

Pattern of invasion by Cameraria ohridella in France: a comparison with Germany

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Cameraria ohridella and other invasive leaf-miners in Europe



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DEVELOPMENT OF THE *CAMERARIA OHRIDELLA* POPULATION IN THE CITY OF ROTTERDAM, THE NETHERLANDS

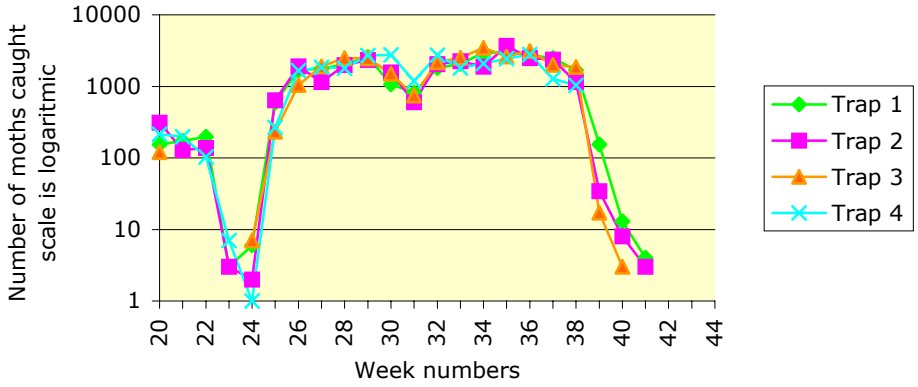
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In 2003 Foreest Groen Consult conducted a field study on the development of *Cameraria ohridella (*horse chestnut leaf miner) in the city of Rotterdam. The study was carried out on a location with 41 horse chestnut trees (*Aesculus hippocastanum "Baumannii"*). In each tree we placed two funnel traps. Four trees got one funnel trap and one delta trap. The traps were placed in the lower part of the crown, next to the stem. Weekly the sticky inlays of the four delta traps were changed and the number of moths was counted.

When changing the sticky inlays weather conditions and abnormalities were written down. Every two weeks a picture was taken from the four trees with the delta traps. After 8 and 16 weeks the pheromone capsules were changed. At this time the funnel traps were cleaned. The graph below show the results of the weekly catch.

Population development of *Cameraria ohridella*
from 12 May to 20 October 2003, Rotterdam



Conclusions

- Three generations of moths develop during one year
- Monitoring the development of the *Cameraria ohridella* with pheromone baited traps is possible
- It seems as if a natural brake on the population development exists
- With pheromone traps large numbers of moths can be caught
- The traps catches no other species than male Cameraria moths
- Funnel traps should be cleaned regularly in order to prevent strong smelling of dead moths

PATTERN OF INVASION BY *CAMERARIA OHRIDELLA* IN FRANCE: A COMPARISON WITH GERMANY

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Although it is likely that the horse chestnut leaf miner *Cameraria ohridella* (Lepidoptera: Gracillaridae) entered France in the course of 1998, its first observation was recorded near Paris in 2000. The rapid spread of the leafminer throughout Europe suggested that the country would be quickly invaded, and surveys were carried out in 2001, 2002 and 2003 to follow its nationwide spread. Surveys aiming at detecting the presence of the pest by visual observation of leaf damages were carried out in late summer/early autumn in the centre and East of the country in 2001, whereas they were extended to the West in 2002 and 2003. The pattern of spread derived from the spatial analysis of these data is compared to the pattern of spread already described for Germany in term of spread rate and possible relationship with human population density.

THREE-YEAR STUDIES ON THE EXISTENCE OF *CAMERARIA OHRIDELLA* (LEPIDOPTERA, GRACILLARIIDAE) IN GREECE: (2001-2003)

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It is well known that the horse chestnut trees leaf minor *Cameraria ohridella* is a new pest for the Europe, which was first detected near Ohrid-Lake in 1985. The year 1996 Skuhravý (1999) found the insect on horse chestnut trees in Florina (North Greece) for the first time in Greece, while in the same publication he reports the oral information about the appearance of the insect on two trees in the garden of a monastery on Pillion (Central Greece) as well.

Based on this little piece of knowledge acquired, the collection of information started in 2001 in order to create a distribution map of this scientifically unknown, concerning the Greek region, insect.
This effort, which was carried on until 2003, was put within the framework of the European Union project CONTROCAM, in which Greece (Department of Forestry at Drama, Technological Educational Institute of Kavala) participates, together with seven other European partners.

The detection of the places, where the insect appears, was realized by visual observations. The results of this work will be summarized in the following part:

- The problem caused by the attack of the infection of horse chestnut trees by the moth seems to be more serious than it was first thought to be. Until now, the insect was found in 65 different places, which were distributed in central and northern Greece. The main distribution area is the mountainous region of Pindos, whose direction is from northern to southern Greece.
- In the time of observations, the unique host plant that was detected is *Aesculus hippocastanum* L. Till now it was not found in Greece another species attacked by the moth.
- The insect was found to be capable of attacking wild as well as artificial horse chestnut trees.
- In any case the wild horse chestnut trees, which were found in 33 different areas of Greece, was attacked by the moth.
- The infestation level on wild as well as on the artificial horse chestnut trees, which were attacked by the moth, fluctuated between some mines per compound leaf to an almost covering of its surface by them.
- The attacked trees were found in different altitudes that varied from 40 to 1400 meters above the see level.
- The longitude of the places with attacked trees fluctuated between 0200 30 710 and 0260 31 998 degrees, while the latitude was between 380 37 345 and 410 30 008 degrees.
- In the future the problem caused by the moth in Greece will become more intense since the horse chestnut tree is one of the most favourite species used in parks, gardens and boulevards throughout the land during the last years. Another reason used to support this argument is the fact that the trees in parks near areas, where heavy infection was found, were not attacked till now, probably because of their geographical isolation.

EFFECTS OF THE HORSE CHESTNUT LEAF-MINER *CAMERARIA OHRIDELLA* DESCHKA & DIMIĆ ON THE FROST HARDNESS OF *AESCULUS HIPPOCASTANUM* L.

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The chestnut leaf-miner (*Cameraria ohridella)* spread out in Europe in few years and causes heavy leaf-symptoms by susceptible species of *Aesculus* every year, especially by *Aesculus hippocastanum*. This has a considerable impairment of the appearance of the chestnut as consequence, leads however also with strong damage of the leaves to a premature defoliate and to an autumnal flowering of the concerned trees. Little secured hints are available up to now that the vitality of this trees is decreased.

In the research-project "BerlinCam" become the population-developments of the pest and his natural opponents, the effect of measures as well as the development of the chestnuts themselves in detail examines. For the first time it could be shown that the release of a second flowering decreases the winter-frost-hardness of the chestnut and damages are evoked with early-frosts at the leaf- and bloom-formation of the trees. So the first frost-days led in October and November 2002 to it that sprouted leaf-buds were damaged locally and sprout out in the following spring small-flaky and deformed. If the characteristic tip of the twigs were sprouted with their bloom, so these were completely frostbitten and remained as mummy until today at the trees. A sprout out in the following spring was possible in these cases no longer.

In such a way damaged chestnuts show a considerable decline in the bloom-formation, chlorosis and deformations of the leaves as well as a declining of the fine-branches, in the first following year altogether. Subsequently, leaf-buds that allow only a low twig development and a weakened new bud-formation, sprout out differently strongly. Also, an attack follows with weakness-parasites. The further consequences are discussed.

MINE DEVELOPMENT OF HORSE CHESTNUT LEAF-MINER (*CAMERARIA OHRIDELLA*) ON LEAFS EXPOSED TO SUNLIGHT OR SHADE

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Introduction

Larval development of *Cameraria ohridella* is rather short and adults appear from pupae within 6 to 8 weeks after deposition of eggs. Due to fast development, *Cameraria ohridella* is able to have 3 to 4 generations per year[1]. As for most insects with short reproduction cycles, climatic factors (temperature) have an important influence on the duration of larval development. Therefore it might be possible, that eggs and larvae on leafs exposed to direct sunlight show a much shorter development because of better microclimatic factors, such as higher temperature

Materials and Methods

To investigation a possible influence of microclimatic differences on mine development, a total of 281 mines on sun exposed leafs (SUL) from the lower outer treetop and 267 mines on shaded leafs (SHL) from the inner lower treetop, were selected, permanently marked and surveyed between 21.05.-10.07.2004. From those mines 31 on SUL and 29 on SHL that covered an area of 1mm^2 or less, were surveyed during their growth until emergence of adults. Every 4 days the circumference of each mine was copied on transparent film. Millimetre graph paper was used for counting out each area. Temperatures were recorded. 28 standardized samples from leafs were taken and dried to estimate their dry weight.

Results

Adults emerged from 21% of the mines on each leaf type during the survey period (21.05.-10.07.2004), whereas pupation still continued in 4% of mines on SUL and in 5% on SHL. Mine growth could be observed until 10.07. in 24 % (SUL) and 31 % (SHL). The majority of larvae died (51% SUL, 43% SHL) soon after mining. 75% of mines, which did not expand anymore within 20 days after occurrence and were therefore defined as dead, covered a maximum area of 2 mm^2.

Mine growth and larval development differed significant, but in an unexpected way. On SUL it took an average of 23 days until mine growth ended, whereas on SHL the average area increment lasted 15 days. Average time for total development (from mines of 1mm^2 or less until emergence of adults) differed between SHL and SUL only 3 days, nevertheless a *t*-test still proofed a significant difference between both leaf types.

In contradiction to these results, the average area covered by mines on SHL (204 mm^2) was twice the area on SHL (102 mm^2). Opposite findings were made for the average dry weight of SUL (7.9 mg/cm^2) and of SHL (3.2 mg/cm^2). Both results were significant

Conclusions

Results above show that larval development on SUL is worse compared to SHL. This might be referred to higher temperatures and a possible lack of water due to direct sunlight, leading to higher transpiration and subnormal conditions for larvae[2], which can explain longer larval development on SUL. Larger mines on SHL can be referred to leaf structure and lower dry weight of these leafs. Lower dry weight results mainly from a higher content of cellulose. From this biological difference larvae of *Cameraria ohridella* can gain no advantage on the one hand, because they do not feed on cellulose. On the other hand denser SUL might explain a slower development on that type of leafs too.

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FORAGING BEHAVIOUR OF TWO RECENTLY COEXISTING PARASITOIDS

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The citrus leafminer (*Phyllocnistis citrella*) is a pest arrived in the 1990's in Europe causing damage to young trees. In Spain, exotic parasitoids were introduced three years after its arrival as biological control agent. One year later, a field survey showed that indigenous parasitoids were dominant in abundance. As parasitoid efficiency largely depends on their host location behaviour, the analysis of the foraging behaviour is a fundamental step to evaluate their potential impact on the host abundance. So, we analysed host location and host selection by two main parasitoids of Citrus leafminer, one indigenous *Cirrospilus* near *lyncus*, one introduced *Quadrastichus* sp. Both are idiobionts, solitary, generalists and ectoparasitoids.

Both parasitoids detect the host in the mine by searching with the antennae and the ovipositor. A few transition probabilities, related to the use of the ovipositor, are significantly different between species. Time management was different with *Quadrastichus* sp. spending three-fold time on its host but the probability of laying an egg while examining the host was 3 to 4 times greater for *Cirrospilus* sp. near *lyncus*. Finally, the parasitism rate was similar for both species. Given that the foraging behavior of *Cirrospilus* sp. near *lyncus* and *Quadrastichus* sp. is similar, differences in abundance in the field is probably due to other processes.

PARASITOIDS WEBS ON A RECENT INVASIVE MOTH *CAMERARIA OHRIDELLA*

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Leafminers are insects recognized to be heavily parasitized. In case of leafminers invasion, two processes are occurring: either the host is arriving with an assemblage of parasitoids or local parasitoids may adapt to this new host. In some cases, local parasitoids are more efficient to slow the progression of the invader than its own train of parasitoids.

Cameraria ohridella, the horse chestnut leafminer, arrived in France in 1998. Our aim was to understand the structure and dynamics of *Cameraria ohridella* parasitoids webs over the course of the invasion. Sampling in 2002 was done along two gradients. The first is a spatial gradient following the arrival of *Cameraria*. The second is a gradient according to habitats: in a city along a street, in an urban park and in a rural place.

Ten species were recovered, 7 belonging to the Eulophidae family. *Minotetrastichus* is the most abundant one. The parasitism rate is very low and quite constant whatever the date of arrival and the habitats, around 3.2% for the first generation and 1.8% for the second.

Parasitoids webs structures (primary, multi, super, hyper parasitism) are more complex in the locations invaded for several years whereas primary parasitism is the only type to occur in the very recently colonized sites. Species richness is higher in rural places and in formerly used sites.

The next step is now to study the parasitoids foraging behaviour, in the hope to explain the low parasitism rate.

AN IPM PROGRAM FOR *PHYLLOCNISTIS CITRELLA* STAINTON IN SICILY CITRUS NURSERIES

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Sicilian citrus nursery growers, since 1995 considered the citrus leafminer *Phyllocnistis citrella* Stainton the most important pest. Plant growth and quality of young trees, cultivated primarily for ornamental finality, can be compromised if leafminer is not managed. For this pest up to 10 generations per year are reported in Sicily[1]. Monitoring of populations showed as the infestation are first detected in nursery trees(May-June) than in mature groves (late June)[2]. For protection of the trees 20-25 applications per season are reported[3]. The most utilized compounds are abamectin, imidacloprid and methomyl. In order to minimize the side effects of theses repeated applications IPM tactics have been investigated in the period 1995-2001. The use of a drench application of imidacloprid at rate of 0.05 a.i. per tree resulted in 4-5 weeks of efficacy[4]. The use of screenhouse for the exclusion of adults was investigated but data showed an harmful increasing of temperature and relative humidity[4].

The two introduced parasitoids *Semielacher petiolatus* and *Citrostichus phyllocnistoides* displaced the indigenous parasitoids[5]. Theses eulofids resulted in leafminer infestation levels lower than those found when the pest appeared initially in Sicily[6,7]. The application of azadirachtin resulted in an appreciable control of *Phyllocnistis citrella*[8]. In 2001, a sampling program reduced the chemical applications respect to the farmer's strategy. Mineral oil and azadirachtin significantly reduced the number of *Phyllocnistis citrella* per leaf, but had more mines than imidacloprid. All treatments had fewer mines parasitized. The mineral oil strategy resulted environmentally not safe[9].

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BIOLOGY AND CONTROL OF *PHYLLOCNISTIS CITRELLA* AND *CAMERARIA OHRIDELLA* IN CENTRAL ITALY

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The citrus leafminer *Phyllocnistis citrella*, native of South-East Asia, was first recorded in Italy (Sardinia) in 1994; in 1995 it was reported in the citrus groves of southern regions and also in ornamental nurseries and gardens of Tuscany[1]. In Tuscany *P. citrella* completes 7 to 8 generations per year between May and December, with the pupa overwintering stage. The development times from egg to adult range from 16 to 18 days in the middle of summer up to 39 days in November. The female oviposites preferably on young leaves close to the midrib on the underside of the leaf. The 1st type larva digs a serpentine mine that involves the epidermis, leaving the parenchimatic tissue intact; instead, the 2nd type larva is aphagous and pupates near the leaf's edge.

While in the citrus cultivation areas of South Italy the numerous native parasitoids and some released species (*Ageniaspis citricola*, *Semialacher petiolatus*, *Citrostichus phyllocnistoides*) are able to control the leafminer, in the ornamental nurseries where the economic thresholds are low due to aesthetic requirements, the defence of potted citrus is presently entrusted to chemicals. The better results were obtained with: a) foliar sprays (lethal effect for 10 days), alternating abamectin (Vertimec 0.6 mL/L) and imidachloprid (Confidor 0.5 mL/L); b) soil application (lethal effect for 30-40 days) of Confidor (0.5 mL/plant in pots up to 30 cm).

Cameraria ohridella, which has been reported in Italy[2] since 1995, appeared in Tuscany in 1999. Here it completes 4 generations/year, plus a 5th when the horse chestnut is in a good vegetative state even in late autumn. In Florence the monitoring of the adult's flights, carried out with pheromone traps, pointed out 4-5 peaks from the beginning of April to the end of November 2001 and 2002. In Lucca, where the first consistent attack by the leafminer occurred only in September 2001, in 2002 population density (4400 adults/trap/10 days) and foliar attack (123 mines/leaf) reached the highest levels recorded in Tuscany.

Up to now, the activity of the entomophagous insects, whose percentage of active parasitization in any case did not exceed 5% in 2002, did not seem to have an influence on the gravity of the infestations. All the 7 species found up to now are indigenous and polyphagous Eulophids typical of leafminers, among which *Minotetrastichus frontalis* and *Closterocerus trifasciatus* prevail. A Braconid, *Colastes braconius*, was also isolated in 2000.

The damage was found to be significantly lower in zones (roads and paved squares) where old leaves under diseased trees are periodically raked up and destroyed, than in grassy gardens and parks where dead leaves remain on the ground. The destruction of the fallen leaves, in which the cocoons of *C. ohridella* overwinter, is a very efficient means for controlling the species in cases of not very extensive infestation. Otherwise, the only possibility for controlling the insect in the urban environment is to apply insecticides to the trunk using endotherapeutic methods.

Tests were performed in May after flowering, using Merit Green insecticide (17.8% imidachloprid) in a 0.7% aqueous solution, in doses of 1 ml of mother solution per 10 cm of trunk circumference: a) under controlled pressure; b) by the natural absorption method. The preliminary results indicated that the method (a) guaranteed a degree of efficacy, 75.4%, more satisfactory than method (b), 46%, even if, in the case of the larger plants, only a part of the branches had healthy leaves at the end of the season.

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ASSESSING THE HOST PLANT RANGE OF THE HORSE-CHESTNUT LEAF MINER, *CAMERARIA OHRIDELLA* DESCHKA & DIMIĆ (LEPIDOPTERA: GRACILLARIIDAE) – A HINT TO THE ORIGIN OF THE MOTH?

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Different host plant specificity tests with ca. 400 potted three- to five-year old trees were employed to investigate the host plant range of *Cameraria ohridella* within the genus *Aesculus*.

No-choice tests as well as field-tests including choice- or colonisation-tests revealed that 21 of the 36 tested *Aesculus* species supported full development of the horse-chestnut leaf miner. These 21 species included the endemic species *Aesculus hippocastanum*, *Aesculus turbinata*, *Aesculus octandra* (= *Aesculus flava*) and *Aesculus pavia*. On *Aesculus chinensis*, also an endemic species, *C. ohridella* could not develop to adults. On the endemic *Aesculus indica*, *Aesculus californica*, and the hybrid *Aesculus.* x *carnea*, drafted on *Aesculus hippocastanum*, the larvae died in the first or second mining larval instar. These instar belong to the sap-feeder type. In the field *Acer pseudoplatanus* and *Acer platanoides* were attacked – in some cases so heavily that up to 100 % of the leaf surface area were destroyed – and full development was observed. The emerging females were fertile and produced offspring.

By no-choice tests only the physiological host plant range can be determined. I.e. *Cameraria ohridella* has the capability to develop on different host plants, which does not mean that the moth would oviposit on the same plants in the field if it had a choice. Still the colonisation tests and preliminary oviposition tests showed that the horse-chestnut leaf miner oviposits relatively indifferent, though preferring normally *Aesculus hippocastanum*.

The potential origin of *Cameraria ohridella* and the possibility of an expansion of the host range of the horse-chestnut leaf miner will be discussed. In short, the eastern North America and East and Southeast Asia cannot be ruled out as the origin. However one hypothesis should not be omitted in the discussion: There is still the possibility that *Cameraria ohridella* is not associated at all with a member of the *Aesculus* genus in its place of origin.

BIOLOGICAL CONTROL OF LEPIDOPTERA THROUGH DICHOTOMOUS SPERMATOGENESIS?

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As a rule, adult males of Lepidoptera bear two kinds of concomitant spermatozoa: haploid-eupyrene and anucleate-apyrene; both kinds reach the spermatheca of the inseminated female[1]. The eupyrene spermatozoa leave this organ and fertilize the eggs. But apyrene spermatozoa remain in the spermatheca where they eventually decay[2]; nevertheless, apyrene spermatozoa do facilitate fertilization. Therefore, several functions have been proposed for the anucleate spermatozoa but none of them has been confirmed. Dichotomous spermatogenesis has not yet been reported for *Cameraria*. However, it is hard to believe that *Cameraria* spermatogenesis might differ fundamentally from that found in the other species throughout the order.

Only one kind of early primary spermatocytes occurs during the dichotomous spermatogenesis but they yield both kinds of spermatozoa: eupyrene spermatozoa at larval stages and apyrene ones in pupae and adults. The shift from eupyrene to apyrene differentiation occurs in the early meiotic prophase. Interfering with the normal course of the shift would cause imbalance between the populations of the two kinds of spermatozoa, which are both needed for fertilization.

The shift is induced by a still unidentified haemolymph apyrene-spermatogenesis-inducing-factor (ASIF), or even by a set of factors, becoming active at the start of pupation. Isolation, identification, and elucidation of the mode of action of the factor(s) inducing apyrene spermatogenesis may open a completely new approach to the control of lepidopteran pests through induced male sterility.

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DISPERSE WITH THE LEAVES AND DEVELOP LOCALLY: A SUCCESSFUL STRATEGY FOR INVASIVE LEAFMINERS ?

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The very fast spread of the horse-chestnut leafminer *Cameraria ohridella* throughout Europe has been mainly attributed to a very high dispersal ability combined with wind or human passive transportation over long distances. However, the species effective dispersal characteristics remain to be better understood. Here we present data of experiments aiming to describe the decrease in population estimate as a function of distance to a population source. Firstly, we analysed the decrease in infestation level on horse-chestnut trees along three transect avenues departing from population source located in green areas in Brussels. Secondly, we describe the decrease in catches of pheromone traps disposed along two transects departing from an isolated group of infested chestnut trees. Results of these two experiments suggest that the active dispersal ability might be very short (approx. 100m), and that the largest part of the dispersal could be the result of infested leaves (or fragments) blown from population source during the winter. The evolutionary perspective of this hypothesis (i.e. do leafminers benefit from being dispersed with their leaves) and applied consequences for pest management at the scale of cities is discussed.

MORTALITY FACTORS AFFECTING THE DIFFERENT DEVELOPMENTAL STAGES OF *CAMERARIA OHRIDELLA* DESCHKA & DIMIĆ IN SWITZERLAND

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The horse-chestnut leaf miner, *Cameraria ohridella* (Lepidoptera, Gracillariidae) was first found in Switzerland in 1998. It has since spread over the whole country reaching Delémont in the years 1999-2000. The study carried out at CABI Bioscience is part of a European research program, CONTROCAM, a multidisciplinary project on integrated management of this pest, involving 9 European teams.

One part of the work carried out at CABI Bioscience Switzerland centre is the evaluation of mortality factors affecting each developmental stage of *Cameraria ohridella* in Switzerland. The study has been carried over the years 2002 and 2003 at two sites and the data obtained will be compiled into life tables. Most mortality factors affecting eggs, larvae and pupae of *Cameraria ohridella* have been identified and quantified. Egg mortality was studied by exposing eggs in the canopy of the trees. Egg mortality reached 30%, but the mortality factors could not be properly identified. A weekly monitoring of mines in the field and fortnightly dissections assessed larval and pupal mortality. Factors acting during the summer season included predators (arthropods and birds), parasitoids and intra- and interspecific competition. Birds have been found to have an increasing action on populations, especially in the late generations of the moth, when density is highest. The parasitoid present were mostly generalist eulophids, such as *Minotetrastichus frontalis* and *Pnigalio agraules*, with parasitism rates staying very low,. Mortality was also studied during the winter months, by following overwintering pupae and trying to determine the effect of earthworms. 80% of the pupae died during the winter.

CAMERARIA OHRIDELLA: PENETRATION INTO EAST EUROPE

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In the territory of Russia for the first time *Cameraria ohridella* was observed on the territory of the Kaliningrad Region in 2003. The attempts to catch butterflies in the pheromone traps in Moscow have proved the absence of this pest there.

At present this pest invaded the west part of Belorussia, the most part of Ukraine, and Moldavia. In the 2004 *Cameraria ohridella* will be observe in Lithuania and in the next year – in Latvia.
But now we have not any data about *Cameraria ohridella* in Armenia, Georgia, Azerbaidjan and east parts of Turkey. Our investigation in the 2003 shown that this species is absent in Teheran.

WHY ARE NATIVE EUROPEAN PARASITOIDS NOT ABLE TO CONTROL THE HORSE CHESTNUT LEAFMINER?

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Parasitoids usually play the most important role in the control of leafminer populations and contribute significantly to overall leafminer mortality. Parasitism rates of more than 50% have been recorded from several leafminer species closely related to *Cameraria ohridella*[1,2,5]. In addition, a lot of leafminer parasitoids are highly polyphagous and parasitize all kinds of leafminers on comparable host plants[2]. This high flexibility enables them to incorporate invasive leafminers into their host spectrum within reasonable timeperiods. Parasitism of invading leafminers is therefore increasing with time[6,7,4] and may reach quantities sufficient for effective natural control.

A three years analysis of the parasitism of *Cameraria ohridella* in various European regions shows that the adaptation of the native parasitoids to the invasive host is very slow. The parasitoid complex of *Cameraria ohridella* is similar to that found in other European leafminers. Nevertheless, parasitism levels remain low, even at places with more than 10 years of continuous *Cameraria ohridella* infestation. Among other reasons, the low parasitism of the horse chestnut leafminer may be due to poor synchronization between the parasitoids and the new host.

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FEMALE MASS TRAPPING – A CONTRIBUTION TO *CAMERARIA OHRIDELLA* CONTROL

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Females of *Cameraria ohridella* Deschka et Dimić (Lepidoptera, Gracillariidae) have been shown to respond in locomotion and oviposition to the lipophilic leaf extract of its host plant *Aesculus hippocastanum* L. Polyethylene surfaces treated with leaf extract from horse chest nut trees were accepted for oviposition, whereas untreated foils were not. Olfactometer studies revealed significant attraction of females towards volatile constituents extracted from host tree leaves. Males, however, showed no response. For incorporation into a mass trapping strategy a series of experiments with adhesive insect traps was performed, testing glue, colour, orientation and position in the tree for their influence on trapping efficacy. Distinctively more moths were caught in traps on the stems of trees in comparison to traps mounted on various positions in the crown. Height above ground had no significant influence. No significant differences between different colours were observed, although yellow had higher catches than others, blue had least catches. On basis on these findings a new adhesive trap was developed with high capacity in mass trapping of leafminers and strongly reduced captures of non-target organisms. The new trap combines a large trapping surface with strong resistance to the impact of wind. Besides, handling for the user in terms of fixation on the tree as well as tidiness was strongly improved in comparison to existing concepts. The properties and limitations of the new trap and its prospects for *C. ohridella* control in combination with pheromones and female attractants will be discussed.

BIOLOGICAL CONCEPTS TO CONTROL THE HORSE CHESTNUT LEAF-MINER *CAMERARIA OHRIDELLA* DESCHKA & DIMIĆ, 1986 (LEP., GRACILLARIIDAE)

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In cooperation with the Official Bureau of Plant Protection Berlin, the University of Applied is developing and testing new strategies to control the chestnut leaf-miner *Cameraria ohridella* in the city of Berlin. Intensive field and laboratory studies, spanning three vegetation periods at about 20 sampling sites throughout Berlin are in progress.

For a possible reduction of the hibernation stages of the moth in the foliage and rotten leaves, first laboratory tests with four different species of nematodes (*Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, *Steinernema feltiae* and *Steinernema kraussei*) were carried out. At a temperature of 12 °C to 22 °C all species were highly efficient in infesting and killing the pupae of *Cameraria ohridella.* At 22 °C the differences in the rate of parasitism among them were very low, at 12 °C *Heterorhabditis bacteriophora* ceased its activities and *Steinernema feltiae* and *Steinernema kraussei* were the most efficient species. Field tests will prove whether under the climatic conditions in North-eastern Germany both species could be used for a possible treatment of the foliage in spring and autumn.

First results in the laboratory and in the field were achieved by a treatment with *Bacillus thuringiensis aizawai*. For a successful large scale application of the Attract & Kill method (pheromone und Appeal®) essential factors have to be optimised, such as the design and the number of traps, their position relative to the infested trees, and the timing and frequency of changing the lure.

In addition to Attract & Kill methods and different strains of *Bacillus thuringiensis* (*Bacillus thuringiensis* var. *aizawai* and var. *kurstaki*), the species composition of parasitoids in the vicinity of Berlin will be examined in detail. There will be an attempt to rear selected native parasitic wasps, e.g. *Pnigalio agraules* [Eulophidae, Chalcidoidea] known to parasitize on a wide range of hosts, on replacement hosts, such as other leaf-mining Lepidoptera occurring on fast growing plants. Laboratory tests with the Eulophidae species *Diglyphus isaea* and *Dacnusa sibirica*, successfully used as natural antagonists in vegetable cultivation, resulted in a rate of parasitism in *Diglyphus isaea* of 3 %.

MECHANISMS OF THE EXOSECT AUTOCONFUSION TECHNIQUE AND APPLICABILITY TO INTEGRATED CONTROL OF *CAMERARIA*

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The ExoSex Autoconfusion system differs fundamentally from all other mating disruption systems by contaminating the target pest with electrostatically charged powder formulated with pheromone or other biologically active materials. Hence the mode of action in disrupting mate location, courtship, and mating, may include a variety of effects, all of which contribute to the efficacy of management of the pest in question. The most important are believed to be the following:

a. False trail following
b. Habituation
c. Trail masking
d. Sensory imbalance
e. Inhibition of courtship
f. Enhancement of predation
g. Delay of mating

Field trials with a variety of orchard and forest pests have shown that ExoSex techniques can result in better control than conventional confusion techniques, although requiring only 25 dispensers and less than 100 mg of pheromone per hectare.

Factors making ExoSex Autoconfusion such a uniquely powerful technology are demonstrated by the following calculations based on the threshold responsiveness of *Cydia pomonella* to pheromone. It should be noted that the thresholds of *Cydia pomonella* are significantly higher than those of *Cameraria*, which should make the latter highly susceptible to this control technique.

One particle of EntoStat® (wax) powder contains on average 67 femtograms (6.7×10^{-14} g) of formulated pheromone, and there are approximately 1.5×10^{10} particles per gram of powder.

One particle resting on the antennae would theoretically release sufficient pheromone to induce habituation of responsiveness to pheromone. Approximately 1800 particles would constitute an attractive source for a male moth in the field. Hence the contents of one ExoSex® dispenser are theoretically capable of contaminating around 1 billion male codling moths with enough pheromone to make them attractive sources to other males.

DEVELOPMENT OF THE HORSE CHESTNUT LEAFMINER (*CAMERARIA OHRIDELLA* DESCHKA ET DIMIĆ) ON THE HORSE CHESTNUT TREES IN LOWER SILESIA, POLAND

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Horse chestnut leafminer was identified in Poland for the first time in 1998 in Lower Silesia[1]. Now, the pest occurs in each region of our country.

The objective of our study was to determine the development of *Cameraria ohridella* mainly on white horse chestnut (*Aesculus hipocastannum* L.).

Observations were carried out at several locations of the horse chestnut in Lower Silesia, in 2000-2003. The moths were counted on tree trunks, 1.5 m. above ground, within a surface of 400 cm^2 (20 × 20 cm), marked on the tree bark. Number of eggs, larvae and pupae was counted on 4 leaves in each location once a week during the whole horse chestnut vegetation period.

The first moths of the horse chestnut leafminer hatching from the overwintering pupae appeared on tree trunks at the end of April or at the beginning of May. Three generations of the pest were recorded in Lower Silesia. Percentage of male and female pupae was similar in each generation. Very low larvae and pupae mortality was observed in our trials. Females laid numerous eggs on *Asculus carnea* leaves but the development of the hatched larvae was strongly restricted on this tree. Raking and disposing of the leaves in autumn substantially reduced the abundance of eggs, larvae and leaf damage of horse chestnut in the next year.

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FUNGAL INFECTION INDUCED VOLATILES INFLUENCE THE BEHAVIOUR OF *CAMERARIA OHRIDELLA*

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The larval stages of *Cameraria ohridella* develop mining in leaves of *Aesculus hippocastanum* and can completely destroy the leaf surface. The Ascomycete *Guignardia aesculi*, which has spread from North America throughout Europe since 1954[1], causes conspicuous necroses like the butterfly. In 1999, another fungus from North America, the powdery mildew *Erysiphe flexuosa*[2] found for the first time in Europe on horse chestnuts. Often, all three diseases occur in parallel at the same leaves. The interaction between host plant, butterfly and fungi concerning host choice of *Cameraria ohridella* was examined.

The volatiles of healthy and differently infested leaves of *Aesculus hippocastanum* were collected and analysed with GC-MS/EAD[3]. More than 30 compounds could be identified. In addition to the typical green leaf volatiles, leaves infested by butterflies emit benzaldehyde, methyl salicylate and phenyl ethanol. These volatiles indicate that the tree recognizes the feeding of larvae and responds with an induced defence reaction. 1-Octen-3-ol was identified as the typical fungus odour caused by *Guignardia aesculi* and 3-octanone by *Erysiphe flexuosa*. Further, the compounds 5-ethyl-2(5H)-furanone and pentyl popanoate appear in connection with fungal infection.

In behavioural assays, the butterflies oviposit significantly less on leaves with the odour of fungi; no significant differences were found between healthy leaves and leaves sprayed with compounds that were identified to be typical for a host defence reaction.

In addition, the seasonal coincidence of leafminer attack and *Guignardia aesculi* infection were examined on different locations in Göttingen in order to assess the probability of interaction under natural conditions. The impact of these results on the design of biotechnical control measures of the moth is discussed.

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IDENTIFICATION OF HOST PLANT VOLATILES AND DETERMINATION OF THEIR ATTRACTIVENESS FOR *CAMERARIA OHRIDELLA*

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Gravid females of *Cameraria ohridella* prefer to oviposit on the white blooming horse chestnut, *Aesculus hippocastanum* L., over the red blooming *Aesculus* x *carnea*. In the search for biologically relevant plant attractants respective repellents we employed GC, GC-EAD and GC-MS analysis of dynamic head-space samples of chestnut tree volatiles. GC-MS showed that no single compound is predominating and characteristic enough to be recognized as typical for *Aesculus hippocastanum* or *Aesculus* x *carnea* odour. The main constituents were fatty acid derivatives (8.5%), terpenoids (69.63%) and aromatics (8.11%). The GC-MS comparison of *Aesculus* x *carnea* and *Aesculus hippocastanum* showed remarkable qualitative similarities of *Aesculus hippocastanum* and *Aesculus* x *carnea* head space. However the compared samples differed quantitatively: *Aesculus hippocastanum* blend contained more ocimenes and certain fatty acid esters. On the other hand *Aesculus* x *carnea* blend was characterised by higher amount of sesquiterpenes.

GC-EAD experiments showed that both sexes are sensitive to host plant volatiles. Female antennae responded to identical compounds in both *Aesculus hippocastanum* and *Aesculus* x *carnea* blends. In order to clarify the role of these compounds in chemical ecology of *Cameraria ohridella*, preliminary field experiments were performed and attractiveness of individual compounds was studied. Surprisingly, the majority of tested compounds were more attractive for males than for females, but the attractiveness was far bellow of female sex pheromone. The role of these active compounds in *Cameraria ohridella* chemical ecology will be discussed.

ALTERNATIVE CONTROL OF THE HORSE CHESTNUT LEAFMINER

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Currently, gardeners were advised to remove the leaf litter immediately after the trees have shed their leaves in autumn, because it was feared that overwintering pupae fall out of disintegrating leaves. However, we demonstrated that horse chestnut leaf litter can be removed at any convenient date until the beginning of March. Nevertheless, our results stress that horse chestnut leaves must be completely removed and that during the removal leaves are not damaged.

Furthermore, people not only have to remove pest-infested horse chestnut leaf litter, they also have to dispose the litter removed. Currently they are advised to destroy the collected foliage in waste incinerators or to deliver it to huge composting plants. Our results showed that *Cameraria ohridella* adults are prevented from emergence when pest-infested horse chestnut leaf litter is covered by a layer of soil or uninfested plant material. In future, horse chestnut leaf litter can be safely composted on small compost heaps saving the money and labour that were previously invested in litter disposal.

However, the removal and disposal of leaf litter does not only reduce the risk of leafminer infestation, it also prevents the emergence of hibernating parasitoids. Thus, the use of mass-hatching devices seemed to be a promising complementary measure to augment parasitoids adapted to *Cameraria ohridella* and thereby preventing the annual disruption of the build-up of parasitoid populations. The set-up of mass-hatching devices in the field significantly increased parasitism rates and the observed parasitism rates were among the highest ever observed in *Cameraria ohridella.*

FINDING THE AREA OF ORIGIN OF THE HORSE-CHESTNUT LEAF MINER. WHERE ARE WE TODAY?

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Twenty years after the discovery *Cameraria ohridella* in Macedonia, its origin remains a mystery. Possible areas of origin include the Balkans, where *Aesculus hippocastanum* comes from and where the moth was first discovered, or other regions in the world (Asia, North America), where other *Cameraria* spp. and *Aesculus* spp. occur. Its host of origin is also uncertain, and it cannot be ruled out that, in its native range *Cameraria ohridella* attacks other ree genera, such as *Acer*. The search for its area of origin requires a multidisciplinary approach, including various strategies.

(1) Studies of the parasitoid complex of *Cameraria ohridella* in the Balkans and of other *Cameraria* spp. in other parts of the world, to observe whether the parasitoid complex of *Cameraria ohridella* is more important and richer in native *Aesculus hippocastanum* forests, and to examine typical parasitoid complexes of *Cameraria* spp. in their native range.
(2) Host tree screening tests, to assess the host range of the moth and help locating possible host trees of origin.
(3) Surveys in the Balkans, Asia and North America through visual examination of leaves and pheromone trapping.
(4) Molecular studies on *Cameraria ohridella* and congeneric species to assess, firstly, whether populations in Europe originate from a single population and, secondly, in which regions and on which trees do the most closely related species occur.

MATING DISRUPTION AND MASS TRAPPING – POSSIBLE TOOLS IN *CAMERARIA OHRIDELLA* CONTROL?

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Study on disruption of *Cameraria ohridella* male orientation was conducted in a wind tunnel. Males were pre-exposed to different doses (100 pg - 10 ng) of synthetic pheromone and were allowed to fly after different recovery intervals (0 - 3 hours). A possibility of increased threshold for response after a pre-exposure (10 pg) was also studied. Males of *Cameraria ohridella* are able of temporal habituation to their sexual pheromone, which is positively correlated with the dose of pheromone and inversely with the period of recovery. Males remained unable to locate source of pheromone up to two hours (according to the dose). There was only slight indication for a shift of threshold for response especially in behavioural category source location

Mass trapping experiment was conducted in isolated area at centre of Karlovy Vary city. The number of trapped males during the flight period was considerably high, though probably a mere fraction of individuals present at the location treated. Under such circumstances, mass trapping had just a slight effect upon the population density (measured as leaf attack) in the spring generation and no effect in the summer generation. The use of large doses of the pheromone as the lure during mass trapping attract-and-kill control of *Cameraria ohridella* increases the naturally present concentration at local scale. Such information pollution can cause also a considerable male confusion effect.

INFESTATION OF WHITE (*AESCULUS HIPPOCATANUM* L.) AND RED HORSE CHESTNUT (*AESCULUS CARNEA* H.) BY THE HORSE CHESTANUT LEAFMINER IN LOWER SILESIA (POLAND)

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The horse chestnut leaf-miner (*Cameraria ohridella* Deschka et Dimić) was observed for the first time in 1998 in South-Western Poland[1]. The pest spread rapidly and after 4 years it was present within the whole area of Poland.

The observations were carried out at several locations of white and red horse chestnut in the city of Wroclaw in 2001-2003. The moths were counted on tree trunks, 1.5 m above ground, within a surface of 400 cm^2 (20 × 20 cm). Number of eggs and larvae was counted on 10 leaves, once a week, from April to October.

Larvae of *Cameraria ohridella* feed leaf tissue and create typical mines. The females laid eggs on the leaves of white and red horse chestnut. The degree of leaf damage of *Aesculus hippocastanum* was considerably greater than that of *Aesculus carnea*. In September 2001 the infestation of *Aesculus carnea* was about 10%. In the year 2002 and 2003 it didn't exceed 0.5%. In the same time in 2001 and 2002, infestation of white horse chestnut surged above 50% and in 2003 above 80%. The variable infestation resulted from the fact that the larvae hatched on *Aesculus carnea* were developing at a slower pace and were dying short after biting into leaf parenchyma. As an effect, there were many small mines observed on the leaves of red horse chestnut, but the resulting infestation was minimal.

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STANDARDIZED PHEROMONE-BASED SYSTEM AND PROPHEROMONES FOR MONITORING THE HORSE CHESTNUT LEAFMINER, *CAMERARIA OHRIDELLA*

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The female sex pheromone of the invasive leafminer *Cameraria ohridella* Deschka et Dimić was isolated and identified in the Institute of Organic Chemistry and Biochemistry (IOCB) in 1999[1]. The pheromone, (8*E*,10*Z*)-tetradeca-8,10-dienal, synthesized in IOCB was used as a lure in traps for monitoring this pest[2]. Development of a standardized pheromone-based monitoring system was the primary task of our working group within the scope of the CONTROCAM project.

Optimization of lure
Doses of 10 to 1500 ng or even up to 5000 ng of the pheromone were used. Two types of pheromone dispensers were compared: "Black rubber caps" (Hevea, Šestajovice, CZ) showed better efficacy than "red rubber septa" (Thomas Scientific, Swedesboro, New Jersey, USA). Differences in 100 ng dispensers "freshly" prepared and "aged" (stored at room temperature for 3 and 6 weeks respectively) were estimated according to their efficacy in field trials. The black cups are efficient for at least 6 weeks. It is not necessary to replace them during the flight of one generation.

Design of pheromone traps
The conventional delta trap (design Biola, Chelčice, CZ, green color) was selected as a standard. Maximal capacity of the trap provided with glue insert (Lonamet, Vetox, Praha, CZ) depending on weather factors reached up to 900, exceptionally 1400 male moths per one 8 × 19 cm insert. Adjusted polyethylene bottles with water, insecticide-treated insert and insecticide vapours as killing agent were used as a tool for precise estimation of peaks of flight (and potentially also for male annihilation assays). Most efficient was the device with dichlorvos (filter paper treated with insecticide Nuvan) - the highest catches reached over 20 000 of male moths per week.

Pheromone trapping in comparison with other monitoring methods
At beginning of the season, the first males were observed in pheromone traps, with a few days delay appeared the first moths in photoeclectors. Appearance of moths sitting on tree trunks is very irregular and weather dependent. The pattern of flight of *Cameraria ohridella* males as monitored by pheromone traps is more illustrative and realistic.

Propheromones
Trimethyl[(1*E*,8*E*,10*Z*)-tetradeca-1,8,10-trienyloxy]silane, the silyl derivative of the pheromone (formulated in black rubber caps) and 2-[(8*E*,10*Z*)-tetradeca-8,10-dienylhydrazinyl]-*N*,*N*,*N*-trimethyl-2-oxoethanammonium chloride (formulated in filter paper) at different doses were tested and compared with pheromone baited black rubber caps on three locations. The silyl propheromone at all tested doses (300 µg to 1500 µg) was equally or more efficient when compared to pheromone baited caps (100 ng and 500 ng) and its efficacy lasted over the whole season. The properties of propheromone lures will be further examined during the season 2004.

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PRESENT STATUS AND FUTURE OF THE HORSE-CHESTNUT LEAFMINER (*CAMERARIA OHRIDELLA*) CONTROL IN POLAND

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The horse-chestnut leafminer appeared in south part of Poland in 1998[1] and distributed very fast over the whole country. Experiments with control of this pest were carried out in urban areas on old tress with circumferences between 60 cm and 330 cm. The first experiment with application of Imicide (10% imidacloprid) according the Mauget system was established in July 2000. Further experiments with product of polish company - Best-Pest from Jaworzno named "Gel to control of *Cameraria ohridella* and *Guignardia aesculi* on horse-chestnut trees using microinjection technique", which contains 12% imidacloprid and 8% tebuconazole were carried out in 2001-2002. This product was applied using injection technique. The holes were drilled in trunks approximately 1 m above the ground at angle of 45°, every 15 cm of circumference. The diameter of holes was 8 mm and they penetrate 70 mm into the xylem and allows to hide 2.7 mL of product inside the trunk. The results of these researches were published earlier[2]. This product on base of these data was registered by Ministry of Agriculture and Village Development in Poland on March 4, 2003. It allowed for application of this product using injection technique to about 20 thousand trees by professional workers from April do May 10, 2003. Efficacy of this product was estimated in sample of 60 leaves taken from every of three treated trees and untreated ones based on the number of large mines (over 0.5 cm long) or percentage of damaged leaves classified in five categories. Effectiveness of treatments checked in June and August, 2003 ranged from 30.0% till 94.9% and from 42.2% till 93.4%, respectively depending on time of application and circumference of trunks. The second polish product - Treex 200 SL (20 g/L abamectin + 180 g/L propiconazole) was tested in 2003. It was applied in April and at the beginning of May using injection technique. The results obtained in August and September were excellent, treated trees were quite green comparing to untreated ones, which lost all leaves. The third polish product - Bioneem 020 SL (20 g/L azadirachtin) will be tested in 2004. The technique of application will be improved by introducing Chemjet tree injector, which allows to decrease the number and size of holes drilled in trunks.

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LOOKING FOR THE ORIGIN OF *CAMERARIA OHRIDELLA* - GENETIC ANALYSIS OF *CAMERARIA* SPECIES

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The origin of *Cameraria ohridella* Deschka et Dimić, 1986 is still unknown. To initiate an effective biological control of the species, the most crucial question of the research work is to determine its origin. The original habitat of the moth was supposed to be co-located with the different chestnut trees' habitats. This view supported Japan, Northern-China, Eastern-India, the Balkans and North-America as being the bedrock of *Cameraria ohridella*[1].

Several species of the genus live in Asia mainly on plants of the *Leguminosae* order[2,3]. Among the species living in Japan, the *Cameraria niphonica* and the *Cameraria acericola* make mines in plants of the *Acer* genus, while a still undescribed species exists on *Aesculus turbinata*.

In North-America more than 50 *Cameraria* species are known, some of them (e.g. *Cameraria aceriella*) live on *Acer*, but one of them (*Cameraria aesculisella*) mines on different North-American chestnut species (*Aesculus glabra, Aesculus flava, Aesculus octandra, Aesculus pavia*)[4].

In our study we compared European, Asian and North-American Cameraria species using mitochondrial DNA (COI) sequences. The presentation will show the first results and will discuss the phylogenetic relationship of the species as well as the possible origin of *Cameraria ohridella*.

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CONTROL OF THE HORSE CHESTNUT LEAF MINER WITH NEEMAZAL T/S

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In tree nurseries the indication "against mining microlepidopteran caterpillars on ornamental plants" or "biting insects (enclosing mining moths) on ornamentals" can get used. This failed in public green areas because of fearing "... in several cases hazardous effects to the health of humans and animals ..." (Act of Plant Protection 1998). It must guarantee an easy and secure reduction. This leaded the author to an insecticide made of the kernels of the neem tree *Azadirachta indica* A. JUSS.

The alley trees, proposed for the test, were situated inside a park of a big city including 90 trees of *Aesculus hippocastanum* in an age of about 80 years up to 25 meters high and a soil surface covering with plants. A strong populations pressure of the leaf miner grew up in 2000 and destroyed the foliage surface up to 95%.

Pheromone traps, depot proves and a phenological monitoring on the trees and the moths determined the time of application "flight of the moth and begin of egg deposition = full flowering of the chestnut". The trial was carried out with NeemAzal T/S in a concentration of 0.3 % under use of a "spray gun" of fire brigade according to the principles of good professional practice.

The activity of the moths never got affected by the application. The treaty worked to the mining over 6 weeks. The 2nd and 3rd generations did not react. The population pressure and the immigration out of the neighbourhood was too high. The aim of the trial "reducing the damage of the leaf surface by horse chestnut leaf miner on white horse chestnut inside urban areas with one application of NeemAzal T/S" succeeded. The efficacy of spraying the pesticide was moderate and not exceeding the 1st generation. One reply of the application could improve the efficiency. A shoot painting with neem on nursery trees was successful.

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RECENT SITUATION OF THE INVASION BY *PHYLLONORYCTER ISSIKII* IN BRANDENBURG

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The microlepidopteran of the family of *Gracillariidae,* subfamily *Lithocoletinae,* came from Japan and got specified in 1963 by KUMATA as a leaf miner on trees of the genus *Tilia*. Crossing the Siberian part of Russia the linden leaf miner arrived Germany for the first time in 2001 in the regions along the river Neisse in Brandenburg and Saxony.

Up to now discoveries are announced from several sites of the eastern and southern parts of Brandenburg and in the north of Berlin at *Tilia cordata* and *Tilia platiphyllos*. Outside the state of Brandenburg detection announced to the authors reaches to the cities of Meissen and Radebeul (Saxony) and self made finding to Vienna (Austria). Leaf symptoms of the moth priority were found on trees of street alleys, parks and in margins of forests, the typical sites of lime trees in the State of Brandenburg. We expect affects of all growing, planted and cultivated *Tilia* species, indigenous and not indigenous.

Typical are the "dispersion steps", here in a direction straight from east to west, and the creation of "bridgeheads" inside a not occupied area, well known from the *Cameraria ohridella.* The regular steps forward of 80 to 100 km, also known from *Cameraria ohridella*, can also be realised with this species.

This miner will get affected by parasites as well as the most of the other more than 70 indigenous and invaded species of the genus *Phyllonorycter*. In 2002 about 50% mines with symptom of parasites were found and in the following year at the same places in autumn 60 ... 90% of the mines presented symptoms of larval parasitism. Thus this species is to evaluate different in relation to their host than *Cameraria ohridella*.

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EFFECTS OF ABIOTIC FACTORS ON OVERWINTERING PUPAE OF *CAMERARIA OHRIDELLA* (GRACILLARIIDAE, LEPIDOPTERA)

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Abiotic factors have an important influence on the survival of overwintering pupae of *Cameraria ohridella* Deschka et Dimić. The aim of this investigation is to get basic information about the tolerance of overwintering pupae of *Cameraria ohridella* on abiotic factors for suitable composting of infested horse chestnut leaves, especially in great cities, as a possibility for cultural measurements against *Cameraria ohridella*.

Laboratory experiments have been carried out to test the effects of temperature, relative humidity and the thickness of soil layers on the emergence of *Cameraria*-pupae. Altogether 1380 *Cameraria*-pupae were isolated from fallen leaves in autumn and put into plastic boxes for the trials. Temperatures between 24 °C and 45 °C (hot variant) and temperatures between 0 °C and -21 °C (cold variant) were tested; every temperature was tested with two different degrees of humidity (30% and 95% relative humidity). Another part of the *Cameraria*-pupae was covered with 3 different layers (thickness of 1 cm, 3 cm and 6 cm soil). In spring the number of emerged and dead moths of every variant was evaluated.

POPULATION DYNAMIC OF *PHYLLOCNISTIS CITRELLA* STAINTON (LEPIDOPTERA: GRACILLARIIDAE) AND PARASITISM LEVELS IN WESTERN SICILY (ITALY)

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Studies on population dynamics, parasitism levels and mortality of *Phyllocnistis citrella* are carried out in some unsprayed citrus orchards of western Sicily (Italy) from June 2002 to December 2003. *Phyllocnistis citrella*, recorded in Italy in 1995[1], showed high infestation levels with 10 generations per year and a life-cycle of 15 days in summer and 134 days in winter[2]. Initially, many indigenous natural enemies were found[3], later on exotic species were introduced in Sicily[4], but in the last five years parasitoid species greatly represented were *Semielacher petiolatus* (Girault) and *Citrostichus phyllocnistoides* Narayanan[5]. Our results show that the active presence of the citrus leafminer was detected in the summer and autumn months of 2002 and 2003 years, with high population densities and simultaneous presence of all different stages. The average number of eggs/leaf was very low and never more than 1 egg/leaf. For first instar larvae the major density was recorded on July 2002 and 2003, for second instar larvae on July 2002 and September 2003, for third instar larvae on Autumn months 2002 and 2003, for fourth instar larvae on November 2002 and August 2003 and for pupae on June-July 2002 and 2003. The parasitoid species more present was *Citrostichus phyllocnistoides* followed by *Semielacher petiolatus*. The major percentage of active parasitism was recorded on December 2002 and 2003 with values up to 75%. Parasitism on first instar larvae was never found. The parasitism level for second instar larvae was more high on November 2002 (about 28%) and on October 2003 (about 33%), for third instar larvae on November 2002 (about 50%) and November 2003 (about 58%), for fourth instar larvae on August 2002 (about 50%), November 2002 (about 40%) and on July 2003 (50%) and on August 2003 (20%) and for pupae on October 2002 (50%) and September 2003 (50%). The correlation between parasitism levels on different stages of *Phyllocnistis citrella* and the average number of each them per leaf was significantly positive for second, third and fourth instar larvae and significantly negative for pupae. The larval mortality rate ranged from 9% (June 2002) to 99.2% (December 2002) and from 16% (June 2003) to 97% (December 2003).

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METHOD TO QUANTIFY CAMERARIA OHRIDELLA LEAF DAMAGES ON *AESCULUS HIPPOCASTANUM* USING IMAGE ANALYSIS

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Quantification of feeding by phytophagous insects is often very difficult and imprecise. There are several methods to estimate damages. For example, counting the number of squares with damages on a grid overlayed on the leaf surface; visual estimating the leaf area by comparison with pictures of leaves with known amount of damage[1,2]. These techniques are simple, but sometimes imprecise. They depends on the personal interpretation of damaged areas and this causes error unavoidable. When there is the necessity for a more accurate estimation (e.g. to improve biological knowledge), other techniques such as the automated image processing are required.

In this study the image analysis software SigmaScan® Pro was used to estimate *Cameraria ohridella* leaf damages. In particular this accurate evaluation was useful to:

- establish the progressive infestation during the year on *Aesculus hippocastanum* L. trees
- define the leaf surface eaten by one larva when its development is completed.

Materials a methods

Aesculus hippocastanum trees were chosen in a public park where *Cameraria ohridella* had been already detected in previous years.

The leaf damage was measured every four weeks between May, when first mines occurred, and the falling of the leaves. Five leaves per tree were randomly taken at three different height: 2, 5 and 10 m. They were taken to the Institute of Entomology of the University of Milan where each one was acquired with a digital camera (300 pixel/inch resolution). Every image was analysed using SigmaScan® Pro. This programme was calibrated and set to calculate the area in cm^2. At first the damaged area was calculated and, then, the entire leaf area. All data were acquired in a Excel file and analysed according to the Analysis of Variance (ANOVA). Then, a Duncan's multiple range test was used to separate means into significant ranges when a significant ANOVA was obtained ($P > 0.05$ used throughout).

To define the leaf area eaten by one larva, every month, 13 mines containing a pupa were individually scanned with a flat bed colour scanner (1200 pixel/inch). The area of every mine was calculated with SigmaScan® Pro.

Results and disscussion

The ANOVA and the Duncan's test gave evidence of significant differences between the three height (Table 1).

Table 1: Results of Duncan's test

	June	July	August
2 m	13.31 ± 2.30 a	13.56 ± 5.29 c	73.70 ± 23.26 e
5 m	16.12 ± 4.31 a	44.82 ± 21.16 d	78.71 ± 22.67 e
10 m	1.49 ± 2.13 b	33.92 ± 8.97 d	65.62 ± 31.39 e

In June, the attack was similar at 2 and 5 m, while the lowest infestation was at 10 m. At this height the mean surface attacked was only 1.49% while at 2 and 5 m was already 13.31% and 16.12% respectively.

In July, damages increased only at 5 and 10 m, while at the lowest height were similar to the values in June (13.56%). In August, the infestation was equally distributed all over the tree and the leaf surface was nearly completely covered with mines. After this check, the leaves felt down and no more monitoring was possible.

These results demonstrates the trend of *Cameraria ohridella* to colonise progressively the plant beginning from the lowest leaves and migrating to the upper part ones. This confirms the leafminer behaviour to move on the trunk cork at the beginning of the spring. The highest colonization at 5 and 10 m in July demonstrates that the leafminer migrates to the upper leaves, even if there is enough food in the lowest zone, probably attracted by light. Only when the food is low the leafminer migrates again in the basal part of the plant.

The mean of the leaf area eaten by a single larva at the end of its development was 2.15 cm^2 with a standard error of 0.60. This means that in summer, when a leaf surface is up to 1000 cm^2 and it is completely damaged, there could be about 465 mines on it.

The image analysis software used provides reliable damage estimates because measurements are automated and do not depend on personal interpretation. Besides, pictures provide permanent record that could be used again, because they are stored on disk.

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HORSE CHESTNUT TREE INJECTION TREATMENTS AGAINST *CAMERARIA OHRIDELLA* AND *GUIGNARDIA AESCULI*

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Treatments in urban areas to control the horse chestnut leaf miner by means of trunk injections are suggested and carried out by several Italian public administrations. To evaluate the efficacy of this technique, two-year trials were conducted. The active ingredients Imidacloprid and Abamectin (MeritGreen® and Vertimec® registered in Italy specifically for tree endotherapy) were compared. Furthermore, since endotherapy is a rather invasive technique, we examined the possibility to control *Guignardia aesculi* adding the fungicide Thiabendazole (Arbotect 20S®). To enhance the plant metabolism we also tested the Siapton® a product with biostimulant effect. The healing of injection wounds was checked at the end of the trials. Both insecticides were able to control *Cameraria ohridella* larvae. Abamectin was dislocated in the plant faster than Imidacloprid and showed higher persistence in control up to the second year after the injection. The efficacy of the fungicide and of the biostimulant was not clearly evinced, and most of the injection wounds were healed by the plants after several months.

FIRST RECORD OF *PHYLLONORICTER DELITELLA* Z. LEPIDOPTERA: GRACILLARIIDE ON *QUERCUS PUBESCENS* WILLD., IN SICILY (ITALY)

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Phyllonoricter delitella Z. (Lepidoptera, Gracillariidae) is a specific leaf miner of the downy oak (*Quercus pubescens* Willd.). It is found throughout Europe. In Italy it has been found wherever there is the host plants but never been found in Sicily. The Gracilariid moth is recorded for the first time in Sicily. It was found at "Pizzo dell'Apa", a wood owned by forest state of Santo Stefano Quisquina. It is a natural wood land 157 ha wide at an altitude between 520 and 890 metres above sea level. Later, *Phyllonoricter delitella* was also found at "Bosco della Ficuzza", a natural wood land 50 km from the first area of finding.

Investigations were carried out, both on the biology of *Phyllonoricter delitella* and on the presence of natural enemies. Particular care was taken regarding the number and duration of the generations per year and the presence of parasitoids.

In order to determine the duration of the life cycle and number of annual generations as soon as the first emerging adults, undamaged oak plants were infested by *Phyllonoricter delitella.* The next generation would then follow on these plants. Plant were infested by closing them up in net bags where 20-30 males and females adults of *Phyllonoricter delitella* had been introduced. At the same time some uninfested downy oak branches were bagged and then infested using the methods, described above, in order to make comparisons. Observations were then carried out to note the biological development.
Sample were collected periodically to locate the presence of parasitoids. The pupae, with part of the leaf, taken in the sample were each putted in glass vials (1 cm ∅ and 5 cm long). The vials were kept in the lab at room temperature until adults emergence.

During the year, from the first ten days of April 2000 until the first then days of April 2001, five generations were recorded. The first generation lasted 49 days, the second 44 days, the third 37 days the fourth 58 days the fifth lasted 160 days.

Several species of parasitoids belonging to the Braconidae (60.6%) and Eulophidae (39.4%) from isolated pupae emerged. From the Eulophidae family the following were: *Cirrospilus vittatus* (Walker), *Sympiesis sericeicornis* (Nees), *Pediobius saulius* (Walker), *Chrysocharis* sp., *Euderus* sp., *Pnigalio* sp, *Aprostocetus* sp.. The species belonging to the Braconidae family are still under identification. During the observation period, the parasitization level ranged from 20% to 30%.

AESCULUS HIPPOCASTANUM WITH RESISTANT BEHAVIOUR TOWARDS THE LARVAE OF *CAMERARIA OHRIDELLA* IN THE CZECH REPUBLIC

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Horse-chestnut leaf miner *Cameraria ohridella* Deschka et Dimić, (Lepidoptera, Gracillariidae) is the most important pest of horse-chestnut *Aesculus hippocastanum* in Europe. The first occurrence in the Czech Republic was in 1993[1].

In 1998 a full-grown tree *Aesculus hippocastanum* significantly less damaged by *Cameraria ohridella* than other horse-chestnut trees in vicinity was found. On leaves were created small, elongated atypical uneven mines and sometime also typical larger mines, but the larvae development in either was not completed. On some leaves typical mines with complete larvae development were found. This characteristic has persisted till present time. This tree (designation HZR1357) was used as donor of scions for grafting in order to obtain plant material for experiments.

Twenty five young grafted plants HZR1357 were used to verify the resistant behaviour observed on the donor tree. The infestation was carried out under artificial conditions in glasshouse as well as in natural conditions under full-grown trees *Aesculus hippocastanum*. Young seedlings of *Aesculus hippocastanum* were used as a control.

In 2001 and 2002 on some of HZR1357 plants were created only atypical mines, on the rest of HZR1357 plants atypical and also typical mines occurred. The overall damage of all HZR1357 tested plants was distinctly lower compared to control seedlings.

In 2003 the experiments were carried out with eight selected HZR1357 plants and the same number of control seedlings under water-stressed conditions. On the control plants there were created only typical mines and the damage reached up to 90% of lamina. All of the tested HZR1357 plants demonstrated similar behaviour as in 2001 and 2002, but on some plants the atypical mines gradually became longer and concentrated mainly along leaf veins. Development of *Cameraria ohridella* in these mines was not completed. Mining damage of these HZR1357 plants increased up to 50% of lamina, but the overall damage was still distinctly lower than on control plants.

The results of experiments in 3 vegetation periods verified the resistant behaviour to *Cameraria ohridella* on young grafted plants HZR1357.

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CHESTNUT PROTECTION AGAINST *CAMERARIA OHRIDELLA* USING IGR

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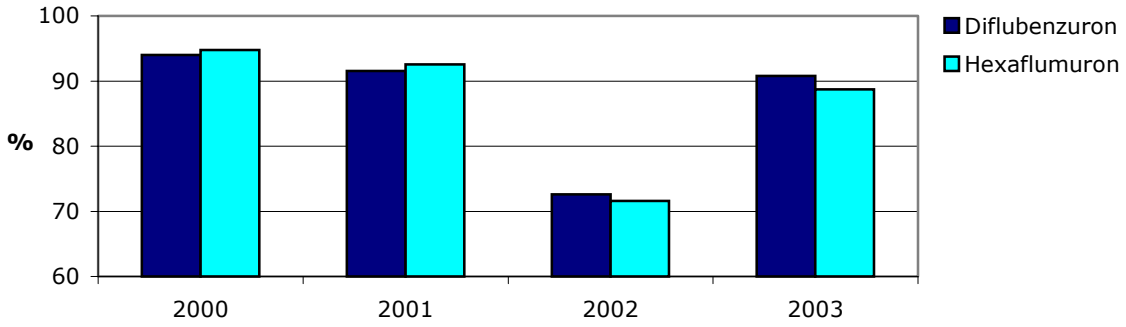
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Over the past about a dozen years in Croatia there has been a considerable increase of damage caused to ornamental plants in urban surroundings. The appearance of *Cameraria ohridella* Deschka et Dimić in 1989 have pointed to the need of a more considerable plant protection in urban surroundings as well. Since horse chestnut is very common in most Croatian cities, damage caused by chestnut miner has provoked the attention of a wide public. This was the reason why other pests attacking the urban vegetation also came into focus.

Cameraria ohridella is in Croatia prevented mostly with knapsack mistblowers. The lacks of this method are the risk of polluting human environment by pesticides and treatment up to only 6-10 m. Still, according to our four-year experience, if applied timely, this constitutes a good protection of horse chestnut against *Cameraria ohridella*. The basic precondition is the monitoring of pest population through pheromone use and visual inspection of plants. In the course of 2003, we have also started the research of endotherapeutic methods in horse chestnut protection against pests.

The only agent today permitted for use in *Cameraria ohridella* prevention in Croatia is the insect growth regulator diflubenzuron, applied through foliar treatment. The research includes only one other insect growth regulator hexaflumuron. During trials, horse chestnut trees 8-15 m high were treated twice a year within terms set according to the monitoring of the pest population dynamics. Dimilin SC 48 (diflubenzuron) was prepared in concentration of 0.05%, and Sonet 100 EC (hexaflumuron) in that of 0.2%. During application, the liquid application rate per tree was 1.5-3 L.

Graph: Efficacy of IGRs recorded in August 2000-2003



The graph shows that there are no statistically significant differences between the efficacy of diflubenzuron and that of hexaflumuron. The application of both insect growth regulators resulted in very good protection against the chestnut leaf miner. Somewhat poorer chestnut protection was achieved in 2002 because the application was too late.

DAMAGE TO LEAVES OF HORSE CHESTNUT TREES INDUCED BY THE HORSE CHESTNUT LEAFMINER (*CAMERARIA OHRIDELLA* DESCHKA ET DIMIĆ)

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The horse chestnut leafminer (*Cameraria ohridella* Deschka et Dimić) was first observed in Slovenia in June 1995 on old horse chestnut trees (*Aesculus hippocastanum*) in Kette Avenue in Novo mesto, Dolenjska, and then in autumn of the same year in the area of Ormož, in the vicinity of the Slovenian-Croatian border. The species has spread very rapidly all over the country since then, and so in 1998 it was found even in the remotest areas in which horse chestnut is present. Therefore it can be concluded that the pest was introduced into Slovenia a year or so prior to the above mentioned find in 1995.

In 2003, horse chestnut trees of Tivoli Park in Ljubljana, the capital of Slovenia, were monitored for the presence of the horse chestnut leaf miner and subsequently induced damage. The monitoring and sampling of infested leaves were conducted on 15 trees of *Aesculus hippocastanum* in three separate groups, that is, 5 trees in each group. The first group consisted of trees 10 - 15 m in height with the mean girth of trunk of 70 cm, 0.5 m above the ground. The second group contained old trees over 25 m high with the mean girth of trunk of 215 cm, and the third group contained young trees 4 to 6 m high with the mean girth of trunk of 27 cm. The sampling was carried out 4 times, in the middle of June, July, August, and September. Each time 4 leaves were collected from each tree, that is, 60 leaves in total. The leaves were encircled onto tracing paper and holes in the leaf surface caused by the horse chestnut leafminer were coloured. The area of holes and that of leaves were measured by means of the computer programme Analysis.

The findings of this study show that three generations developed in this year. Moths of the first generation emerged from the end of April to the end of May, moths of the second generation emerged from mid-June to mid-July, and moths of the third generation were present abundantly in August. Larvae of the third generation cause most damage due to their large numbers, which was also confirmed by the measurement of damage to leaves. In June, 1% of leaf surface on average was adversely affected, in July 3%, in August 24%, and in September 68%. As early as mid-September, desiccation of leaves and leaf fall were observed particularly in leaves, which were almost completely covered with boreholes. An essential difference in damage was also noted between the different groups of trees that were investigated.

USE OF PHEROMONE TRAPS FOR SEASONAL MONITORING OF *PHYLLONORYCTER PLATANI* (LEPIDOPTERA: GRACILLARIIDAE) IN BULGARIA AND IN THE CZECH REPUBLIC

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Phyllonorycter platani (Staudinger, 1870) is an occasional pest on *Platanus* spp. It was described from northern Italy, but its autochthonous area reaches east of the Balkans up to Central Asia. During the 20th century it invaded nearly the entire Central and Western Europe up to Denmark and the southern parts of England and Sweden. The species was introduced also in the United States.

Sticky Delta pheromone traps lured with the female sex pheromone of *Phyllonorycter platani*, *Z*10-tetradecenyl acetate, were used for seasonal monitoring of the pest in two sites in Bulgaria and one site in the Czech Republic in 2003.

In Bulgaria the first *Phyllonorycter platani* male catch was registered on April 21. The flight of the first (overwintered) generation lasted almost till the end of June. The flight of the second (first summer) generation started at the beginning-middle of July and lasted till the beginning of August. The flight of the third (second summer) generation was not registered - only single moths were caught at the end of August and the middle of September.

In the Czech Republic the start of the flight of *Phyllonorycter platani* was registered a week later than in Bulgaria - on April 28. The flight of the first (overwintered) generation terminated before the middle of June and the second (first summer) generation commenced about a week later with a peak between the end of June and the middle of July. The regular flight continued till the beginning of August. Only single moths of the third (second summer) generation were caught during August with the last specimen caught in August 28. The early occurrence and termination of the third generation was due to the unusually hot summer in 2003.

The catches from the second and particularly of the third generation were lower than the catches from the first generation in both countries. This is in some contradiction with the higher number of moths observed on the trunks of trees for the summer generations. Some hypothesis explaining this phenomenon and possibilities for optimisation of the pheromone traps for monitoring *Phyllonorycter platani* are discussed.

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RESIDUES OF DIFLUBENZURON ON HORSE CHESTNUT LEAVES AND EFFICACY OF INSECTICIDES AGAINST THE HORSE CHESTNUT LEAFMINER (*CAMERARIA OHRIDELLA*) WITH NOTES ON ITS PARASITIZATION

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Residues of diflubenzuron on horse chestnut leaves were determined using simple extraction procedure with methanol followed by HPLC with UV detection on octadecyl-modified stationary phase. Linear gradient of methanol in 0.01 M ammonium acetate was used for the separation of the pesticide from other components of the extract. To quantify the amounts of diflubenzuron in the extracts, calibration curve was measured in the concentration range 0.1 to 15 μg/mL. All calibration solutions were prepared in methanol extract of diflubenzuron-free horse-chestnut leaves to standardize matrix effects. Detection and quantification limit for diflubenzuron was found to be 44 ng and 74 ng, respectively, in injected volume. Using this method, residues of the insecticide on trees treated with Dimilin® 48 SC (a. i. diflubenzuron) were quantified. After 127 days from "run-off" application of Dimilin, 38% of the diflubenzuron still remained on the leaves. Analytical data showed long-term persistence of diflubenzuron on the horse chestnut leaves, that is supported also by biological data.

Biological efficacy of diflubenzuron was estimated by counting the mines on the leaves simultaneously used for residue analysis. Irrespective of the term of infestation by the leafminer moths (50, 63 and 127 days after treatment) only two small (< 2mm) mines were found on treated leaves in comparison to 254 - 348 large (<9 to >9 mm) mines on the respective untreated leaves. According to the experiments with potted horse chestnut seedlings the development of the leafminer progeny was affected probably also by contamination of the moths caged on plants freshly treated (one day earlier) by Dimilin.

In similar experiments, when only the lower part of the plant was treated by Confidor (imidacloprid) or Calypso (thiacloprid), the development of the leafminer progeny was almost completely stopped on the treated leaves and to some extent inhibited also on untreated leaves. This can be explained by translocation of the insecticides in plant tissues. The unexpectedly high occurrence of hymenopteran parasitoids in leafminer mines was found in these experiments.

The same phenomenon, increased parasitization, was observed when scoring the size of mines, leafminer larvae and pupae on Mospilan (acetamiprid) treated and untreated *Aesculus hippocastanum* and on untreated *Aesculus carnea* trees in one locality characterized by high population density of *Cameraria ohridella*. In relation to the number of emerged imagoes, the number of mines with parasitoids was low on untreated *Aesculus hippocastanum*, higher on treated *Aesculus hippocastanum* and extremely high on *Aesculus carnea.* These preliminary data on parasitization of horse chestnut leafminer population stressed by unfavourable conditions (insecticide, nutrition?) are presented with the aim to focus interest to these questions.

IMPACT OF *CAMERARIA OHRIDELLA* ON WATER RELATIONS AND PHOTOSYNTHETIC PRODUCTIVITY OF *AESCULUS HIPPOCASTANUM*: SCALING FROM SINGLE LEAF TO WHOLE TREE

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The mining of leaves of *Aesculus hippocastanum* (horse chestnut) caused by the larvae of *Cameraria ohridella* leads to precocious defoliation of trees. Damage to plant productivity was estimated in terms of the photosynthetic performance as well as of leaf water relations and hydraulics of increasingly mined leaves from infested plants in comparison with the same variables measured in non-mined leaves (controls). Measurements revealed that photosynthetic rate of green portions of mined leaves was close to that of non-mined leaves, so that a 1:1 relationship existed between photosynthesis loss and loss of green areas. In other words, damage to the functional integrity of the photosynthetic system did not extend beyond the mines. Stomata below the mines were functional but leaf conductance to water vapour and transpiration rate were 60% lower in mined leaf areas with respect to controls and green portions of mined leaves. Leaf water potential was insensitive to the amount of mined leaf area and so was leaf hydraulic conductance. Anatomical observations of leaf minor veins revealed that they were structurally and functionally intact even in leaves with 90% mined surface area.

In order to assess the whole-plant loss of productivity caused by the parasite, we monitored seasonal changes of leaf gas exchange and leaf area losses in horse chestnut trees (growing in Trieste, North-eastern Italy) freely infested or chemically treated to prevent moth infestation. Data were integrated in a model and the annual loss of net primary productivity (NPP) was calculated for infested trees with respect to controls. Measurements showed marked vertical stratification of *Cameraria ohridella* attacks, with lower crown strata being more infested than higher ones. Leaf gas exchange was maximum between May and early June, but it strongly decreased starting from mid-June even in controls. Model calculations showed that NPP loss of infested trees was about 30% in year 2003 (when the first moth attack was recorded at the end of April). Model simulations showed that postponing the start day of attack would have important positive effects on plant's NPP. For example, if the start day of attack were postponed to May 20th, the annual loss of NPP would be about 15%.

Our study suggests that *Aesculus hippocastanum* trees attacked by *Cameraria ohridella* are not facing serious risks of decline, especially if cultural methods are adopted to postpone the start day of attack (e.g. removal and destruction of fallen leaves in autumn). Our data do not support the view that plants need to be totally protected from the parasite by application of insecticides.

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LEAF MINERS AND THE EPPO PROJECT ON QUARANTINE PESTS FOR FORESTRY

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EPPO is an intergovernmental organization responsible for international cooperation in plant protection in the European and Mediterranean region. In the sense of the International Plant Protection Convention, it is the regional plant protection organization for Europe. Founded in 1951 with 15 member governments it now has 44 member governments including 15 countries of the European Union and 29 non-EU countries.

The EPPO Secretariat proposed and the EPPO Council of 1999 adopted a new Project on Quarantine Pests for Forestry, which would systematically evaluate the phytosanitary risks for the EPPO region (mainly from pests occurring on the territory of the former USSR) and would take account of the special nature of forestry phytosanitary problems to develop procedures to reduce the risk of spread of pests. It was also decided by the Council to create a new EPPO Panel on Quarantine Pests for Forestry for the implementation of this Project.

Under the Project, the EPPO Secretariat collected data on the distribution of 653 species of main forest trees (196 coniferous & 457 deciduous) and 1342 species of forest pests (1142 insects & 200 pathogens) occurring on the territory of the former USSR. Information on most of them exists only or mainly in Russian. In 2000 – 2003 EPPO held 8 meetings of the Panel on Quarantine Pests for Forestry (in Finland, Russia, Croatia, Lithuania, Latvia, Italy and France - twice). The Panel have been prioritising insect pests and performing the Pest Risk Analysis (PRAs) (according to the EPPO standards) for the most important pests on the base of datasheets collected by the Secretariat. This work will be continued in 2004 when one more Panel meeting is planned (in Sweden). The main criteria being used for the prioritisation are: major importance on the territory of the former USSR, damage to plants important within the EPPO region, absence from the non-Asian part of the EPPO region, availability of trade pathways.

27 species of insect pests have been analysed for phytosanitary risk by 2004. Sixteen of them have been proposed by the Panel as quarantine pests for the inclusion into the EPPO lists, including the lime leaf miner *Phyllonorycter issikii* Kumata. Pest specific phytosanitary requirements (PSPRs) were elaborated for these pests. At the end of the Project, commodity standard for wood will be prepared. It is planned to publish all databases, data sheets, PRAs, PSPRs and other results obtained under the Project. The specificity of leaf miners and their phytosanitary significance are discussed.

SYSTEMIC APPLICATIONS OF BIOINSECTICIDES FOR CONTROL OF *AESCULUS HIPPOCASTANUM* AGAINST *CAMERARIA OHRIDELLA*

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The insecticidal properties of neem bioinsecticides were evaluated against the horse-chestnut leafminer, *Cameraria ohridella* (Lepidoptera, Gracillariidae) on the horse-chestnut trees (*Aesculus hippocastanum* L., Hippocastanaceae). The insecticide was applied by means of the systemic tree injection tube inserted into trunks.

On 20.4. 2003, undiluted formulation NeemAzal-T (contained 5% azadirachtin) or NeemAzal-U (contained 16 % azadirachtin) was injected into horse chestnut tree (15 – 18 cm diameter at breast height) at 0.08, 0.15, 0.25 g of active ingredient per 1 cm of diameter at breast height (hereafter AICDBH). After 60 h all of the contents had entered the trees.

The effective dosage of azadirachtin was obviously lower than 0.15 g AICDBH as comparable foliage protection was achieved at 0.08 g AICDBH that resulted in 78.6% (for 1st generation) and 68.6 % (for 2nd generation) pupae reduction. These systemic injections with formulations containing azadirachtin provided long-lasting insecticidal activity. A dosage of ≥0.15 g AICDBH in the horse-chestnut tree was effective against 1st; 2nd and 3rd generations for at least 23 weeks, and dosages 0.25 and 0.15 g AICDBH that resulted in 100 and 99.7 % (for 1st generation) and 100 and 99.1 % (for 2nd generation) pupae reduction, respectively. For 3rd generation and dosages 0.25 and 0.15 g AICDBH was expressed as the number of pupae/ leaf for 0.0 and 0.2 pupae, respectively.

INVESTIGATIONS ON *CIRROSPILUS TALITZKII* BOUČEK (HYMENOPTERA EULOPHIDAE) NEW PARASITOID OF *CAMERARIA OHRIDELLA* DESCHKA ET DIMIĆ (LEPIDOPTERA GRACILLARIIDAE) IN ITALY

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Investigations on spread and biology of *Cirrospilus talitzkii* were conducted during 2003. This parasitoid was detected in 2002 for the first time in the urban area of Bologna on *Cameraria ohridella*. Last year it was found also in one locality on the Apennines Mountains and in one area in Lombardia region (personal communication). It seems that the parasitoid can spread following the horse chestnut leaf miner diffusion. In the surroundings of the first collection site, *Cirrospilus talitzkii* was well established and its population density had increased more than of the other *Cirrospilus ohridella* parasitoid species. The parasitoid emerged from summer and overwintering generations of *Cirrospilus ohridella*. New data on its parasitization behaviour on *Cirrospilus ohridella* third instar larvae was video-recorded. Drumming, probing, host feeding, ovipositing and preening were observed.

PHENOLOGY OF *CAMERARIA OHRIDELLA* DESCHKA & DIMIĆ (LEPIDOPTERA: GRACILLARIIDAE) IN BULGARIA

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Cameraria ohridella Deschka et Dimić produces a variable number of overlapping generations per year in different areas in Europe. Some pecularities, like the presence of a facultative summer diapause observed in the development of the moth, make difficult the exact evaluation of the phenology of the pest by using only one method, e.g. pheromone trapping.

For overcoming these difficulties, three methods were used to study the phenology of *Cameraria ohridella* in Bulgaria. Flight activity of moths was studied by pheromone traps and photeclectors, and the development of preimaginal instars was followed by dissection of 500 mine every two weeks.

The compilation of these three methods showed that three generations of *Cameraria ohridella* occur in the region of Sofia. As estimated by pheromone traps, the flight of the first generation began at the middle - end of April, the flight of the second (first summer) generation began in third decade of June, and the flight of the third (second summer) generation began in second decade of August. Dissection of mines, made at the end of the first summer generation, showed that there are regions in Bulgaria with different development of moth's population. The development was much faster in some regions, like Shumen, Sandanski, Tzarevo etc. In the latter regions, the flight of the first summer generation began 2-4 weeks earlier than that from other parts of Bulgaria and this makes possible development of a partly fourth (third summer) generation.

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SEMIOCHEMICALS IN CONTROL OF CAMERARIA OHRIDELLA?

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Identification of female sex pheromone[1] for *Cameraria ohridella* opened the possibility for effective monitoring of insect populations under diverse environmental conditions in whole Europe[2,3]. Recently developed pro-pheromone-based monitoring system provided stable trap catches over the whole season when formulated onto rubber dispensers.

The challenge we are facing now is to develop the effective control method for regulation of the pest population based on this sex pheromone. We investigated two basic control strategies 1) mass trapping and 2) mating disruption. In mass trapping experiments using several trap designs we attempted to reduce the number of males within the population and consequently reduce the number of mated females able to establish the next generation. Though the number of retained males by mass trapping was extremely large (50-90 000 males/season), the reduction of population density of the next generation (estimated as leaf infestation) was negligible. Another possibility tested was mating disruption using large doses synthetic pheromone or ExoSect™ autoconfusion method. The disorientation was very effective under laboratory conditions and short exposure of males to very low doses of pheromone significantly reduced their ability to find the female in wind tunnel experiments. However, when tested in field, this method did not reduced population density. The results will be discussed with respect to potential use of sex pheromone in environmentally safe control measures within IPM.

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EXPERIENCE OF USAGE TREE INJECTION AGAINST *CAMERARIA OHRIDELLA* IN HUNGARY

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The vitality of trees planted in urban areas has been deteriorating since 1990s in Hungary. The major insects responsible for this deterioration are the horse chestnut mining moth (*Cameraria ohridella*) and the sycamore leaf-bug (*Corycthuca ciliata*). Due to technological shortage of spraying technologies we tried to solve the problem with tree-injection technology. According to our results we are able to protect the trees up to full height against damage of 2 or 3 generations applied one treatment. The 90% of the assimilation surface has not been deteriorated during the vegetation period. The other advantage that leaves were at trees more time and could give their nutrient contents to trees before they fall down.

The major results experienced in horse chestnut were used for the registration procedure of the technology. The callus creation of the wounds caused by drilling has been also investigated in three years long trial.

RECENT ESTABLISHMENT OF HORSE CHESTNUT LEAF-MINER, *CAMERARIA OHRIDELLA*, IN THE UK

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The horse chestnut leaf-miner, *Cameraria ohridella*, was first found established in the UK in the London borough of Wimbledon in July 2002. High densities of leaf mines, and large numbers of adult moths, were found on street trees and in gardens in residential areas of Wimbledon, and on a line of horse chestnuts planted along the edge of Wimbledon Common. Small numbers of leaf mines were also found on trees up to 1 - 2 km from the main area of infestation.

During 2003, *Cameraria ohridella* was recorded from the neighbouring London boroughs of Merton, Kingston and Richmond, and from the more distant boroughs of Ealing, Westminster, Kensington and Camden. Leaf mines of *Cameraria ohridella* were also found for the first time at Ashtead (14 km from Wimbdledon), Leatherhead (16 km), Weybridge (16 km) and West Byfleet (22 km) in Surrey; at Sevenoaks (33 km) and Tonbridge (43 km) in Kent; and at Oxford (80 km). At all of these new sites, densities of leaf mines were low.

The trees at Wimbledon that were heavily attacked in 2002 were also severely infested in 2003, and suffered about 60% defoliation by late September. Monitoring of the population indicated that 2 - 3 generations of the moth occurred during the summer, with the development times of the second and third generations overlapping considerably. The exceptionally hot and dry weather during 2003 probably aided population development and further establishment, and will have assisted dispersal.



Location of current records of *C. ohridella* in the UK

EFFECT OF IMIDACLOPRID INJECTIONS TO THE BARK OF HORSE CHESTNUT ON LEAF INJURES BY *CAMERARIA OHRIDELLA*

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Experiments were carried out in two public parks in Warsaw in 2003. Injured area of horse chestnut leaves by larvae of *Cameraria ohridella* was estimated on 82 trees previously treated and untreated with imidacloprid. Percent of injured area of 50 leaves of each group was estimated using 10-injury scale. Development of mines caused by the larvae of *Cameraria ohridella* was also observed. Significant differences in the level of leaf injury between treated and untreated trees were found. Number and size of mines were strongly reduced on treated trees as compared to not treated.

PHENOLOGY OF PARASITOIDS ON *CAMERARIA OHRIDELLA* DESCHKA ET DIMIĆ (LEPIDOPTERA: GRACILLARIIDAE) IN NATURAL HORSE-CHESTNUT STAND IN BULGARIA

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As a part of European research program, CONTROCAM, parasitoids of horse-chestnut leafminer *Cameraria ohridella* (Deschka et Dimić) were studied in reserve "Dervisha" a natural stand of *Aesculus hippocastanum* L. in Bulgaria.

The study was conducted during 2001-2003. Abundance of the moth and its parasitoids were studied during three seasons with different infestation level. First season – before the total defoliation of the reserve, second season - total defoliation of whole reserve in July and third season – after the total defoliation of the reserve during previous season. Flight activity of the moth and its parasitoids was studied by photoeclectors. The development of immature stages was followed by regular dissections of mines.

The information is given on the percentage of parasitism, relative abundance of parasitoids of each moth generation during the course of the investigation and on the emergence pattern of the dominant parasite species involved. The phenology of the parasitic wasps is compared with the occurrence of *Cameraria ohridella.*

The impact of parasitoids on each moth generation is discussed. The observation suggests that infestation level of the moth as well as immigration of parasitoids from another leafminer hosts influence the species composition and relative abundance of the parasitoids on *Cameraria ohridella.*

PARASITOIDS OF *PHYLLONORYCTER PLATANI* (ST.) (LEPIDOPTERA: GRACILLARIIDAE) IN BULGARIA

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The parasitoid community of *Phyllonorycter platani* (St.), an invasive leafminer in Europe was studied in Bulgaria during 2001 - 2003.

Parasitism of overwintering generation was studied in 23 sites with different climatic conditions. Parasitoids attacking summer generations were studied in Sofia. Mines of the leafminer have been dissected and parasitized stages of the moth have been reared in laboratory.

A total of 14 parasitic species belonging to the superfamilies Ichneumonoidea and Chalcidoidea emerged from mines of *Phyllonorycter platani* on *Platanus* spp. in Bulgaria. All of them are polyphagous on leafminers.

The level of parasitism varied from 18% up to 65 %. The relative abundance of parasitoid species differed between sites. The endoparasitoid on pupae *Pediobius saulius* Walker was the dominant species, attacking overwintering generation in most sites. It was found as an ectoparasitoid on larvae from summer generations too. *Minotetrastichus platanelus* (Mercet) was the most abundant in the summer generations. The encyrtid *Holcothorax testaceipes* (Ratzaburg) was found only in region of South - West Bulgaria where it was the most abundant parasitoid.

PARASITOIDS OF INVADING LEAFMINER MOTHS (LEPIDOPTERA: GRACILLARIIDAE) ON BLACK LOCUST IN REGION OF SOFIA

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Parectopa robiniella (Clemens) an invasive leafminer moth in Europe on black locust *Robinia pseudoacacia* L. was first reported in Bulgaria in 1993. For the period of next two – three years the moth has caused premature defoliation of host plants. *Phyllonorycter robiniella* (Clemens) was found in Sofia ten years later. At present black locusts in Sofia are heavy infested by *Ph. robiniella* but is difficult to find any mine of *Parectopa robiniella*.

Being important part of mortality factors the parasitoids attacking both leafminer species were studied in Sofia during 2002- 2003. Regular dissections of mines have been carried out. Parasitized stages of the moths have been isolated in separate tubes and reared in laboratory.

Infestation level of the moths, list of parasitoids, percent parasitism and relative abundance of parasitic wasps attacking both leaf - miners are presented. Impact of parasitism among the other mortality factors is discussed.

NATURAL ENEMIES ASSOCIATED WITH THE EXOTIC CITRUS LEAFMINER *PHYLLOCNISTIS CITRELLA* (LEPIDOPTERA: GRACILLARIIDAE) IN SPAIN

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The citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is a pest native to Southern Asia which spread to all citrus-growing areas worldwide during the last decade of the XX century. When the CLM arrived in Spain, it was rapidly accepted as a host by some indigenous parasitoids of other leafminers. Thus, in 1994, four different eulophid species were identified on *Phyllocnistis citrella*: *Pnigalio* sp. (later identified as *Pnigalio pectinicornis* L.), *Cirrospilus pictus* Nees, *Cirrospilus vittatus* Walker and *Sympiesis sandanis* (Walker). By 1997, seven more species were added to the list. These were the eulophids *Cirrospilus brevis* Zhu, LaSalle and Huang (= *Cirrospilus* nr. *lyncus* Walker), *Sympiesis gregori* Bouček, *Diglyphus isaea* Walker, *Chrysocharis pentheus* (Walker), *Neochrysocaris formosa* (Westwood) and *Ratzeburgiola cristata* (Ratzeburg) and a pteromalid species. The larval, solitary, idiobiont ectoparasitoids *Cirrospilus brevis* and *Pnigalio pectinicornis* were the most abundant species found on *P. citrella*, representing more than 95% of total parasitoid fauna. In the Mediterranean Basin as a whole, about 30 different species have been recorded since 1994 that parasitize the CLM. Furthermore, lacewings, ants, spiders, minute pirate bugs, thrips, and ladybirds have been cited in the Mediterranean as possible predators of *Phyllocnistis citrella*.

Opportunistically recruited entomophagous insects had a significant and practical effect on CLM populations. Contribution of indigenous natural enemies was important and gave us the chance to become aware of the existence of an important guild of undetermined generalist predators, and opportunistic parasitoids.

Classical biological control programs against this pest were also extensively developed in Mediterranean countries. In Spain, eleven species of parasitoids were imported and six of them were released in the field: the encyrtid *Ageniaspis citricola* (Logvinosvskaya), and the eulophids *Quadrastichus* sp., *Semielacher petiolatus* (Girault), *Galeopsomyia fausta* LaSalle, *Cirrospilus ingenuus* (Gahan), and *Citrostichus phyllocnistoides* (Narayanan). *Citrostichus phyllocnistoides* has been able to exert a substantial control of *Phyllocnistis citrella*. Since its introduction in summer 1998, this parasitoid has expanded to all citrus areas in Spain, and has become the most abundant parasitoid. As a consequence, parasitism rates have increased and damage to citrus foliage has decreased.

References, information and pictures are available on-line at:

http://www.ivia.es/~aurbaneja/imag&present/imag&prese.htm
http://www.seea.es/conlupa/AlbertoWeb/portadanova.htm

ADOPTION OF TWO DIFFERENT INVASIVE GRACILLARIIDAE BY NATIVE PARASITOIDS: WHY SUCH DIFFERENCES?

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The parasitism of two Gracillariidae species, *Cameraria ohridella* and *Phyllonorycter robiniella*, has been investigating in the Czech Republic in the last three and two years, respectively. Both species arrived in the country almost at the same time, about 10 years ago. The results indicate that there are strong differences in the level of parasitism. In *Cameraria ohridella*, the level of parasitism is very low, often less than 1 %. In contrast, in *Phyllonorycter robiniella*, parasitism rates are much higher. Similar variations are found in other parts of Europe. The reason for these differences remains unclear, but some possibilities are discussed.

EFFECTS OF DISTANCE AND MECHANICAL METHODS ON POPULATION DENSITY OF *CAMERARIA OHRIDELLA* (LEPIDOPTERA, GRACILLARIIDAE): AN EXAMPLE OF A SMALL TOWN

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The effects of leaf litter removal and distance on population density of *Cameraria ohridella* were examined in an isolated population of horse chestnut trees for the first time. The possible long distance migration is probably limited to roads and railways because the town is surrounded by forests.

In the small urban area of Mosina (Wielkopolska region, Poland) we checked all horse chestnut trees and all the trees were mined. However, the infestation level was different in individual trees. The main factors determining the extent of the leaf destruction were the procedure of leaf litter removal (ANOVA, $F_{1.\ 136} = 123.06$, $P < 0.001$, Fig. 1a) and the number of subsequent generation of the leaf-miner on a given tree (ANOVA, $F_{2.\ 136} = 65.11$, $P < 0.001$, Fig. 1b). Also, the interaction between these two factors was significant (ANOVA, $F_{2.\ 136} = 22.59$, $P < 0.001$), which means that in the localities with leaf litter removal the rate of leaf damage in the time of mining by subsequent generations was smaller.

We have also tried to establish the influence of the mean distance to places with no litter removal on the infestation level of trees where leaves were removed. We have checked the effect of distance to the nearest site without litter removal too. No statistically significant correlation was found (Spearman correlation, $P > 0.1$, in both cases). The growth of infestation level of removal sites was noticeable only for the third generation of larvae (Fig. 1B) - probably as a result of dispersion from no removal sites in conditions of high competition for nutrients.

The effects of leaf litter removal on the infestation level we found are similar to those reported by Gilbert et al. (2003) and Pavan et al. (2003) for open populations of horse chestnut, but we have shown that the similar processes occurred in isolated population. The procedure of leaf raking is recommended to reduce the population density of horse chestnut leaf-miner and could be especially effective in isolated areas if done properly.

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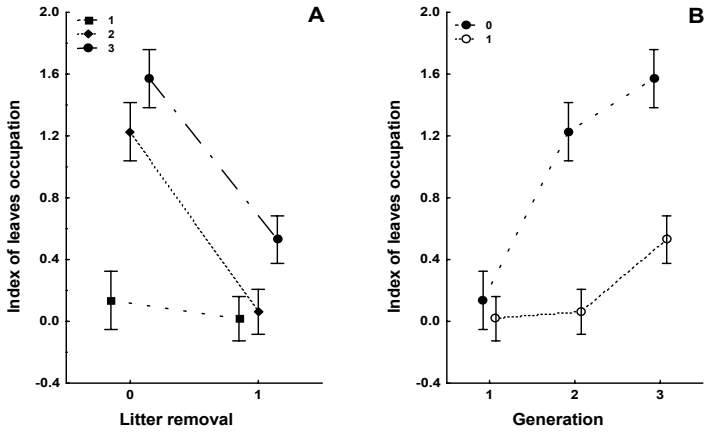


Fig.1
Influence of leaves raking on infestation level of horse chestnut (as percentage of leaves destroyed by larvae (**A**) and growing the leaves destroying by larvae between following generations in relation to raking (**B**). Explanations: 1, 2, 3 – no. of generations; 0 and 1 – no raking and raking areas, respectively. Means are given with 95% confidence limits.

MONITORING OF *CAMERARIA OHRIDELLA* IN THE PARK GROUNDS OF THE CASTLE SANSSOUCI, POTSDAM, GERMANY

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First evidence *of Cameraria ohridella* was found in Brandenburg in 1997/98. Until 2000 the state was colonized region-wide, in 2002 severe damage attracting public attention appeared. As Potsdam and its castles attract thousands of tourists every year, municipal authorities and the park management are trying to find concepts dealing with *Cameraria*.

In spring 2002 the University of Potsdam started a monitoring programme to record ecological data of Cameraria and infested chestnut trees. The following aspects have been investigated:

1. Recordal of the activity of *Cameraria ohridella* by means of pheromone traps
2. Supervision of damage of chestnut trees using a newly developed digital sampling and evaluation method
3. Measurement of physiological parameters in the metabolism of damaged leaves
4. Compilation of a GIS-based land register of the different chestnut tree species and hybrids in the city of Potsdam
5. Testing the effectiveness of the preparation Neem-Azal on single trees

First monitoring results and deriving questions for future studies are presented.

Research is made in cooperation with the municipal authorities of Potsdam, the foundation "Preussische Schlösser und Gärten" (Prussian Castles and Gardens) Berlin-Brandenburg and the Department of Consumer Protection and Agriculture Brandenburg.

THE EVOLUTION OF HOST PLANT USE IN *PHYLLONORYCTER* LEAF-MINING MOTHS

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We used nuclear DNA sequence data to estimate the phylogeny of 77 leaf-mining *Phyllonorycter* (Gracillariidae) moth species. There was strong support for both the monophyly of *Phyllonorycter* and the placement of the genus Cameraria as its sister group. Host plant use was mapped onto the moth phylogeny and investigated statistically in several ways. First, we show that the estimated level of co-speciation between leafminers and their host plants is not greater than expected by chance, despite the physical intimacy of the association. Nevertheless, the pattern of host plant use is far from random, with closely related *Phyllonorycter* species generally feeding on closely related plants. However, while *Phyllonorycter* species from a given host plant tend to form distinct clades, there is also statistical support for multiple independent colonisations of some host plant taxa (e.g. the order Rosales and the genus *Corylus*). Despite numerous host shifts, most *Phyllonorycter* species feed on trees and the few species that attack shrubs or herbs have mostly acquired these habits independently. Similarly, most species mine the lower surface of leaves but the few upper surface miners have each evolved the habit independently. Finally I address the question of how molecular phylogenies can help us to understand the evolution of host use of the genus *Cameraria* and the area of origin of *Cameraria ohridella* in particular.

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The meeting is sponsored by **Klinge Pharma**. **Klinge Pharma** GmbH is a pharmaceutical company located in Munich. The main product of Klinge is **Venostasin® retard** - an approved drug for the treatment and prevention of the chronic venous insufficiency. **Venostasin® retard** is a herbal medicine extracted from horse chestnut seeds. For the horse chestnut leaf miner endangers the health of horse chestnuts, Klinge feels debt to support this symposium.

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