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BIOLOGICAL PECULIARITIES OF *CYDALIMA PERSPECTALIS* (WALKER, 1859) IN THE CONDITIONS OF THE REPUBLIC OF MOLDOVA

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ABSTRACT

The box tree moth *Cydalima perspectalis* (Walker, 1859) (Lepidoptera: Crambidae), dangerous pest boxwood plants in East Asia (China, Japan and Korea), is currently officially present in 35 countries of the Europe. In the plantations of *Buxus sempervirens* in the central zone of the Republic of Moldova, a new invasive pest *C. perspectalis* was revealed in several localities. As a result of studying the biological peculiarities, it was found that the phytophage develops in the republic in three generations, wintering at the stage of larvae of 2-3 ages. The flight of imago in spring begins in May, the next generations appear in the middle and end of summer. Flight of imago and oviposition can continue until September-October with overlapping generations. The development of eggs at a temperature of + 25 ± 2 ^{o}C continued 3-5 days on average, the development of larvae was 20-36 days, the stage of the pupa – 12 ± 1.0 days. Lowering the temperature increased the duration of each stage. Moldovan populations box tree moth, as well as all the other populations described earlier from Asia and Europe, have several different types of wing colouring – typical (white), intermediate and melanic morphs. Pest monitoring is required to control population density. To manage the density of pest populations, it is necessary to use both traps with sex pheromone and microbiological and chemical preparations, depending on the situation.

Key words: *Cydalima perspectalis (Walker, 1859), invasive species, biological peculiarities, life cycle, monitoring pest density.*

INTRODUCTION

The natural range of the box tree moth *Cydalima perspectalis* (Walker, 1859) (Lepidoptera: Crambidae) is the humid subtropical regions of East Asia: in China (Walker, 1859), Japan (Inoue, 1982) and Korea (Park, 2008). In addition, Hampson (1896) mentioned its occurrence in the Indian state Himachal Pradesh but this was the only record for India and therefore it is listed as unreliable and currently absent to India (CABI, 2020). Some authors point out that the Russian Far East – Primorsky Krai (part of it) is a native range for *C. perspectalis* (Kirpichnikova,

2005). However, there is an opinion that, apparently, the moth is not native there, because boxwood plants were also introduced into the region at one time (CABI, 2020). In Europe this pest was firstly registered in 2007, in Southern Germany. Imago and larvae *C. perspectalis* were detected when they fed on *Buxus* plants near the port of trans-shipment in Weil am Rhein (Baden-Württemberg) (Billen, 2007). Since the infection was significant and spread over a large area, it was suggested that the invasion occurred several years earlier – probably 2-3 years before its detection (Billen, 2007; Krüger, 2008). The author assumed that the possible pathway of the introduction is the Rhine port in Weil am Rhein and nearby Basel in Switzerland where large shipments of Chinese imports are arriving regularly. At the April of 2020, i.e. thirteen years after the first record in Germany and Switzerland, *C. perspectalis* was officially present in 35 countries of the Europe (Feldtrauer et al., 2009; Mitchell, 2009; Muus et al., 2009; Aistleitner, 2010; Iamandei, 2010; Mally & Nuss, 2010; EPPO, 2011, 2016; Safian & Horvath, 2011; Hizal, 2012; Koren & Črne, 2012; Pastorális et al., 2013; Gninenko et al., 2014; Ostojić et al., 2015; Strachinis et al., 2015; Bury et al., 2017; Bakay & Kollar, 2018; Plant et al., 2019; CABI, 2020). It is known, that imago the box tree moth are good flyers and can fly long distances. But most researchers believe that infested *Buxus* plants are the main pathway and source of infection, because eggs and larvae, especially of younger instars, are closely related to the host plant and are difficult to visually determine (Leuthardt et al. 2010; van der Straten & Muus 2010; John & Schumacher 2013; Nesterenkova et al., 2017). Thus, in Russia, in the Caucasus region *C. perspectalis* also probably brought with plants imported from Italy for landscaping the Olympic village in Sochi (Gninenko et al., 2014). In Europe, the pest was widespread due to the fact that the European Union is a free market for living plants and *C. perspectalis* was not included in the EU Plant Health Directive or was not classified as a quarantine pest by the European and Mediterranean Plant Protection Organization (EPPO, 2016). In the Republic of Moldova, this species was first registered in the private sector in 2015 on *Buxus sempervirens* plants (ANSA…, 2018). At the same time, we also detected the settlement of boxwood ornamental plantations in the central zone of the Republic of Moldova – in Chisinau city and its environs. In some places, the pest density was so high that it led to complete defoliation of *Buxus* plants and even to complete drying out of large sites of boxwood plantations. The exact invasion routes of *C. perspectalis* into the Republic of Moldova have not been identified. However, in all probability, this species was imported from Europe along with planting stock. In order to organize successful qualified control over the population density of a new dangerous pest, it is necessary to carefully study its biological special aspects in the new conditions. The aim of this paper is to describe the biological particularities of box tree moth with prognosis of its future spread and damages in the Republic of Moldova.

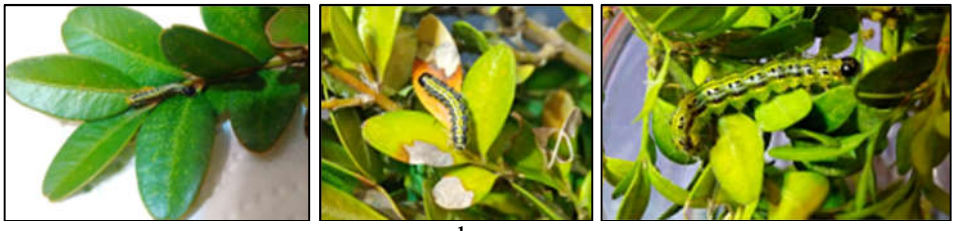
MATERIALS AND METHODS

Observations were carried out in the central zone of the Republic of Moldova – mun. Chisinau during 2016-2020. The number of pests *Cydalima perspectalis* on

boxwood plants (*Buxus sempervirens*) was recorded using the sequential method with an interval estimation of population density. In this regard, boxwood twigs were periodically cut off – 10 samples per 1 meter of boxwood hedge were taken. Larvae of all instars were brought to pupation under laboratory conditions at the +24±2 °C, 16-h day length, in plastic and gaze cages (10 x 10 x 10 cm). Fresh of *B. sempervirens* branches, partly immersed in water, were used as food for larvae of box tree moth. The adults were identified according to Mally and Nuss (2010).

RESULTS AND DISCUSSIONS

As a result of the surveys, we found that the pest *Cydalima perspectalis* overwinters in the conditions of the Republic of Moldova mainly at the stage of larvae of about the third instar (Fig. 1 a). The measurements showed that the length of the larvae was on average 0.9 cm and fluctuations from 0.6 to 1.2 cm. The width of the head capsule averaged up to 1 mm. From November to December, we also found a few cocoons with wintering larvae of younger ages, who started eating when transferring them to heat. Further laboratory observations of the larvae collected on boxwood plants during the winter showed that they molt another three to four times (less often five) and then pupate (Fig. 1 b, c; Fig. 2 a, b). The size of the larvae before pupation in most cases was 2.6-3.4 cm (maximally reached 4.0 cm), the size of the head capsule reached 2.5-3.0 mm. However, it was noted that the larvae before pupation could reach and only 2.2-2.4 cm in length. As a rule, the larvae of the wintering generation have the smallest sizes before pupation. Summer generations are most often significantly larger. Again, this also depends on humidity – the worse the quality of the feed, the smaller the larvae.



a b c

Figure 1. Larvae of *Cydalima perspectalis* of various instars (a – wintering larvae of the youngest instars, collected from *Buxus sempervirens* plants in January-February, b & c – larvae of the older instars), Republic of Moldova, 2020.

a b c d

Figure 2. Stage of puppies of *Cydalima perspectalis* (a – larvae in the woven cocoon, b –pupa in the first hours after molting, c & d – side and top view of pupa)

The newly formed pupa is light green, attached (not always upside down, often just lying in a woven cradle) with a cobweb to the boxwood branch (Fig. 2 b-d). Most often, before pupation, the larva weaves a dense cocoon of leaves. However, if the foliage is strongly damaged, the larva can be fixed before pupation with the help of a twisted cocoon between any branches. The length of spring pupae reached 2.2-2.3 mm.

Over time, the cocoon changes its color and, depending on the morphs of the imago, acquires its characteristic peculiar color (Fig. 4, a-e). The cocoon is attached by a web to the leaves of the plant, in the place of attachment the head capsule of the larva remaining after molting is visible. At a temperature of +22…+24 ^{o}C, pupal development continued 12±1.0 days. With a decrease in temperature to +15…+18 ^{o}C, the stage of development of the pupa increased to 17-20 days or more. Our data are similar to those obtained previously by other authors (Göttig, 2017; Nesterenkova et al., 2017). Thus, according to Göttig (2017) the pupal stage at a temperature of +25 ^{o}C lasts 9±0.5 days, at +20 ^{o}C – 17±1.0 days; according to the Nesterenkova (2017) the pupal stage at a temperature of +23 ^{o}C lasts 9 days. We assume that the development of pupae depends not only on the sum of the effective temperatures and photoperiod, but also on the quality of the feed consumed by the larvae before pupation.

It is well known that *C. perspectalis* populations have several different types of wing coloration. White wings with a dark brown margin and small characteristically crescent-shaped white marks on it characterize representatives of the typical, most common morph (Fig. 5, c-d). The body of the imago is white with a brownish abdominal segment. The melanic morph, has both a body and wings of almost the same dark brown color, with the exception of two white marks (Safian & Horvath, 2011; Szekely, 2011 & Göttig, 2017). In addition, some authors note that exists an intermediate phenotype with an extra brown margin at the forewings, which is also recognized in China (Pan et al., 2011). The moldavians' populations also have several different types of wing coloration – typical (white), intermediate and melanic morphs (Fig. 5, a-d). Our studies have confirmed the opinion of other authors (Göttig, 2017) that melanic morphs can be identified already at the pupal stage (Fig. 4, c-e).

a b c d e

Figure 3. Pupae of *Cydalima perspectalis* before the imago fly (a, b, c – top, side and bottom view of the cocoon white morphs, d – top view of the cocoon intermediate morphs, e – side view of the cocoon dark morphs

a b c d

Figure 4. Imago of *Cydalima perspectalis* top and bottom view (a, b – female intermediate morph, c, d – male typical white morph)

The moths of *C. perspectalis* are active at twilight, lead a nocturnal (and crepuscular) lifestyle. The life cycles these pest occurs completely on *Buxus* plants, were oviposition, larval development, pupation and overwintering take place. Basically, in nature, females lay eggs on overleaf. It is very difficult to find eggs on the boxwood branches, as freshly laid flat eggs are pale green, almost transparent, with a diameter of only about 1 mm. With advancing age, the eggs turn yellow, a dark head of a developing larva begins to shine through the shell. In the laboratory, egg laying can occur not only on the back side of the green leaves of the *B. sempervirens*, but also on the upper side of the leaf, as well as on other objects in the cages. The number of eggs in a clutch varies from a few to several tens, and depends on many factors – on the age of the females *C. perspectalis*, and on the conditions of rearing and feeding. In natural egg-laying, there was also a large discrepancy in the number of eggs, but in most cases it was from about 8-13 to 17-20 eggs in a clutch (Fig. 5, a). The duration of the egg stage in the laboratory was 3-5 days, in the nature its depended on the temperature conditions. Hatching larvae reach about 1.5-2 mm in length (Fig. 5, b, c). The duration of larvae development since hatching from eggs in the laboratory conditions ranged from 20 to 36 days, depending on the generation and sum of effective temperatures.

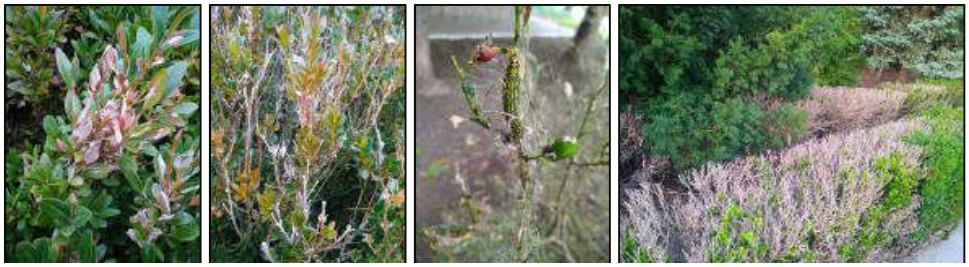
a b c

Figure 5. Eggs and new hatching larvae of *Cydalima perspectalis* in the laboratory conditions (a – ovipositions, b, c – hatching of larvae and newly hatched larvae)

Larvae of the youngest instars feed aggregated, causing damage on the exterior leaf layer. Later they spread over the plant, weave a cocoon and feed separately, typically sparing the vein and often additionally attacking the bark. Different authors point to a different number of instars for larvae *C. perspectalis* – from six to eight, in depending on the host species *Buxus* plants (Maruyama and Shinkaji, 1991; Göttig, 2017; Nesterenkova et al., 2017). We counted six and seven instars in the laboratory, when feeding the larvae with *B. sempervirens* plants. However, we believe that the number of moults may vary depending on external factors, and maybe for other reasons. Our observations have revealed that if larvae were collected from December to February in third instar, then under laboratory conditions they molted another three to four times. We also revealed a dependence of the development (however, as in many species of insects) of all stages *C. perspectalis* from egg to pupa on temperature. The lower the temperature, the longer the development time of each stage. With a decrease in temperature from 30 to 15 ^{o}C, the duration of the larval stage increases by more than 4 times, eggs and pupa stages – two to three times. Consequently, our studies of the development of *C. perspectalis* eggs, larvae and pupa showed a pronounced temperature dependence, which is confirmed by other authors (Maruyama and Shinkaji 1987; Leuthardt, 2013).

The Box tree pyralid *C. perspectalis* is an herbivorous insect that is highly monophagous and specialized on various species of plants of the genus *Buxus* – *B. microphylla, B. microphylla* var. *insularis, B. m.* var. *japonica, B. m.* var. *faulkner, B. sempervirens, B. sempervirens* var. *sempervirens*, *B. s.* var. *rotundifolia*, *B. s.* var. *argenteovariegata*, *B. s.* var. *aureovariegata, B. sinica* (Maruyama, 1992; Maruyama and Shinkaji, 1991, 1993; EPPO, 2011; Leuthardt and Baur, 2013; Leuthardt et al., 2013; Plant et al., 2019). Although some authors point to damage by larvae and some other plant species – *Euonymus alatus* (Thunb.) Siebold, *E. japonicus* Thunb. (Celastraceae), *Ilex chinensis* Sims (Aquifoliaceae) and *Murraya paniculata* (L.) Jack (Rutaceae) (Bury et al., 2017). Our observations over five years have shown that in the conditions of Republic of Moldova, the phytophagus has so far been found only on *B. sempervirens* plants. We found several foci of infection of the *B. sempervirens* plants with a larvae

C. perspectalis (Fig. 6, a-c). For five years, various degrees of infection were observed in different places, with 3 overlapping generations. As a rule, the beginning of the flight month of the first generation in the conditions of the Republic of Moldova occurs in May, the second – at the end of June-July, and adults of the third generation appear in August-September.



a b c d

Figure 6. Damage caused by larvae of *Cydalima perspectalis* to the *Buxus sempervirens* plants (a – by larvae of younger instars c – by larvae of older instars, d – irreparable damage), mun. Chisinau, Republic of Moldova, 2018-2019.

Within three years after the pest was found in the Republic of Moldova, in those foci of infection where at least several treatments were not performed during the spring-summer season, the phytophage completely destroyed boxwood plants, which subsequently dried up and almost completely died (Fig. 6 d). With timely chemical treatments, the phytophage abundance was significantly suppressed, which kept the plantations of the *B. sempervirens* plants in excellent condition. At the same time, it was established that when viral preparations were used in combination with sex pheromone traps, over time, both the number of the pest density decreased and the number of various entomophagous accumulated on the plants, and as a result, there is a death of about 10-20% of the *C. perspectalis* larvae from diseases, probably of viral origin.

Until now, it is not clear how the pest entered the territory of the Republic of Moldova. Many authors in their scientific papers put forward the hypothesis that the phytophage was introduced with plant material (Leuthardt et al., 2010; van der Straten and Muus 2010; John and Schumacher 2013; Gninenko et al., 2014). We also do not exclude this possibility. Moreover, in the Republic of Moldova there is a fairly wide range of imported plants, which are very popular in landscaping the territory of private companies, various enterprises and organizations, as well as private sites.

At the same time, a number of authors (Plant et al., 2019) indicate good flying qualities of *C. perspectalis* imagoes. And also, the species probably to be capable of bearing fairly low temperatures and spreading naturally in the continent (Feldtrauer et al., 2009; Muus et al., 2009; Bakay and Kollar, 2018). It is possible that climate change and human activities are associated with the independent expansion of many species of insects. And *C. perspectalis* is not an exception, but one of the species that have independently reached Moldova. Unfortunately, due to

its small economic importance, the species may be left without due attention from various services and institutes of plant protection. And it is not known in the future what will be the fate of boxwood, used for decorative and landscaping purposes.
Some authors (Buchsbaum and Segerer, 2013) do not consider the invasion of the *C. perspectalis* to be an event requiring urgent measures and special attention, since boxwood is not an autochthonous plant for many regions of Europe. They consider this only enrichment of biota. It is difficult to say who is right in this matter. However, we believe that monitoring populations in the conditions of the Republic of Moldova is relevant and of interest both from a scientific point of view and from a practical point of view, for managing population density and containing damage to boxwood ornamental stands.

CONCLUSIONS

In the conditions of the Republic of Moldova pest *Cydalima perspectalis* development on the *Buxus sempervirens plants* in 3 generations. Imago fly from May till September-October. Moldovan populations box tree moth, as well as all the other populations described earlier from Asia and Europe, have several different types of wing colouring – typical (white), intermediate and melanic morphs. Phytophage overwinters mainly at the stage of larvae of 3-rd instar, but it is able to hibernate in the climate of the Republic of Moldova at the second instar. The duration of the egg stage at the optimal temperature +25±2 ^{o}C consist in average 3-5 days, larval development – from 20 to 36 days, pupal stage continued 12±1.0 days. The development of all stages depends primarily on the sum of effective temperatures, as well as on other factors (host plant, etc.). With decreasing temperature, the duration of each stage increases, with increasing (up to a certain limit) it decreases. Consequently, climate change undoubtedly contributes to an increase in the number of populations of the invasive harmful species. To control the number of pests, it is necessary to carry out several treatments with systemic insecticides per season. If it is impossible to use chemical preparations, preference should be given to pheromone traps and microbiological preparations, especially of viral origin.

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