Life Cycle of *Cydalima perspectalis* (Walker) (*Lepidoptera: Crambidae*) in Craiova Area

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Abstract

Buxus sempervirens Linné is one of the most cultivated ornamental species, but in recent years it has faced a dangerous pest, *Cydalima perspectalis* (Walker) (*Lepidoptera: Crambidae*) native to the Asian Continent (China, Japan, Korea). In 2019 we conducted some investigations on the life cycle of this pest. The research was carried out both in the laboratory and in the field, in the 'Al. Buia' Botanical Garden from Craiova. Under laboratory conditions, this species has developed four complete generations, and the fifth generation, partial. The temperature in the laboratory was between 23.5°C-26°C, and the photoperiod during the study was between 12-15 hours of light and 12-9 hours of darkness. In the field this pest has developed three complete generations and the fourth was partial. The number of days for the generations developed in the laboratory is 272 days, and for the field population 286 days.

Keywords: *Buxus* spp., *Cydalima perspectalis*, evolutionary cycle

Introduction

The Box Tree Moth - *Cydalima perspectalis* (Walker) (*Lepidoptera: Crambidae*) is originating from the Asian Continent (China, Japan, Korea) (Inoue, 1982; Park, 2008). On the European Continent this pest was first detected in 2007 in Germany (Billen, 2007), it is considered that the species was introduced to the area in 2005 or even earlier. Van der Straten and Muus (2010), note that the first reports of larval attack are from Kehl as a possible infestation in 2006 and safe reports of the species in 2007 from Kehl and Weil-am-Rhein to near Basel, spreading further in the country.

In 2008 the species managed to spread to Netherland, France, Switzerland, Austria and the United Kingdom, and in the following years managed to colonize more than half of the countries on the European Continent (Bella, 2013; Feldtrauer *et al.*, 2009; Hizal *et al.*, 2012; Korycinska and Eyre, 2009; Leuthardt *et al.,* 2010; Matošević, 2013; Mitchell, 2009; Muus *et al.,* 2009; Ostojić *et al.*, 2015; Sáfián and Horváth, 2011; Salisbury *et al.*, 2012; Seljak, 2012). In Romania, this pest was first reported in 2010, in three different locations from Bucharest (Iamandei, 2010), but also in 2011 being present in the northwestern part of Bucharest and in the yard of a kindergarten (Szekely *et al.*, 2011), in Timisoara the first larvae were observed in 2013 in a park (Fora and Poşta, 2015), in other localities in Timiș county (Gugea and Vîrteiu, 2017), in Constanța (Skolka and Zaharia, 2014), in Cluj (Bunescu and Florian, 2016; Oltean *et. al.*, 2017), in Arad county (Don *et. al.*, 2016), in Satu Mare (Katona *et. al.,* 2016), in Sălaj (Katona *et. al.,* 2016), and in Craiova was reported in 2018 (Mitrea and Stan, 2018).

Since its arrival in Europe, the taxonomy of this pest has been repeatedly disturbed, being associated with several genera: *Palpita*, *Diaphania*, *Glyphodes*, *Neoglyphodes*, *Phakellura* (Kawazu *et al*., 2007, Korycinska and Eyre, 2009; Maruyama and Shinkaji, 1987, 1991, 1993; Szekely *et al*., 2011), but Mally and Nuss, (2010), clarified the situation in a paper on the phylogeny and nomenclature of this species by classifying it as *Cydalima.*

Symptoms of infestation include damage caused by larvae feeding with leaves, which can leave only the skeleton of the foliage (Leuthardt and Baur, 2013). The larvae can completely defoliate the host plant and eventually lead to its death.

Cydalima perspectalis Walker, is a monophagous species, (Van der Straten and Muus, 2010; Leuthardt and Baur, 2013; Wan *et al.,* 2014), but in Asia it has been reported on other species such as: *Buxus sinica* Rehder & E.H. Wilson, *Buxus microphylla* Siebold & Zucc., *Buxus microphylla* var. *insularis* Siebold & Zucc., *Buxus microphylla* var. *Japonica* Siebold & Zucc., *Buxus rugulosa* Hatusima, *Ilex purpurea* Hassk., *Euonymus japonicas* Thunberg, *Euonymus alatus* (Thunb.) Siebold, *Murraya paniculata* (L.) Jack. (Wan *et al*., 2014; Wang, 2008).

In Europe, the larvae feed on several species of *Buxus*: *Buxus microphylla, Buxus sempervirens, Buxus sinica* and *Buxus sinica* var cultivars *Insularis* and *Buxus sempervirens Rotundifolia* (Mally and Nuss, 2010; Korycinska and Eyre, 2009; Leuthardt *et al.,* 2010; Székely *et al.,* 2011; Hizal, 2012). The number of generations of this lepidoptera changes depending on the climatic conditions of the region (Maruyama and Shinkaji, 1987; Wan *et al.,* 2014). In Asia, 3-5 annual generations appear (She and Feng, 2006, Wan *et al.*, 2014) (in Japan, three generations from May to September), in Central Europe, Nacambo *et al*. (2014) reported only two generations per year. In northeastern Italy, Santi *et al*., (2015), found that *C. perspectalis* completes three generations per year. On the European Continent this species develops 2-3 generations, and in some areas with a warmer climate it can develop even a fourth generation (Leuthardt *et al*., 2010; Brua, 2013, 2014). In Romania in Cluj, *Cydalima perspectalis* Walker develops two complete generations, and the third generation is partial (Oltean *et al*., 2017).

For the developmental stages of egg, larvae and pupae, the temperatures varies between 8-12°C, depending on the environmental factors as well as the geographical position of the researched population (Maruyama and Shinkaji, 1987; Nacombo *et al*., 2014). To determine the larval stages, Maruyama and Shinkaji (1991), performed measurements of the cephalic capsules of the larvae, thus determining 7 larval instars. The average values for the development period of hibernating larvae are 44.6 days at a constant temperature of 15°C, 25.8 days at 20°C, 15.7 days at a temperature of 25°C and 15.0 days at 30°C. Complete larval development lasts between 17-87 days, depending on temperature (Maruyama and Shinkaji, 1987). Maruyama and Shinkaji (1991) reported that in the laboratory at 25°C the larvae of *C. perspectalis* developed 6 instars, instars 1-5 developed in 3 days and the 6th stage developed in 8 days. In areas of origin, full development for the second and third generation of the same year occurs in 24.9 ± 2.89 days at a temperature of 25°C (Maruyama and Shinkaji, 1987), and in North China the second and third generations of the same year completed their development within 24.9 ± 0.73 days at a temperature of 27°C (Zhang *et al.*, 2007). The mean period for the pupae stage is 38.4 ± 1.49 days at a constant temperature of 15°C, 17.3 ± 0.78 days at 20°C, 10.0 ± 0.36 days at 25°C and 7.1 ± 0.43 days at 30°C (Maruyama and Shinkaji, 1987).

Adult mating occurs only once in their lifetime and lasts about 2 hours (Cheng, 2005). The mean values for the preoviposition period were 5.5 days at 15°C and 3.2 days at 20°C, 2.3 days at 25°C and 2.2 days at 30°C (Maruyama and Shinkaji, 1987). Female fertility depends on the generation, decreasing from the hibernating generation to the third generation, from about 500 to 200 eggs (Wan *et al*., 2014). The eggs are laid on the underside of the leaves of the host plant, in groups of about 20-30 eggs (Bella, 2013). The lifespan of females is 8.02 ± 0.18 days and 8.69 ± 0.12 days for males (Zhou *et al*., 2005).

Studies conducted in Japan have reported a different duration of development of *Cydalima* eggs at different temperatures, so it has been shown that with increasing temperature, development (in days) decreases. At a temperature of 15°C the egg development period lasts 15.3 ± 0.64 days, at 20°C it lasts 7.1 ± 0.23 days, at 25°C it lasts 4.0 ± 0.15 days, at 30°C it lasts 3.0 ± 0.10 days (Maruyama and Shinkaji, 1987). Diapause is induced by a length of day less than 13.5 h (Maruyama and Shinkaji, 1993; Xiao *et al.,* 2011), so the life cycle of the Box Tree Moth includes a diapause of 6-8 weeks at low temperatures (Nacombo *et al.,* 2014).

In China most larvae overwinter in the 3rd or 4th instar (She and Feng, 2006, Xiao *et. al*., 2011), and in Japan they enter to diapause in the 4th or 5th instar (Maruyama and Shinkaji, 1991). In Europe this species overwinter in the third larval stage (Nacombo *et al*., 2014). In the spring, the feeding activity restarts.

Materials and methods

In 2019 we performed some investigation regarding the life cycle of *Cydalima perspectalis* Walker. The researches were conducted both in the laboratory and in the field in the 'Al. Buia' Botanical Garden from Craiova. For the research carried out in the laboratory, the biological material was collected from the field in February, before the beginning of the pest activity and taken to the Entomology Laboratory of the Faculty of Horticulture and placed in breeding cages (Fig.1). In the laboratory we monitored the average temperature and the photoperiod to which the larvae were exposed, and in the field we monitored the average temperature and precipitations. During the research, the following aspects were noted: the beginning of the hibernating larvae activity, the succession of developmental stages and number of generations per year.

Results and discussions

The experimental results obtained in 2019 following the research conducted in the laboratory of the Faculty of Horticulture and the 'Al. Buia' Botanical Garden regarding the succession of generations of The Box Tree Moth are presented in Table 1.

In laboratory conditions the overwinter generation started its activity on March 4, and in the field on the March 8. The feeding period of the hibernating larvae until the appearance of the first pupae was 24 days at an average temperature of 23°C, in the field the development of the larvae was prolonged for a period of 41 days, due to low temperature and precipitation during this period (11.0°C average monthly temperature and 24.0 mm average rainfall in March and 11.9°C average monthly temperature and 42.0 mm average rainfall in April) (Fig. 2). In the laboratory the first pupae were observed on March 28, the pupation period lasted 10 days, and the first adult appeared on April 7 (Fig. 3), after 3 days the eggs were laid.

In field the first pupae were observed on April 18, the period of the pupation was 15 days. And the first adults were observed on May 3. The minimum development period of the hibernating generation in the laboratory was 34 days and in the field was 56 days. The preoviposition period was 3 days in the laboratory, and the incubation period was 6 days, the larvae of the first generation started their feeding activity on April 16, their evolution period

Figure 1. *Buxus sempervirens* Linné, bushes (left) and *C. perspectalis* adults in growth box (right)

Table 1. The evolutionary cycle of the species *Cydalima perspectalis* Walk. in 2019 in Craiova area

Development stages	Laboratory		Field - 'Al. Buia' Botanical Garden	
Winter generation				
Larval stage:	04.03	01.04	08.03	27.04
minimum development period	24 days		41 days	
Pupae stage: first appearance	28.03	15. 04	18.04	10.05
Adult stage: first appearance	10 days		15 days	
	07.04	25.04	03.05	29.05
The first generation				
Egg stage:	10.04	27.04	07.05	05.06
incubation period	6 days		7 days	
Larval stage:	16.04	20.05	14.05	25.06
development period	29 days		36 days	
Pupae stage:	15.05	30.05	19.06	07.07
development period	10 days		12 days	
Adult stage:	25.05	12.06	01.07	26.07
The second generation				
Egg stage:	27.05	17.06	03.07	28.07
incubation period	5 days		6 days	
Larval stage:	01.06	02.07	09.07	19.08
development period	28 days		32 days	
Pupae stage:	28.06	11.07	10.08	30.08
development period	9 days		10 days	
Adult stage:	07.07	23.07	20.08	12.09
The third generation				
Egg stage:	09.07	27.07	22.08	16.09
incubation period	5 days		6 days	
Larval stage:	14.07	17.08	28.08	10.10
development period	29 days		36 days	
Pupae stage:	12.08	29.08	03.10	21.10
development period	8 days		12 days	
Adult stage:	20.08	10.09	15.10	***
The fourth generation				
Egg stage:	22.08	12.09	18.10	***
incubation period	5 days		7 days	
Larval stage:	27.08	30.09	25.10	wintering
development period	28 days			
Pupae stage:	24.09	10.10	***	***
development period	10 days			
Adult stage:	04.10	27.10	***	***
The fifth generation				
Egg stage:	07.10	30.10	***	***
incubation period	6 days			
Larval stage:	13.10	wintering	***	***
development period	***			

Figure 2. Cocoon hibernating larvae in the field (left) and larvae in the field (right)

Figure 3. First pupae in the laboratory (left); First adult of the population raised in the laboratory (right)

was 29 days. In the field the preoviposition period was 4 days, and the incubation period was 7 days, the larvae of the first generation appeared on May 14 and had a period of development of 36 days. The period of the pupae stage in the laboratory was 10 days, the first adults being observed on May 25. The minimum development period of the first generation of The Box Tree Moth was 45 days. In the field, the first pupae were reported on June 19 and the pupation period was 12 days. The first adults appear on first of July. The period of the evolutionary cycle of the first generation is 55 days.

The second generation appeared on May 27, the incubation period being one day shorter than the first generation. The evolution period of the larvae is 28 days, and the pupation period is 9 days. The first adults were observed on July 7. The development period for the second generation is 42 days. In the field the second generation appeared at the beginning of July, period of development of larvae instars was 32 days, the pupae stage was 12 days, and the period of the evolutionary cycle of this generation is 48 days, 7 days shorter than the first generation.

In the laboratory, the third generation laid the eggs on July 9, the first larvae started their activity on July 15, the larval development was 28 days, and the pupae stage was 8 days at a temperature of 26°C, the first adults being observed on August 20, the evolutionary cycle for the third generation in the laboratory was 42 days. In the field the third generation was observed on August 22, the larval development period was 36 days. During the research in the field we noticed that there were larvae that entered to diapause in the 4^{th} instar, others continuing their development. The first pupae were observed in the first decade of

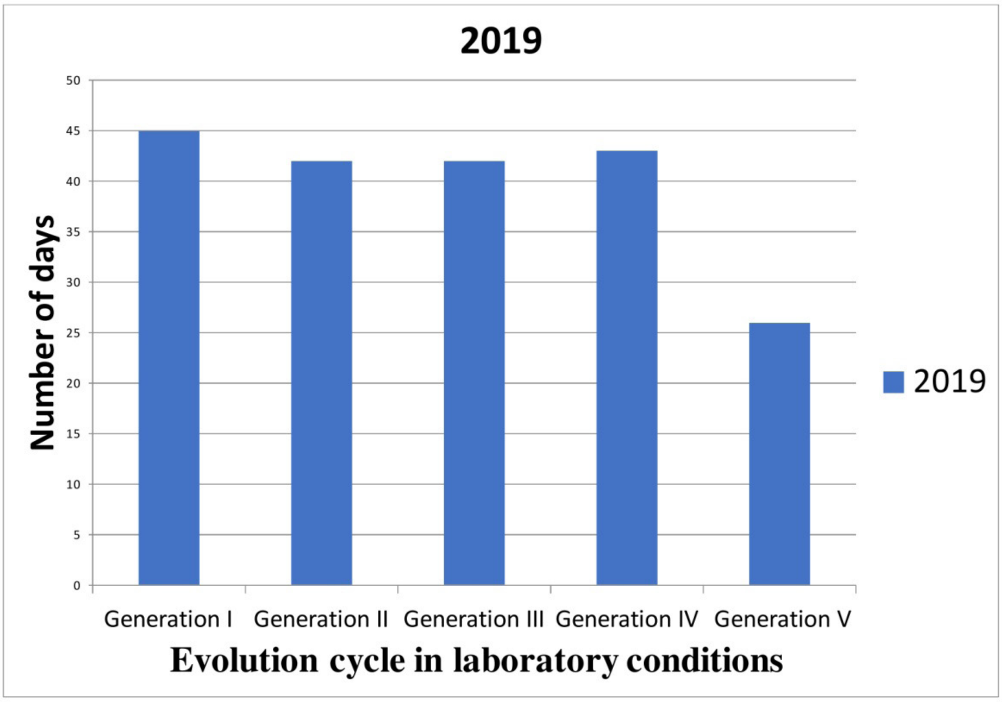

Figure 4. The evolutionary cycle developed in the laboratory / the period of the evolution of each generation (days)

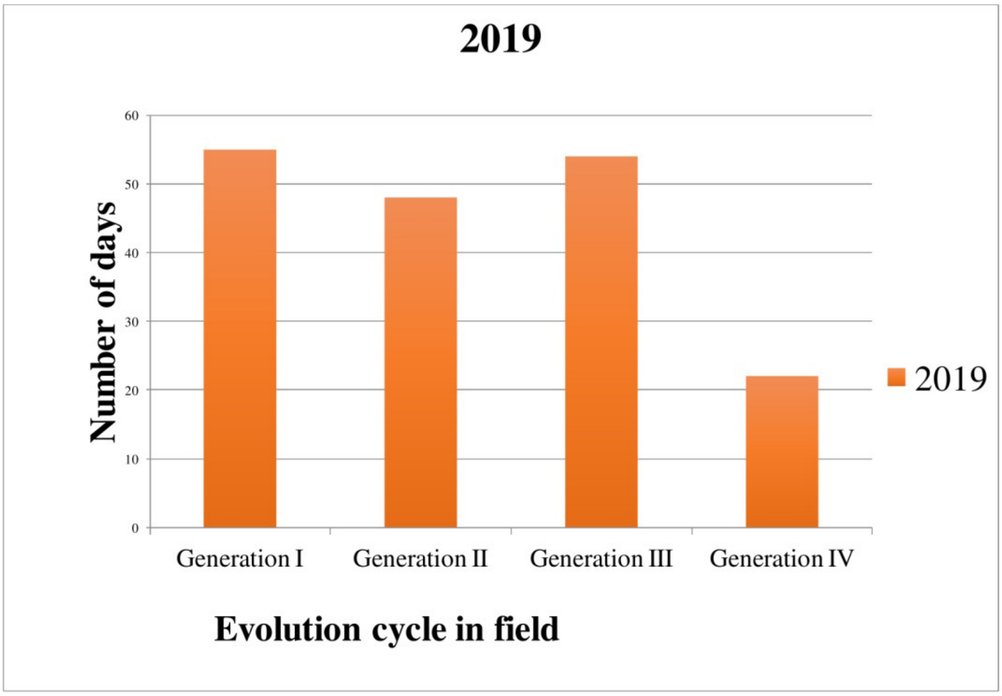

Figure 5. The evolutionary cycle of the species *Cydalima perspectalis* Walker developed in the field in 2019

October, and the pupae stage was 12 days, the first adults were observed on October 15, and the evolutionary cycle of the third generation totaled 54 days.

The fourth generation in the laboratory appeared on August 22, the larval development was 28 days, and the pupae stage was 10 days. The first adults appeared on October 4, the preoviposition period was 3 days, and the incubation period was 6 days. The duration of the complete development of the fourth generation in the laboratory was 43 days. In the field the fourth generation is incomplete, the eggs were observed on October 18, and the first larvae on October 25, after a short period of feeding the larvae entered to hiemal diapause, at this point the larvae were in second and 3rd instar.

The first eggs of the fifth generation appeared on October 7, and the first larvae appeared on October 13, most of the larvae overwinter in 4th instar. In laboratory conditions this species has developed 4 complete generations, and the 5th generation is partial, the larvae of this generation overwinter in 4th instar of development. (Fig. 4).

Following the observations made in the field, it was found that this species has developed 3 complete generations, and the 4th generation is partial (Fig. 5).

The prolonged period of development of hibernating larvae was caused by low temperatures and the amount of precipitation, which slowed down and affected their growth and development. The hibernating generation in the laboratory totaled 34 days, and the field generation 56 days. The number of days of the generations developed in 2019 for the laboratory population is 272 days, and for the field population 286 days.

Comparing these data it is observed that the population grown in the laboratory is 14 days shorter than in the field, but the number of generations is different, in the laboratory the fourth generation was complete, thus giving birth to a fifth generation, and in the field the fourth generation was incomplete. The difference of one generation for the population raised in the laboratory conditions resulted from the climatic conditions, food and light conditions and all these factors producing an environment conducive to a prolonged evolutionary cycle.

Conclusions

Under laboratory conditions *Cydalima perspectalis* Walker has developed four complete generations, and the fifth generation was partial. In the field this pest has developed three complete generations, and the fourth is partial.

The number of days of the generations developed in 2019 for the laboratory population is 272 while for the field population is 286 days. The development period for the hibernating generation was 24 days in the laboratory at a temperature of 23°C, and in the field it was 41 days at an average temperature between 11.0°C - 11.9°C for March and April. The average period of larval development was between 28-29 days for the generations in the laboratory, respectively 32-36 for the generations that evolved in the field. Like the other stages of development, the pupation stage is also influenced by temperature, so the pupation period for the laboratory population varied between eight days (at a temperature of 26°C) and ten days (at a temperature between 23°C - 24°C). In the field, the period of population depending on the generation varied between 15-10 days. The preovipsition period lasted between 2-4 days. Depending on the generation, the incubation varies between 5-6 days for the laboratory population and 6-7 days for the field population.

The difference of one generation for the population raised in the laboratory conditons resulted from the climatic conditions, food and light conditions, all these factors producing an environment conducive to a prolonged evolutionary cycle.

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