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Development of *Cameraria ohridella* DESCHKA & DIMIĆ, 1986 (Lepidoptera, Gracillariidae)

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Abstract

The paper deals with the life cycle of *Cameraria ohridella* DESCHKA & DIMIĆ, 1986 (Lepidoptera, Gracillariidae), the leaf miner of the wild chestnut.

Zusammenfassung

In der vorliegenden Arbeit wird der Lebenszyklus von *Cameraria ohridella* DESCHKA & DIMIĆ, 1986 (Lepidoptera, Gracillariidae), der Kastanien-Miniermotte, dargestellt.

Introduction

The leaf miner of wild chestnuts, *Cameraria ohridella* DESCHKA & DIMIĆ, 1986 (Lepidoptera, Gracillariidae) was observed for the first time in the region of Ohrid (Macedonia) in 1985 (SIMOVA-TOŠIĆ & FILEV 1985, DESCHKA & DIMIĆ 1986). Over the past few years it has spread rapidly northwards across all chestnut-populated stretches of land in Serbia (DIMIĆ & MIHAJLOVIĆ 1993), thus becoming a new species in the fauna of Serbia. The miner has so far spread far beyond the boundaries of its initial sighting (Macedonia, Serbia, northern Bosnia, Hungary, the Czech Republic, Austria). The species is new and not yet sufficiently studied. Furthermore, the miner as a rule occurs massively, causing premature defoliation of chestnut trees in wide areas. Defoliated chestnut trees completely lose their ornamental and other characteristics that make the species popular in urban surroundings worldwide. In addition to aesthetic qualities, however, the species' great importance in ecological, sanitary, health and economic respect should never be neglected. Chestnut protection against noxious leaf miners is therefore evidently needed. However, the fact that wild chestnut trees are mostly grown in parks, rows along thoroughfares and gardens in human settlements of all sizes (JOVANOVIĆ 1950) makes their protection highly delicate and, if carried out chemically, highly dangerous. Insight into the pest's development and its monitoring is therefore crucial for successful chestnut protection (i.e. the choice of adequate method, time of application and chemicals). It is also important to monitor the varying ecological factors of the environment. This is the basis of modern, successful and rational plant protection against pests in general.

Location, material and method

Cameraria ohridella has been investigated continuously for more than ten years. Previous investigations had been carried out in Sarajevo over the 1985-1992 period in natural environment, but in strictly controlled conditions, because the species had not been registered as occurring naturally in the region of Sarajevo. Since 1993, the development of *C. ohridella* has been investigated at several locations in the Belgrade region, in conditions of fully natural environment and in controlled natural conditions (in isolated or closed systems) simultaneously at Banovo brdo (Faculty of Forestry, Belgrade), Topčider and Zemun (Institute for Plant Protection and Environment, Belgrade), Smederevo (Plavinac), Čortanovci (Novi Sad), Obrenovac, ect.

The original investigation material (chestnut leaves infested by miners, their caterpillars and pupae) was collected at several locations in a wide area of Belgrade and each sample was investigated separately. The course of moth eclosion was also investigated on the material (100 chestnut leaves inhabited by miners) collected by using entomological cages (30 x 30 x 60 cm) at a number of localities. For investigation of the course of eclosion, photoeclectors of different sizes and shapes were used. It should be pointed out, however, that they proved unreliable in exact investigations despite their adaptability (a large number of moths died without ever appearing in the test tube or flacon). Photoeclectors may therefore be used for investigations of miner parasites.

Wild chestnut seedlings intended for isolating miners were planted into pots of adequate dimensions (20 cm deep, upper diameter 18 cm, lower diameter 12 cm) to fit comfortably into entomological cages or cylinders. Over the entire vegetation period, young chestnut plants were cultivated in isolation inside entomological cages (30 x 30 x 60 cm) and glass cylinders (50 x 22 cm) topped by thick metal (brass) network. It is possible to use a sleeve-like isolator of fixed dimensions (mostly 60 x 20 cm), made of dense perlon tulle, marquisette or organdy. These isolates are normally placed onto selected branches of older trees, which is the simplest method in practice, but we did not apply it for several reasons.

C. ohridella moths were released into the cages and cylinders in certain numbers and the entire process of development was monitored (copulation, oviposition, heatching of caterpillars, development of caterpillars, transition into pupal stage, moth eclosion). To avoid probable confusion and mistakes, only the moths emerged on the same day were released onto the plants. For the same reason, each generation of moths was always isolated on completely new plants free of mines from the previous generation (i.e. grown protectedly in cages or cylinders). After moth emerging, the leaves with mines were removed from chestnut seedlings. They were conserved until the following spring in natural environment, in glass jars of different sizes closed by perlon tulle, to enable the monitoring of moth emerging. Leaves taken from each cage were put into separate jars, and this method has proved fully justified. Firstly, it prevented erroneous results and incorrect conclusions. Besides the expected results, this method also provided an excellent opportunity for distinctive traits of the investigated species to become manifest, registered and investigated, producing also some unexpected results.

Results

C. ohridella is a monophagous species living solely on the leaves of the wild European chestnut (*Aesculus hippocastanum* L.).

The results of the investigation of *C. ohridella* leaf miners, conducted in the natural environment and controlled natural conditions, enable the application of some highly successful measures of wild chestnut protection, which was the primary objective of our research. Trials carried out in controlled conditions in the natural environment (not in laboratory) provided precision in work (by eliminating factors such as parasites, etc.), and precluded incorrect conclusions (which are quite frequent in investigations of polyvoltous species in fully natural conditions). In addition, they enabled that some very important details regarding the species' development and the distinctive traits of its development be fully registered. The development of *C. ohridella* has been recognized as highly distinctive.

The results of several years of investigation showed that *C. ohridella* invariably gave three generations annually (calendar of *C. ohridella* development) in the entire area of current distribution and outside of it (Sarajevo), Belgrade included, regardless of the locality, the variation of ecological conditions in it and the year of investigation. Hence, the species has a constant number of generations in the entire area of distribution. Ecological factors, temperature in particular, manifestly exerted their influence only on the development duration and the time of the early signs of the species' development stages (e.g. beginning and course of moth emergence). According to hitherto results, the emerging of moths inside a single region begins in the area of Smederevo, moving to Belgrade and then to Obrenovac. The beginning and duration of emergence may vary significantly depending on the location. Significant differences were also observed depending on the year of investigation (variation of ecological factors - e.g. moth emergence of the overwintering generation started in Belgrade on April 19, 1993 and on March 31, 1994). The beginning and course of moth emergence is therefore influenced by ecological factors (primarily temperature variation) and the year of investigation. All this indicates a need to monitor the species' development annually and at each locality in order to provide its successful control.

The emerging of the third, overwintering generation of moths in the region of Belgrade occurred in April, starting most often on April 5. The results indicate, however, that the time of the beginning of emergence may vary considerably. The emerging of this generation lasted for about 30 days (28 days in 1994), longer than other generations, which is understandable considering the period of the year. The spring emergence (beginning and course) is simultaneous for all pupae of the first, second and third generation and it produces a new generation, the first of that year. It has never happened that some pupae remain in diapause up to the moment of the emergence of the next generation or the next year. The beginning and course of the flowering phenophasis of wild chestnuts greatly varied and was generally not simultaneous for all trees in a location. The investigation proved that the first moths appeared always, without exception or variation (locality, year), simultaneously with the first chestnut blossoms. Moth emergence from the overwintering pupae is always synchronized with the blossoming of wild chestnut. The first generation, which is the most important, cannot and should not therefore be controlled by insecticides on the account of the protection of pollinating insects. To control other generations, the flight of moths needs to be monitored, which has so far been possible only using the methods decribed in the chapter "Location, Material and Method".

It is characteristic of the moths of all three generations to stay predominantly on the bark of chestnut trunks, rather than on other trees in the neighbourhood. Merely a few hours after the emergence (i.e. after straightening up and drying their wings to be able to fly), the first moths copulated and immediately after that (over the initial 24 hours) the females started to oviposit. Other individuals copulated and oviposited later, over a long period. They oviposited into the settled parts near the secondary nerves on the upper side of the leaf because otherwise they could easily fall off the leaf surface. After 6-10 days, which is the period of embryonic growth, caterpillars hatched and immediately bored into the leaf epidermis cells underneath chorion, without ever emerging on the leaf surface.

The first instar caterpillars fed exclusively on the cellular sap from the leaf face epidermis ("Sap feeder") and formed tiny white line-shaped mines whose length rarely exceeded one milimeter. At the next stage, the second instar, contrary to the position of the chorion, the larva added to this hall a small round part of the mine measuring 0.6 - 1.0 mm in diameter. In this phase, therefore, the larva did not enlarge the primary hall of the mine, as other leaf miner species do. In later instars, the larva enlarged the mine at the peripheral part, creating an irregular blotch-mine field (Platzmine). Leaf face epidermis separated from the palisade tissue underneath, turning greenish yellow and soon brown at the central part. Older caterpillars not only widened the mines but also deepened them all the way to the epidermis of the leaf backside, feeding on the cells of the palisade and sponge-like parenchyma of the leaf ("Tissue feeder"). This is where the mine became transparent. The mines took various shapes: round, elliptic, amoebic, sometimes quite elongated into a kind of a hall (narrow and long). At first impression, the damage caused by miners seems similar to diseases caused by pathogenic microorganisms, e.g. *Gvignardia aesculi* STEWART (MILATOVIĆ 1956), and they are often and unjustifiably confused with them in practice. The diameter of the formed mine (after caterpillar has completed growth and passed into pupa stage) also varied considerably, ranging from 16.9 to 56.8 mm, but most often it is about 24 mm. The mines were placed densely together, with mine fields overlapping and creating large mine blots. Many of the caterpillars died at this stage. Besides, the entire leaf surface was at the time of moth eclosion often occupied by mines of the previous generations (late July-early August, when chestnuts are already completely brown), so that moths of the first and especially of the second generation had a very slight chance of reproducing that year. A considerable and as yet unaccounted loss occurred in the first instars of caterpillar. According to our observations over several years, those losses made a regular or normal phenomenon not only in natural surroundings but in isolators as well, reaching about 20 % (mortality of the first instar larvae in Belgrade was 19,11% in 1993 and 23,01% in 1994). However, the mortality of caterpillars occasionally rose sharply, influenced by different factors that have not been fully identified (over-moistured insides of the mine over the rainy period, ect.). The mortality of the larvae was the highest on the outermost leaflets (left to right: leaflets 1, 2 and 7) although the number of mines there was the lowest. The lowest mortality was on the big central leaflet, although there was the highest number of mines. A larger number of caterpillars was often found inside joint mines but canibalism was not proved. Mortality occurred as a result of intraspecific competition, i.e. due to a lack of food. The larvae had 8 and 9 instars. In all instars the caterpillar was dorsally and ventrally very flattened, with a prognath head position. Larval excrement was liquid and sticking to mine walls, i.e. leaf epidermis. The existance of excrement pellets could not be found inside the mine. Before going into the pupal stage, the larvae formed densely spinned cocoons of regular round, discus and lens-like shape. The cocoons were fixed only to the lower leaf epidermis. The upper part of the cocoon near the leaf epidermis was also thickly upholstered. The discus-like shape of the cocoon caused visible bulge on the reverse side of leaf epidermis.

It was characteristic of the pupae not to have chremaster. Segments of the pupa abdomen (second to sixth) had each a pair of inward-bended thorn-like growths serving to fix the cocoon and facilitate moth eclosion. During the eclosion of moths, the face side of the leaf epidermis was opened without exception. Pupal exuviae remained in the hole of the epidermis, so that the course of emergence could be easily monitored.

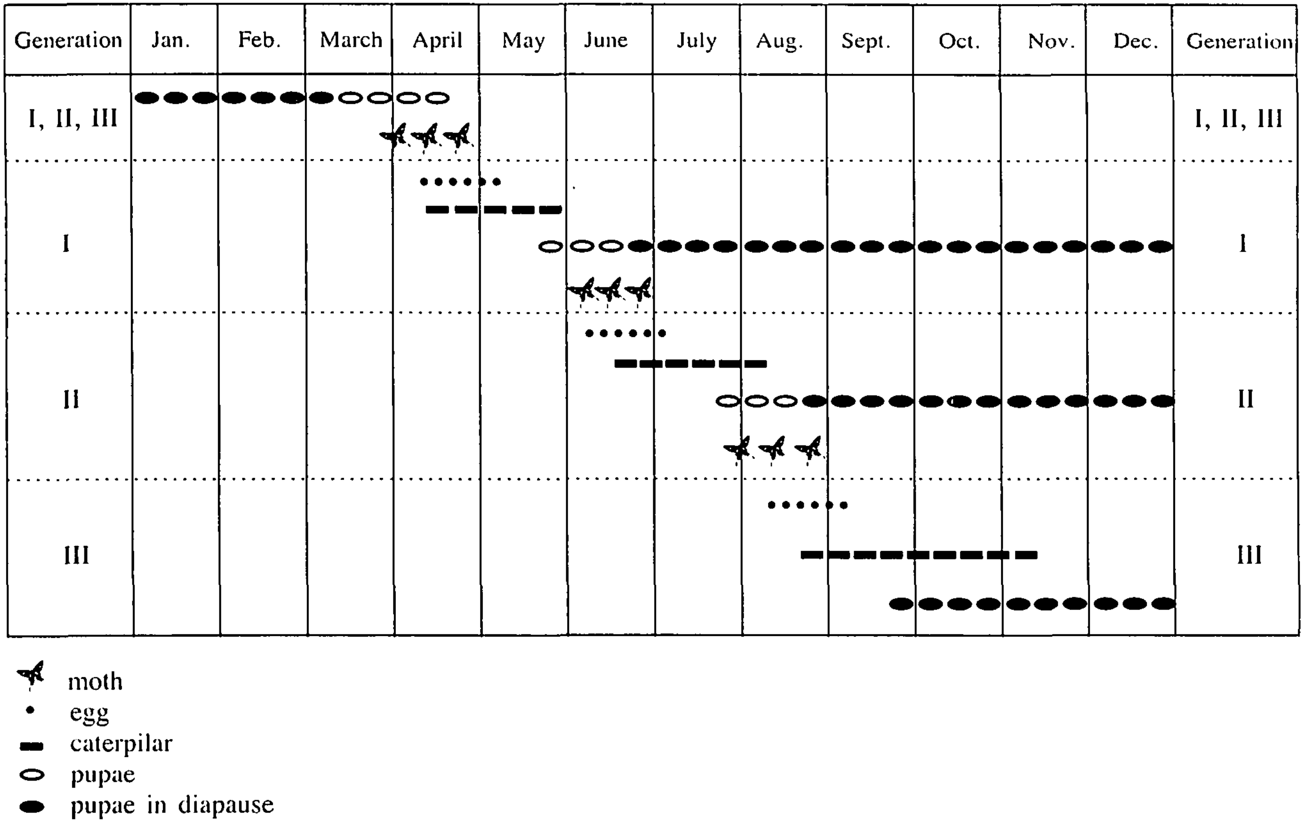
Unlike previous findings (DESCHKA & DIMIĆ 1986), we established that the intensity of infestation was the highest at the base and lowest at the top of the crown. In subsequent generations, the pest moved toward the top part of the crown, attacking and damaging top leaves. As we mentioned previously, the attack of the first generation was often strong enough to cover the whole leaf surface by mines. Due to special characteristics of the species, this phenomenon had no significant effect on population density the following year.

The eclosion of the first instar moths occurred in June. In the region of Belgrade it began in the first decade of June (June 8, 1993) and a little later (on June 22, 1993) in the region of Obrenovac near Belgrade. Flying out lasted three weeks (22 days in 1993 and 1994).

The second generation of moths first appeared in the last week of July and their eclosion ended well into the second half of August (in the region of Belgrade in 1993: three weeks, i.e. 23 days).

C. ohridella overwintered exclusively at the pupal stage, in round disc-like cocoons inside mines of the fallen leaves. This is a critical moment in the species' development. It enables chestnut protection over the autumn-winter period by a mechanical method involving no pesticides. The development of *C. ohridella* is distinctive. All individuals (pupae) of the third, overwintering generation remained in diapause, waiting for the usual favorable environmental conditions of the following spring. However, pupae of the first generation (26% on the average) and the second generation (45% on the average) also remained in diapause. This phenomenon has not been registered so far for other species belonging to the group of leaf miners. It ensures an unfailing and fully safe survival of *C. ohridella* in unfavorable conditions such as the lack of food for the next generation, which

Life cycle of *Cameraria ohridella* Deschka & Dimić



has become a regular occurrance in recent years (premature defoliation of trees caused by various factors: agents of diseases, drought and the miner itself). The number of pupae remaining in diapause varied within the same generation. Individuals originating from earlier egg deposits went to diapaus in the lowest percent. Their number gradually increased and was at the highest for individuals hatched from the latest deposits of eggs in a generation. With the exception of the pupae in diapause, *C. ohridella* generations did not overlap so that no problem of precise identification and possible confusion may arise in this respect.

Investigation of the dynamics of infestation intensity per generation in fully natural environment in the area of Belgrade over the period of last two years showed that, in cases of severe attack, population density increased from generations one to two, decreasing in the third generation due to their own abundance and a lack of free leaf surface. From each of the 100-leaf samples taken from the infested wild chestnut trees (at the locality of Batajnica) flew out 1783 moths of the first generation, 3609 moths of the second and 1299 of the third generation (a decrease was registered although individuals of all three generations were found in the third generation sample - diapausing pupae). Besides the lack of food, other biotic and abiotic factors, their variations and extremes in winter also had considerable effect on the reduction of population density of the overwintering generation.

The recommended mechanical and other non-pesticidal methods of protection occasionally meet strong opposition, reserve or distrust among those who are not sufficiently informed and who mostly allege their concern for the survival of beneficial organisms - parasites and predators. Nevertheless, this method, carefully applied as it may be, still enables the survival of a sufficient number of individuals of the pest being controlled and its natural regulators of population density. This is precisely its greatest advantage, compared to the chemical method of protection that does not allow either to survive. The entire concept of integrated protection is based on the principle of the inclusion of all available methods into plant protection practice, aiming to preserve as far as possible the beneficial organisms, i.e. the pests' natural enemies.

Conclusions

C. ohridella is a monophagous species of leaf miners. It inhabits exclusively the leaves of the wild European chestnut (*Aesculus hippocastanum* L.).

The development of *C. ohridella* is highly distinctive.

In the entire area of distribution, this species has a constant number of three generations per annum.

Moth eclosion of the third generation occurs in April, the first in June, and the second from late July to mid-August.

The miners overwinter at the pupal stage, in round disc-like cocoons inside mines made in fallen leaves. Besides the pupae of the third generation, those of the first generation (26% on the average) and second generation (45% on the average) also remain in diapause. This enables the application of mechanical control of the pest by way of collecting and destroying fallen leaves with mines and the inhabiting pests.

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