The recent spreading of *Cameraria ohridella* (Lepidoptera: Gracillariidae) in Belgium

J. DE PRINS, W. DE PRINS & U. DALL'ASTA

Summary

Since the discovery in Belgium of *Cameraria ohridella* DESCHKA & DIMIC, 1986, the species has spread all over the country at an enormous speed and it now occurs in all provinces, sometimes in huge numbers, causing severe damage to chestnut trees (*Aesculus hippocastanum*) the larval foodplant.

Keywords: *Cameraria ohridella*, *Aesculus hippocastanum*, faunistics, Belgium.

Samenvatting

De recente verspreiding van *Cameraria ohridella* (Lepidoptera: Gracillariidae) in België.

Sinds de ontdekking van *Cameraria ohridella* DESCHKA & DIMIC, 1986 in België, heeft de soort een explosieve verspreiding gekend. Ze komt momenteel voor in alle provincies van het land, soms zeer talrijk, en daardoor veroorzaakt ze enorme schade aan de paardekastanjes (*Aesculus hippocastanum*), de voedselplant van de rups.

Trefwoorden: *Cameraria ohridella*, *Aesculus hippocastanum*, faunistiek, België.

Introduction

Cameraria ohridella DESCHKA & DIMIC, 1986 was described from specimens collected in the surroundings of Lake Ohrid in Macedonia. By anthropogenic means, it was later introduced into the regions of Linz and Vienna in Austria where the species found an empty niche in the chestnut trees (*Aesculus hippocastanum*) planted in large numbers in the streets and parks of both cities. During the last decade of the 20th century, the species spreads, mainly induced by aerial factors, in all directions but especially westwards (see map in ŠEFROVÁ & LAŠTŮVKA 2001). Towards 1995, *C. ohridella* was well established in all Central European countries and in some of these regions, it was very abundant, causing important damage to the local chestnut trees. Towards the end of the 90's, the species had reached the western part of Germany and in 1999, several mines were observed at Tervuren.

C. ohridella (cover illustration) was first mentioned for the Belgian fauna by DE PRINS & PUPLESIENE (2000) and a more detailed account of its recent distribution was given by DE PRINS & DE PRINS (2001). The species has spread over the whole Belgian territory at an enormous speed and in huge numbers. Within one year, its presence was established in all Belgian provinces, sometimes in very high numbers, causing panic reactions to some foresters and responsible persons for the plantations in parks and along roadsides. In some cases, it was decided to simply cut down all chestnut trees (*Aesculus hippocastanum*), the larval foodplant of *C. ohridella*, in the infested zone.

In 1999 the west border of the species' distribution was situated at Brussels and the eastern part of the province of Antwerp. No information about the situation in the south of Belgium was available then. In and around Antwerp city, and more westwards, no chestnut trees were infested, though many parks were visited. In 2000 however, the distribution in Belgium of *C. ohridella* had quite extended towards the west. The species was observed in almost all localities visited in vain before, and it was furthermore recorded in different places by various entomologists who sent detailed information. In the western part of its distribution area, the species was certainly less abundant than in the central part, where some chestnut trees had almost no leaf areas containing chlorophyll in late summer. In the cities close to the seacoast, only individual mines were reported. During 2001, the occurrence of *C. ohridella* in the western part of the country increased enormously, and in the central part – especially the zone between Antwerp and Brussels – the species caused complete decoloration of many chestnut trees towards late summer, leaving hardly any leaf area for the autumn generation of females to deposit their eggs. The physiology of many chestnut trees was completely out of balance causing some specimens to start blossoming in late autumn as was observed at Tervuren.

Until now, the species has been recorded from following localities in Belgium (see also fig. 1):

- Antwerpen: Antwerpen-City, Beerse, Berchem, Boechout, Boom, Borgerhout, Brecht, Deurne, Edegem, Ekeren, Geel, Kalmthout, Kapellen, Kasterlee, Lier, Mechelen, Merksem, Mol, Mortsel, Nijlen, Oevel, Olen, Postel, Ranst, Tongerlo, Turnhout,

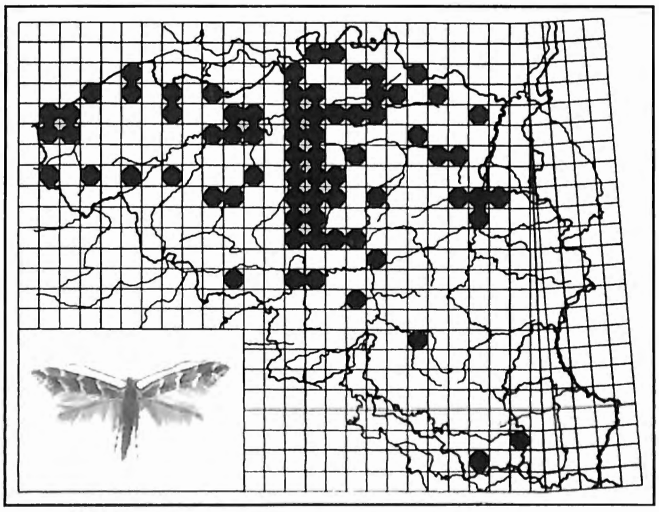

Fig. 1 — Distribution of *Cameraria ohridella* in Belgium (data until mid 2001).

Wechelderzande, Westerlo, Wijnegem, Willebroek, Wilrijk, Wommelgem, Zwijndrecht;
- Brussel: Brussel, Laken, Ukkel;
- Henegouwen: Charleroi, Mons, Mont-sur-Marchienne;
- Limburg: Beringen, Diepenbeek, Genk, Hasselt, Lommel, Opoeteren, Tessenderlo, Tongeren, Voeren;
- Luik: Luik, Visé;
- Luxemburg: Arlon, Virton;
- Namen: Belvaux, Denée, Han-sur-Lesse, Namen, Maredsous;
- Oost-Vlaanderen: Aalst, Assenede, Beveren, Dendermonde, Eeklo, Gent, Kruibeke, Kruishoutem, Lokeren, Ninove, Sint-Niklaas, Waasmunster, Wetteren, Zelzate, Zomergem;
- Vlaams-Brabant: Aarschot, Geraardsbergen, Hofstade, Kampenhout, Leuven, Meise, Oudergem, Sint-Lambrechts-Woluwe, Sint-Martens-Latem, Tervuren, Vilvoorde, Zaventem;
- Waals-Brabant: Braine-l'Alleud, Genappe, Jodoigne, La Hulpe, Louvain-la-Neuve, Nivelles, Ottignies, Perwez, Rixensart, Walhain, Wavre;
- West-Vlaanderen: Brugge, Damme, De Panne, Diksmuide, Ieper, Knokke, Kortrijk, Nieuwpoort, Oostende, Poperinge, Veurne.

Biology

C. ohridella lives only on *Aesculus hippocastanum*, although it has been recorded also from *Acer pseudoplatanus* and *A. platanoides* (GREGOR *et al.* 1998, KREHAN 1995, PSCHORN-WALCHER 1997, HELLRIGL 1999). Sometimes, *Aesculus carnea* is also, though in a lesser degree, infested by *C. ohridella*. Probably due to high population density, the females of the summer generation have difficulties in finding suitable places for ovipositing. In such cases they might lay eggs on other species and races of *Aesculus* and even on other plant species, e.g. *Spiraea* sp., *Fraxinus* sp. and *Symphoricarpos* sp. (SKUHRAVÝ 1999, STIGTER *et al.* 2000). The larvae on these plants, however, can not complete their development and die.

The females oviposit about 30 single eggs on the upper side of chestnut leaves. The eggs hatch after about 10 days and the young larvae immediately enter the leaf through the lower egg surface. The initial mines are comma-shaped and change to more or less rounded mines with an area of 4 to 8 cm^2 (SKUHRAVÝ 1998). The mines are visible as whitish to brownish blotches on the upper side of chestnut leaves, and by holding the leaf towards the sun one can easily check the presence of a *C. ohridella* larva. Older mines colour brown and especially in this stage they can easily be confused with the necrotic pattern caused by *Guignardia aesculi*, a fungal disease of the chestnut tree.

In normal circumstances, the mines do not cross the central nerves of the leaf, but when the population density is very high, they do, and they overlap with other mines. In this way, even the complete leaf surface can be covered with mines, not leaving any space for ovipositing females of the next generation. A larva has 6 instars (fig. 2), a prepupal and pupal stage. Before pupation, the larva constructs a round cocoon inside the mine. Especially the cocoon of the last overwintering generation is very apparently visible through the leaf and dark brown coloured.

In Central Europe, *C. ohridella* has 3 to 5 generations per year, according to the climatic circumstances. In Belgium, there are 3 generations per year. Adults are on the wing in April-May when the chestnut trees are blossoming. They can be found resting on tree trunks of preferably old trees. Mines are observed from the end of May till July, producing the second generation from the end of June till August. This generation is the most abundant. The moths of the third generation fly from the end of August till October. They are less common. The pupae produced by this generation hibernate in their cocoon inside the fallen leaves. In some favourable years, with warm temperatures in October-November, a (partial) fourth generation of *C. ohridella* can develop.

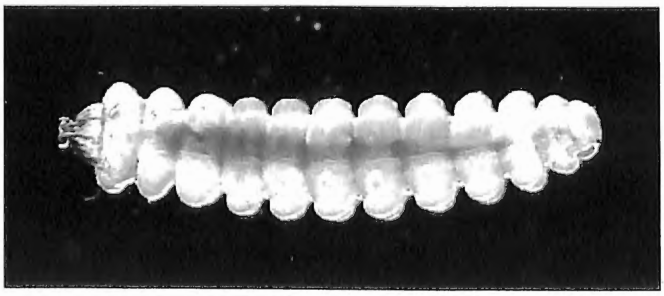

Fig. 2 — Last instar larva of *C. ohridella*.

Parasitism

Parasitism rate is very low in *C. ohridella* and this led to the assumption that *C. ohridella* is not native to the European fauna, although its type locality is Macedonia, but that it dispersed into the western Palaearctic from another area (STIGTER *et al.* 2000, ŠEFROVÁ & LAŠTŮVKA 2001). Anyway, some parasitoids have been recorded from *C. ohridella*, mainly species that are known to parasitise European *Phyllonorycter* species. Thus far, 19 species of Eulophidae, 1 Eupelmidae, 2 Pteromalidae and 2 Ichneumonidae have been mentioned in publications (HELLRIGL 1999). In other European countries the parasitation rate is very low also. In Austria, no more than 1 to 5 % of the larvae and pupae were parasitised in the studied populations of *C. ohridella,* which was established there already for 4 years. The most common parasites were *Pnigalio agraulus* WALKER and *Minotetrastichus ecus* WALKER (LETHMAYER & GRABENWEGER 1997). In the Czech Republic, the parasitation rate was maximum 7 % (SKUHRAVÝ 1999), which is also a very low number, taken into consideration that in most species of European *Phyllonorycter* the parasitation rate extends from 30 % to well over 50 % and sometimes even much higher as in the case of *Phyllonorycter leucographella* (ZELLER, 1850), found at Antwerp where about 100 mines produced the same amount of hymenopteran parasitoids. In Belgium, the parasitation rate is less than 10 % as was observed during several rearings from mines collected through 1999-2001.

A hymenopteran species not yet recorded as a parasite of *C. ohridella*, is *Itoplectis alternans* (Ichneumonidae, Pimplinae, det. C. ZWAKHALS). A female specimen emerged after hibernation on 10.02.2000 from a leaf mine of *C. ohridella*, collected at Tervuren (Belgium, Vlaams-Brabant) in late autumn of 1999. The specimen of *I. alternans* was twice as small than usual sized specimens, because the caterpillar of *C. ohridella* contains much less nutritients than the normal hosts of this hymenopteran species. This case, however, shows that *C. ohridella* can be attacked by a variety of parasites normally not connected to it.

The chromosomal aspects of *Cameraria ohridella*

Cameraria ohridella has recently been karyotyped (DE PRINS, DE PRINS & DALL'ASTA 2002), however we would like to provide some evidents on the peculiarities in chromosome behavior in male and female meiosis. In this species, as it is normal for Lepidoptera chromosomes, the absence of localized centromeres in male meiosis was ascertained and the chiasmatic nature of male and achiasmatic nature of female meiosis was confirmed. Spermatogonial and oogonial haploid chromosome complements were studied. The haploid chromosome number of *Cameraria ohridella* is determined as 30, which is the characteristic and modal number for Gracillariidae (PUPLESIENE & NOREIKA 1993). The karyotype is symmetric with the bivalents forming a desceding series in size, thus confirming the ancestral state of karyotype structure in Lepidoptera in general (ROBINSON 1971 & 1990, LORKOVIÆ 1990). The DNA investigation did not show any significant differences among the populations studied (KOVÁCS, STAUFFER & LAKATOS 2000).

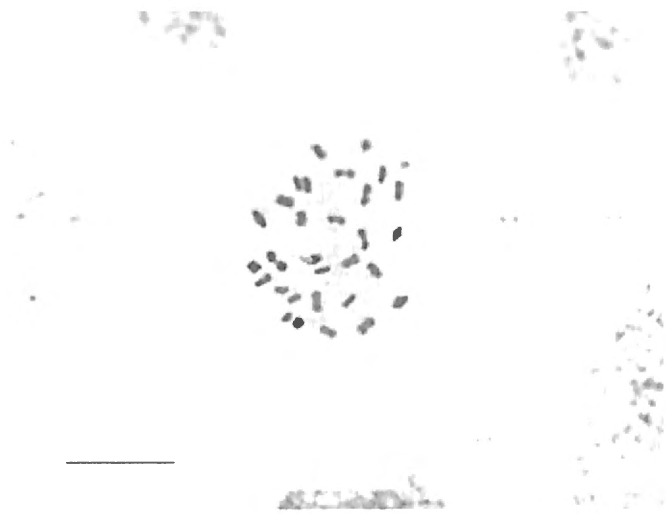

Fig. 3 — A cyst containing spermatocytes in metaphase I of meiosis in *Cameraria ohridella* male pupa (Feulgen-Giemsa method). Scale bar 10 µm.

Meiotic chromosomes in C. ohridella males

Metaphase I of male meiosis is the most suitable stage for counting chromosome numbers in Lepidoptera. Bivalents in this stage are maximally condensed. Three cysts were found in three specimens containing spermatocytes in pachytene, diplotene, diakinesis, prometaphase I and metaphase I (fig. 3).

30 bivalents were aligned in the metaphase I plate. All the bivalents in *M*I are dumb-bell shaped or elongated. The largest bivalent is situated close to the center of the metaphase plate and shows preceding separation of homologues. Most of the bivalents, including the largest one, are homochromatic. However, the midsize bivalent in a descending series shows a distinguishable positive pycnosis. This bivalent of more or less round shape is situated at almost the edge of the metaphase plate, and consists mainly of C-heterochromatin. It could be a sex chromosome. However, in the earlier stages of meiosis as pachytene, diplotene and even prometaphase I, we did not interprete this formation of bivalent as heteropycnotic. This bivalent tends to show a facultative heteropycnosis. The other midsized bivalent is delayed in chiasmata terminalization and in *M*I still shows two chiasmata. Two compacted bivalents in *M*I show a nonhomologous association by their lateral parts, while the other ones are free and radially aligned, keeping a different orientation towards the axis of the meiotic plate. No B chromosomes

and no polymorphism in chromosome number was observed.

Meiotic chromosomes in C. ohridella females
Female meiosis was followed from early pachytene in medium sized eggs till metaphase I in mature eggs. 30 females and 49 oocytes in meiotic divisons were studied. During pachytene stage the bivalents consist of long and wide structures with an exceptionally clear chromomere pattern (fig. 4). A bivalent chromosome having V form was found and thus we identify it as ZW. Therefore, the sex determination mechanism in *Cameraria ohridella* is ZZ? - ZW?. After pachytene, the chromosome condensation proceeded without diplotene and diakinesis stages, showing achiasmatic oogenesis which is typical for Lepidoptera. The condensed achiasmatic bivalents in *M*I of oogenesis are isopycnotic. Nonhomologous telomeric associations forming bivalent chains were found (fig. 5). The largest bivalent showed high condensation degree in *M*I. Intrapopulational polymorphism in B chromosomes was observed.

Damage and control measures

Especially the second-generation larvae of *C. ohridella* cause a lot of damage to the leaves of *A. hippocastanum*. They can be so numerous, up to 200 mines per leaf, that the whole leaf area is covered. This causes the leaves to fall already in July, which in its turn is responsible for an important distortion of the tree's sap stream towards the buts and towards the root system (STIGTER *et al.* 2000). Although "an apocalyptic extinction of the chestnut tree", as announced on several occasions in regional newspapers in Belgium, will probably never occur, while even after several years of heavy infestation in the Vienna area (Austria), no individual case of the death of a chestnut tree caused by *C. ohridella* has been fully documented, it is highly probable that old trees which are also infested by other diseases like *Guignardia aesculi*, die sooner when they are additionally infested by *C. ohridella*. But not just the death of the chestnut trees is the worry of the foresters, instead the esthetic aspect is. Chestnut trees are planted all over Europe not for their economic importance as fruit or wood producers, but for their majestic appearance in parks, gardens and lanes. It is exactly this esthetical importance that gets lost all together with the decoloration and contortion of the leaves and the early leaf fall.

Several measures have been proposed to diminish the damage caused by *C. ohridella*. The use of insecticides has been put forward, but in many cases it has not been accepted as a desirable method, because of the secondary effects chemicals have on the environment. Indeed, many other organisms (not target species) are killed as well by most of the proposed insecticides and residual chemicals always end up in the upper earth layers, which is almost never taken into account by the decision making authorities. In Italy, for instance, different trials with products like imidacloprid (active ingredient of e.g. Admire, Confidor, or Orbid) and abamectin (active ingredient of e.g. Vertimec) were injected into the truncs of chestnut trees, giving very good results in the control of *Cameraria ohridella*, but these measures raised at the same time a big clamour on the use of these endoxilematic methods. Similar tests with Confidor were carried out in Germany (FEEMERS 1997). Fortunately, in many areas in Belgium, e.g. the Brussels district, the use of chemicals against *C. ohridella* is completely prohibited.

A much nature friendlier method is the use of pheromones. The female sex pheromone of *C. ohridella* has recently been isolated (SVATOS *et al.* 1999a & 1999b) and

Fig. 4 — Pachytene in female meiosis of *Cameraria ohridella*. An arrow indicates the bivalent sex chromosome. Scale bar 10 µm.

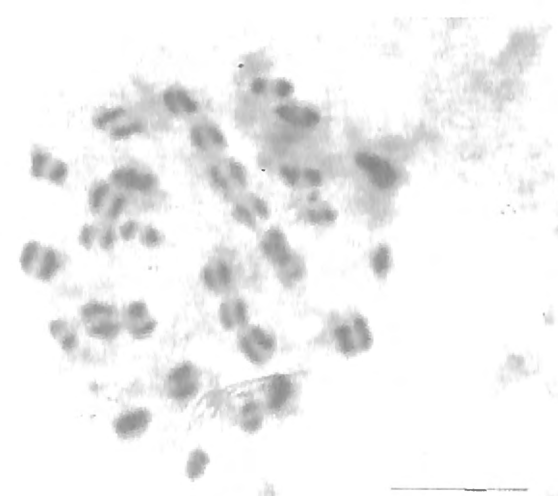

Fig. 5 — Metaphase I in female meiosis of *Cameraria ohridella*, $2n$=60. Nonhomologous telomeric associations forming bivalent chains are seen. Scale bar 5 µm.

it can now be used as an attractant for male specimens. *C. ohridella* imagos like to rest on tree trunks, mainly at lower altitudes (from 1 to 3 m), and by applying glue strips on the trunks, or by placing the pheromone capsules directly into pheromone traps which contain sticky cards, thousands of males can be collected and killed. It is improbable that with this method alone, the infestation rate of *C. ohridella* will decrease drastically. The immediate population density will decrease with the destruction of many males, but enough specimens will remain to fertilize the unaffected females. The use of pheromones is very useful to establish the flight period of the several generations.

The most successful method developed thus far is the removal of all chestnut leaves during winter and early spring. Together with the leaves, also the hibernating pupae of *C. ohridella* are removed. Compostation of the leaves or just putting them in large heaps causes the death of almost all pupae. Population density decreases with 90 %, and, according to studies at the University of Brussels, even with 99 % on the condition that all leaves are removed from the infested area. Until present, this removal of leaves is the most accurate method to keep *C. ohridella* at low densities, though it is rather labour-intensive.

Attempts to apply the use of natural enemies of *C. ohridella* have been unsuccessful thus far and the development of a commercial breeding system of parasitoids in large quantities takes a lot of time and energy. Anyway, a programme of the European Commission has been developed in order to co-ordinate all studies regarding this matter and attempts made to control the damage caused by *C. ohridella* on chestnut trees.

Acknowledgements

We would like to thank P. Berx, D. Boeye, J. Bosselaers, Dr U. Dall'Asta, ir D. De Meyere, G. De Prins, T. Garrevoet, H. Henderickx, J. Possemiers, G. Rappé, and P. Roskams for the information on the occurrence of *C. ohridella* in Belgium, and Dr C. Zwakhals for the identification of *I. alternans*. We are indebted to Dr Luc De Bruyn (Brussels, IN) and Dr Jan Scheirs (Antwerpen, RUCA) for providing the facilities for chromosome research.

References

De Prins, J., De Prins, W. & Dall'Asta, U., 2002. The karyotype of *Cameraria ohridella* (Lepidoptera: Gracillariidae). *Phegea*, 30 (1): 5-10.

De Prins, W. & De Prins, J., 2001. The occurrence of *Cameraria ohridella* in Belgium (Lepidoptera: Gracillariidae). *Phegea*, 29 (3): 81-88.

De Prins, W. & Puplesiene, J., 2000. *Cameraria ohridella*, een nieuwe soort voor de Belgische fauna (Lepidoptera: Gracillariidae). *Phegea*, 28 (1): 1-6.

Feemers, M., 1997. Versuche zur Bekämpfung von *Cameraria ohridella* Deschka & Dimic mittels Stamminjektion (Präparat: Confidor). *Forstschutz Aktuell*, 21: 24-25.

Gregor, F., Laštůvka, Z. & Mrkva, R., 1998. Klínenka jírovcová (*Cameraria ohridella*) napadá také javor [*Cameraria ohridella* also on *Acer* spp.]. *Plant Protection Science*, 34 (2): 67-68. [in Czechian].

Hellrigl, K., 1999. Die Verbreitung der Roßkastanien-Miniermotte *Cameraria ohridella* Deschka & Dimic, 1986 (Lepidoptera, Gracillariidae) in Südtirol. *Veröffentlichungen des tiroler Landesmuseums Ferdinandeum*, 79: 265-300.

Kovács, Z., Stauffer, C. & Lakatos, F., 2000. Genetic study on the spreading of *Cameraria ohridella* Deschka et Dimic 1986, Lep. Lithocolletidae in Europe. *Növényvédelem*, 36(6): 288-290.

Krehan, H., 1995. Roßkastanienminiermotte *Cameraria ohridella* - Befallssituation in Österreich. *Forstschutz Aktuell*, 16: 8-11.

Lethmayer, Ch. & Grabenweger, G., 1997. Natürliche Parasitoide der Kastanienminiermotte (*Cameraria ohridella*). *Forstschutz Aktuell*, 21: 30.

Lorkovlæ, Z., 1990. The butterfly chromosomes and their application in systematics and phylogeny. *In*: Kudrna, O. (ed), Butterflies of Europe, vol. 2. Aula Verlag: 332-396.

Pschorn-Walcher, H., 1994. Freiland-Biologie der eingeschleppten Roßkastanien-Miniermotte *Cameraria ohridella* Deschka & Dimic (Lep., Gracillariidae) im Wienerwald. *Linzer biologische Beiträge*, 26: 633-642.

Pschorn-Walcher, H., 1997. Zur Biologie und Populationsentwicklung der eingeschleppten Roßkastanien-Miniermotte, *Cameraria ohridella. Forstschutz Aktuell*, 21: 7-10.

Puplesiene, J. & Noreika, V., 1993. A brief karyological review of the Gracillariidae (Lepidoptera). *Phegea*, 21: 55-63.

Robinson, R.,1971. Lepidoptera Genetics. Pergamon Press: 687 pp.

Robinson, R., 1990. Genetics of European butterflies. *In*: Kudrna, O. (ed), Butterflies of Europe, vol. 2. Aula Verlag: 234-306.

Šefrová, H. & Latůvka, Z., 2001. Dispersal of the horse-chestnut leafminer, *Cameraria ohridella* Deschka & Dimic, 1986, in Europe: its course, ways and causes (Lepidoptera: Gracillariidae). *Entomologische Zeitschrift*, 111 (7): 194-198.

Šefrová, H. & Skuhravý, V., 2000. The larval morphology of *Cameraria ohridella* Deschka & Dimic compared with the genus *Phyllonorycter* Hübner (Lepidoptera, Gracillariidae). *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 48 (4): 23-30.

Skuhravý, V., 1998. Zur Kenntnis der Blattminen-Motte *Cameraria ohridella* Desch. und Dim. (Lep., Lithocolletidae) an *Aesculus hippocastanum* L. in der Tschechischen Republik. *Anzeiger für Schädlingskunde, Pflanzenschutz und Umweltschutz*, 71: 81-84.

Skuhravý, V., 1999. Zusammenfassende Betrachtung der Kenntnisse über die Roßkastanienminiermotte, *Cameraria ohridella* Deschka & Dimic (Lep., Gracillariidae). *Anzeiger für Schädlingskunde, Pflanzenschutz und Umweltschutz*, 72: 95-99.

Stigter, H., van Frankenhuyzen, A. & Moraal, L.G., 2000. De paardekastanjemineermot, *Cameraria ohridella*, een nieuwe bladmineerder voor Nederland (Lepidoptera: Gracillariidae). *Entomologische Berichten, Amsterdam*, 60: 159-163.

SVATOŠ, A., KALINOVÁ, B., HOSKOVEC, M., KINDL, J. & HRDÝ, I., 1999a. Chemical communication in Horse-Chestnut Leafminer *Cameraria ohridella* DESCHKA & DIMIC. *Plant Protection Science*, 35: 10-13.

SVATOŠ, A., KALINOVÁ, B., HOSKOVEC, M., KINDL, J., HOVORKA, O. & HRDÝ, I., 1999b. Identification of a new lepidopteran sex pheromone in picogram quantities using an antennal biodetector: (8*E*,10*Z*)-Tetradeca-8,10-dienal from *Cameraria ohridella*. *Tetrahedron Letters*, 40: 7011-7014.

Jurate DE PRINS
Ugo DALL'ASTA
Royal Museum for Central Africa
Leuvensesteenweg 18
B-3080 Tervuren

Willy DE PRINS
Nieuwe Donk 50
B-2100 Antwerpen