larvae distributed more evenly across L3 to L6 stages, and the first pupae are observed, indicating the beginning of pupation. This marks the transition toward adulthood.

Pupation and adult emergence

By May 19, the first adult moths (1 male and 2 females) emerge from pupae, signaling the start of the adult phase. The number of pupae and adults continues to rise over the following weeks. On May 26 and June 1, more adults are observed, with a peak of 11 adults (7 males and 4 females) recorded on June 19. Egg-laying activity begins on June 1, with 110 eggs observed during this first-generation reproductive period. The pattern of increasing pupation followed by adult emergence shows that most larvae complete their lifecycle by mid-June. The peak of reproductive activity coincides with this period, with decreasing egg numbers observed after the initial surge.

Second generation development

On 21 June a new generation of L1 larvae (225) marks the beginning of the second generation of the pest. These larvae follow a similar course of development to the first generation, with observations on 28 June and 7 July showing a gradual increase in the later larval stages (L2 to L5). At the end of July, the first pupae and adults of the second generation are observed, albeit in much smaller numbers than in the first generation. The emergence of adults of the second generation is significantly lower. On 5 August, only two moths (1 male and 1 female) were counted. This lower number of adults suggests that the second generation may be exposed to greater environmental or biological pressure, resulting in lower overall reproductive success. On September 1, it was observed that the 24 caterpillars in stages L3 through L5 stopped their development and were entering diapause.

The visualization of development of two *Cydalima perspectalis* generations and their overlapping is presented in Figure 3.

Mortality rates and sex ratios

Of the 292 larvae collected after diapause, only 82 developed to the pupal stage, and 63 successfully emerged as adults, resulting in an overall mortality rate of 78.4% from the first larval stage to adulthood. The pupal mortality rate alone was 23.2%, highlighting a significant loss during this stage. Among the adults, the sex ratio was 1.33 males for every female (36 males and 27 females). This ratio is crucial for understanding population management, as it influences mating dynamics and egg-laying potential, which are essential for predicting population growth and implementing effective control measures.

Egg-laying patterns

Laying activity is concentrated in the first generation, with a peak of 110 eggs observed on 1 June. The number of eggs decreases over time, with 80 eggs on 9 June and 64 on 14 June. At the end of June, only 71 eggs were recorded. In contrast, the second generation shows a significantly lower reproductive performance. The maximum number of eggs observed in the second generation is 44 on 13 August, falling to 34 by 19 August. Developed moths did not copulate, and no egg-laying activity was observed after August 19. Additionally, the eggs and pupae did not develop further after August 25.

Upon investigation, it was determined that the development of the first-generation spans between 60 and 70 days. The duration from oviposition to larval eclosion is approximately four to six days.

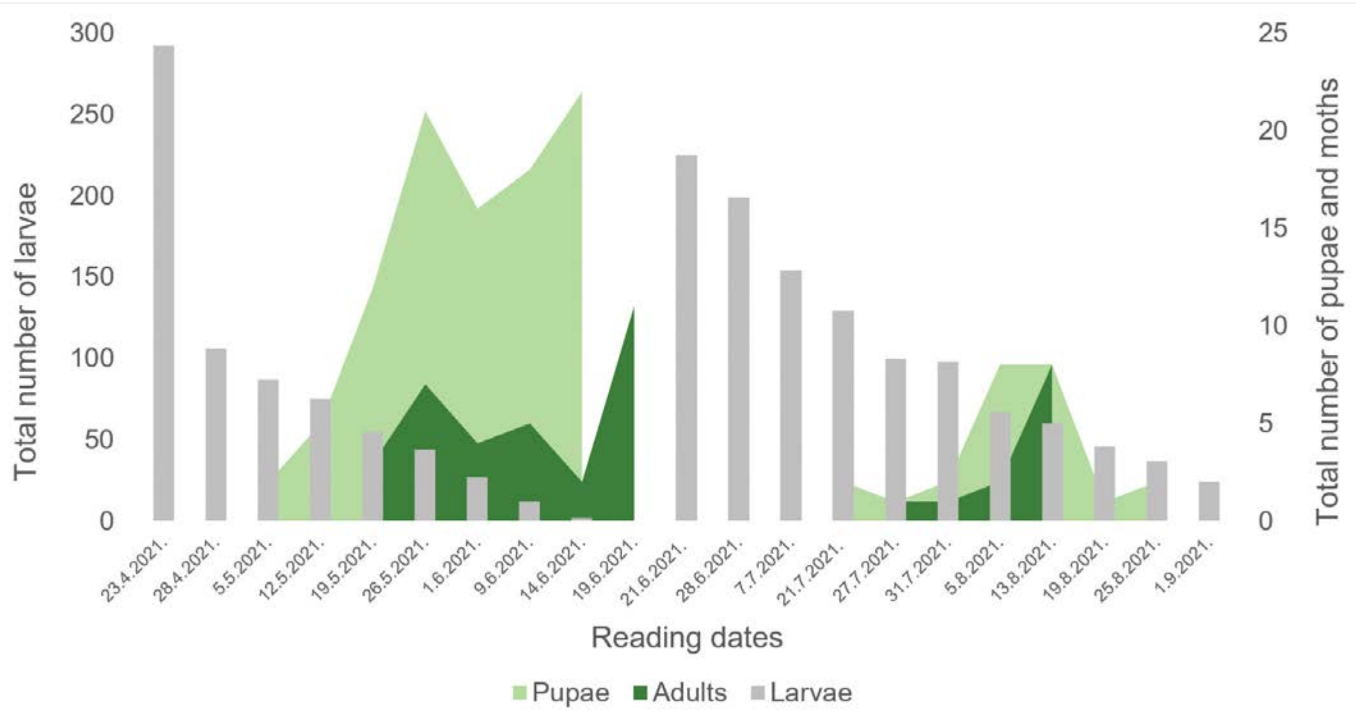

Figure 3. Development and overlapping developmental stages of *Cydalima perspectalis* across two generations

The complete larval development, from the first to the sixth instar, prior to pupation, lasts between 30 and 35 days. Following pupation, the transition to adult moths takes 10 to 15 days. The adult lifespan, defined as the period from eclosion to death, ranges from nine to twenty days. Table 3 provides a detailed overview of the duration of each developmental phase.

Figure 4 presents the degree day (DD) accumulation required for the development of *C. perspectalis* across its two generations, with DD calculations starting from January 1. However, it is important to note that the second generation begins its development in the late summer or autumn of the previous year, affecting the DD accumulation. The first generation requires 560–640 DD, while the second generation needs 530–730 DD to complete its development. For the first generation, larvae are observed after an accumulation of 53 to 302 DD, with pupae appearing between 106 and 378 DD. Adult moths emerge between 174 and 443 DD, and egg-laying is recorded between 242 and 413 DD. For the second generation, larvae appear between 478 and 1419 DD, while pupation occurs between 905 and 1362 DD. Adult emergence is observed between 989 and 1238 DD, and egg-laying is recorded between 1238 and 1316 DD.

Table 3. Duration of each developmental phase of *C. perspectalis*

Stage of the caterpillars	Duration of development until the second development phase /days
L2 – L6 (after diapause)	5 – 7 (per phase)
Pupae	10 - 15
Adults	10 - 20
Eggs	4 - 6
L1	2 - 5
L2	2 - 6
L3	2 - 5
L4	2 - 5
L5	3 - 6
L6	4 - 10
Pupae	15 - 20
Adults	8 - 15

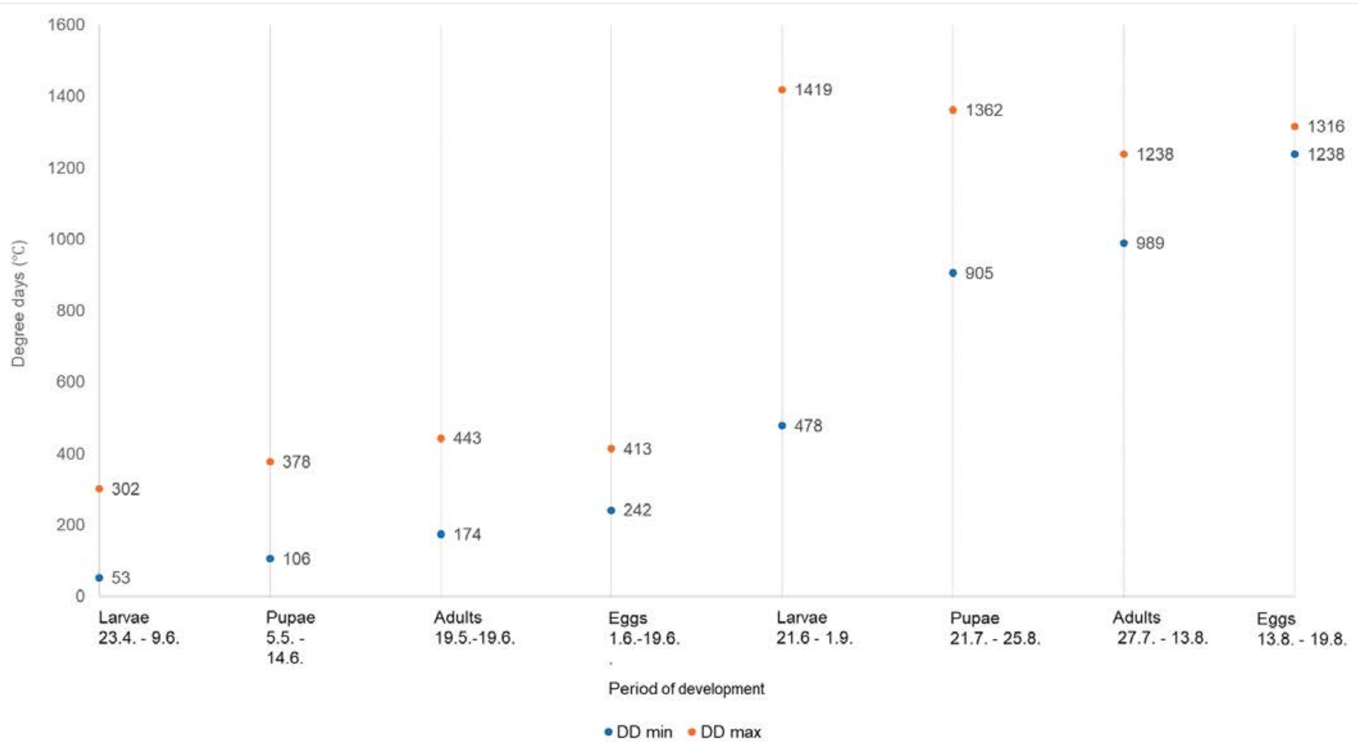

Figure 4. Degree day accumulation and developmental stages of *Cydalima perspectalis* across two generations

Optimal timing for pesticide application

Based on the developmental data of *C. perspectalis* larvae and the findings from degree day (DD) accumulation, the optimal time periods for pesticide application can be determined by targeting the early instar stages (L1–L3) when larvae are most susceptible. Most larvae of the overwintering generation are in the L1, L2 and L3 stages between 23 April and 5 May, which corresponds to an accumulation of over 53 DD. This makes late April to early May the ideal time for treatment as the larvae are actively feeding and have not yet transitioned to the later, more resistant stages. Similarly, the second generation begins around 21 June with a initial population of L1 stage larvae at an accumulation of 478 DD. Pesticide applications should be made between 21 June and 7 July when the larvae are still in the early stages. This is when larvae are most vulnerable, and early intervention maximizes efficacy by reducing larval populations before they move to later stages or enter the pupal phase.

DISCUSSION

The observations presented in this study offer a comprehensive analysis of the developmental timeline and reproductive patterns of *C. perspectalis* in central Croatia, highlighting two distinct generations. The first generation exhibited more robust development, with higher numbers of adults and greater reproductive output, while the second generation was smaller, with fewer adults emerging and reduced egg-laying activity. These findings underscore the importance of timing pest control interventions to coincide with critical developmental stages. Targeted management strategies during the early developmental periods, particularly targeting L1 to L3 instars, can significantly reduce overall population levels and limit the reproductive success of the species. The mortality rates observed during the larval and pupal stages provide further insight into the species' vulnerabilities, presenting opportunities for more effective control measures.

The reduced reproductive activity in the second generation could be influenced by ecological or biological factors, including resource availability, climatic variability, or population stress (Skendžić et al., 2021 a, b). Increased summer temperatures or reduced humidity may have contributed to slower development or lower reproductive success in the second generation. Additionally, competition for resources or population density effects may have played a role in reducing fecundity (Skendžić et al., 2021 a, b). These findings highlight the dynamic nature of the population and the need for well-timed interventions.

This study confirms the presence of two generations per year on the Croatian mainland and provides detailed insights into the length of development for each generation. The development cycle of one generation in this study took between 60 and 70 days, which is slightly longer than the 39–62 days reported by Cook et al. (2020). The extended development cycle allows for the overlap of different developmental stages, contributing to prolonged periods of damage. The second generation began developing at the end of June and continued until the end of August when diapause commenced. Despite earlier reports of three generations per year in Croatia (Matošević et al., 2017), our study did not observe a third generation, possibly due to climatic or local ecological conditions.

Comparative studies from other regions demonstrate varying numbers of generations per year. For instance, in China, up to four generations have been recorded, with the first generation hatching in mid-May and subsequent generations following in July, September, and November (Wang, 2008). In Japan, adults of the overwintering generation emerge in mid-May, with second and third generations appearing in late July and late August, respectively (Maruyama and Shinkaji, 1987). Similarly, in northwestern Switzerland, the first adults emerge in late June, peaking in July, with the second generation hatching from mid-August to early October (Nacambo et al., 2014). These differences likely reflect regional climatic variations, which impact the developmental cycle length and the number of annual generations. In Croatia, the extended development cycle and overlapping stages emphasize the need for carefully timed pest management interventions to minimize damage over the course of the growing season.

Our study revealed that pupae and adult emergence occurred earlier than reported by Weisner et al. (2021) in Ontario, where pupae appeared in the second week of June, and by Matošević et al. (2017) in Croatia, where pupae appeared at the end of May. This suggests a possible variation in the developmental timeline of the local population in Croatia. The earlier emergence aligns more closely with findings by Stan and Mitrea (2020) in Romania, where pupae appeared as early as the end of March in laboratory conditions and in mid-April in field conditions, indicating that the developmental cycle begins earlier in Romania. This variation may reflect different introduction routes or adaptations of local populations, but further genetic analysis would be required to confirm this hypothesis.

Our study observed that diapause in Croatia began in the second half of August, when temperatures ranged from 13 °C at night to 27 °C during the day, with larvae in stages L1 to L4 entering diapause. These findings are consistent with López and Eizaguirre (2019), who reported diapause initiation between mid-August and early September on the Iberian Peninsula. The timing of diapause appears to be influenced by regional temperature patterns, as noted by Poitou et al. (2020), who highlighted that both absolute temperatures and thermal variations can act as triggers. Similarly, in our study, the fluctuating August temperatures likely played a key role in prompting the early onset of diapause in central Croatia.

Korsakova et al. (2022) observed that larvae emerged from winter diapause within 3–4 days, with adult flight occurring within 9–10 days. Similarly, Elistovetskaya et al. (2020) reported that egg development lasted 3–5 days, larval development lasted 20–36 days, and the pupal stage lasted 11–13 days, with lower temperatures extending these durations. Our findings support these observations, with first-generation larvae reaching the L4 stage in 8–20 days. Most second-generation larvae entered diapause, with only a few completing developments. Additionally, second-generation adults had a short life span and did not lay eggs, potentially due to photoperiod or temperature-related factors.

In July, the day length during the emergence of the second generation in Croatia was approximately 15 hours, corresponding to a 15:9 light-to-dark photoperiod. López and Eizaguirre (2019) demonstrated that larval development is faster under longer daylight conditions, with a 16:8 photoperiod yielding quicker development compared to shorter day lengths. This suggests that the 15-hour day length during the second generation in Croatia may have contributed to slower development. Furthermore, our study found that many larvae pupated at the L5 stage, which is consistent with Maruyama and Shinkaji (1991), who observed that larvae often skip the L6 stage at lower temperatures. In our study, pupation occurred at temperatures between 21 °C and 23 °C, further supporting the temperature-dependent variation in developmental stage progression.

Our findings, combined with developmental data and degree day (DD) accumulation, provide insights into optimal periods for pesticide applications. Most larvae of the overwintering generation are in the L1–L3 stages between 23 April and 5 May, corresponding to over 53 DD (counting from January 1). This period, when larvae are actively feeding and have not yet reached the resistant stages, is ideal for pesticide application. The second generation begins around 21 June, with a large population of L1 larvae at an accumulation of 478 DD. Pesticides should be applied between 21 June and 7 July, when larvae are still in early stages and most vulnerable. Early intervention during this window can reduce population levels before larvae enter later stages or pupate.

Lastly, it is important to monitor early developmental stages, as caterpillars begin feeding in early spring and can cause significant damage. Visual inspections and early interventions should be conducted if high numbers or severe damage are observed. Additional control efforts should focus on the initial developmental stages of the summer generation, as larvae at this stage are more susceptible to treatments and are responsible for the next year's infestation rate. However, effective control is challenged by the limited availability of approved insecticides (Somsai et al., 2019). Early intervention before larvae form dense silk webs is crucial, as these webs complicate treatment and make larvae less responsive to chemical control.

CONCLUSION

This study confirms the hypothesis that the lifecycle of *Cydalima perspectalis* in central Croatia differs from established knowledge, with earlier pupal and adult emergence and an extended development cycle suggesting local adaptations. By establishing the degree day (DD) requirements for the first (560–640 DD) and second (530–730 DD) generations, the research highlights the influence of environmental factors such as temperature and photoperiod on development.

Targeted pest control strategies should focus on early larval stages (L1–L3), when larvae are most susceptible, to mitigate damage and reduce population levels. Incorporating degree day models into pest management provides a reliable tool for optimizing interventions and addressing the specific conditions of central Croatia effectively.

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