The Horse Chestnut Leafminer *Cameraria ohridella*: Chemical Control and Notes on Parasitisation

Jelena KULDOVÁ[1], *Ivan HRDÝ*[1] *and Petr JANŠTA*[2]

[1]*Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic;* [2]*Faculty of Natural Sciences, Charles University, Prague, Czech Republic*

Abstract

Kuldová J., Hrdý I., Janšta P. (2007): **The horse chestnut leafminer** *Cameraria ohridella***: chemical control and notes on parasitisation.** Plant Protect. Sci., **43**: 47–56.

The expected high efficacy of Dimilin but also good protection by treatment with Confidor and Calypso were demonstrated. Mospilan was less effective. A high mortality of ultimate larval instars of the leafminer and their substantial parasitisation was observed in experiments with potted seedlings on Confidor and Calypso treated leaves of horse chestnut, *Aesculus hippocastanum.* The parasitoids found in mines with larvae and pupae of *C. ohridella* on *A.* × *carnea* and *A. hippocastanum* were recorded and determined. The most abundant species was *Minotetrastichus frontalis* (Chalcidoidea, Eulophidae). The possible effect of insecticide treatments on parasitisation of the horse chestnut leafminer is discussed.

Keywords: *Cameraria ohridella; Aesculus hippocastanum*; *A.* × *carnea*; chemical control; insecticides; parasitisation

At present, the horse chestnut leafminer *Cameraria ohridella* Deschka et Dimić (Lepidoptera, Gracillariidae) has spread over most of Europe (Guichard & Augustin 2002; Kindl *et al.* 2002). Laštůvka *et al.* (1994) reported the first occurrence of *C. ohridella* in the Czech Republic in 1993. This monophagous species almost exclusively develops on white-blooming horse chestnut trees, causing serious damage. As the horse chestnut is one of the favourite ornamental trees in parks and other public areas, the aesthetical impact of damage together with environmental impact of prospective chemical treatment is apparent. Considering the importance of this pest, the European research project CONTROCAM was initiated, in the framework of which several studies have been carried out in the Czech Republic (Svatoš *et al.* 1999a, b; Šefrová 2001; Šefrová & Laštůvka 2001; Kalinová *et al.* 2003). The present work follows our previous one (Najmanová *et al.* 2006) that dealt with residues of diflubenzuron and their efficacy on *C. ohridella*. The effect of diflubenzuron (Dimilin) was reported and confirmed by several authors (e.g. Buchberger 1997). With the persistence of diflubenzuron and possible selection of resistance of the repeatedly treated pest in mind, the possible efficacy of three other insecticides was examined.

MATERIALS AND METHODS

Experiments on horse chestnut seedlings. The samples of Dimilin 48 SC (a.i. diflubenzuron), batch # 41851001, from Crompton Uniroyal Chemical

Partially supported by research project CONTROCAM QLK5-2000-01684 of the 5th Framework Programme of the European Community (the authors' conclusions do not necessarily reflect the Commission's view and in no way anticipate its future policy in this area), IOBC ASCR, Prague, and by the Ministry of Education, Youth and Sports of the Czech Republic (Project No. MSM 0021620828).

Export (Middlebury, CT, USA), Calypso 480 SC (a.i. thiacloprid), batch # PF90017231 and Confidor 70 WG (a.i. imidacloprid), batch # 233014124 from Bayer Crop Science (Bayer Aktiengesellschaft, Leverkusen, BRD) were used in experiments on potted horse chestnut seedlings 80–100 cm high. The upper parts of the plants (approximately half of the whole foliage) were left untreated while the lower parts were sprayed to run-off, using a hand-operated sprayer, with water emulsions of 0.2 mg/ml Dimilin, 0.1 mg/ml Calypso and 0.1 mg/ ml Confidor at three terms: May 29, June 20 and July 10, 2003. The control group was sprayed with water. Three plants were used for each treatment and date. On July 11, i.e. 1 day, 21 days and 43 days after treatment, all experimental seedlings were infested with seven females and three males from field catches; the sex ratio was in accordance with that of the field population at the time of infestation. The plants were covered with sleeves of nylon mesh from early spring on to prevent natural infestation, and they were kept in a garden at Prague-Zbraslav, far from horse chestnut trees. The sleeves were removed on August 5. The effect of all treatments was scored on September 3, i.e. 54 days after infestation. The number and size of mines (each of four categories corresponding to first and second, third and fourth, fifth and lastly to sixth instar), live or dead penultimate and ultimate larvae in large mines, nondiapausing and diapausing pupae and enclosed imagoes were recorded on each particular plant.

Experiments on full-grown trees. The efficacy of routine treatment (by the firm Gartensta) at the New Jewish Cemetery, Prague, was observed in 2003. Mospilan 20 SP (a.i. acetamiprid) at concentration 0.25 mg/ml was applied by backpack sprayer (motor-assisted) on May 12. The scoring of leafminer was done on leaves randomly collected (at least five to six leaves from three different trees per each variant of experiment) from treated and untreated foliage of *A. hippocastanum* at three terms: September 9, September 23 and October 14, 2003. On the same days the infestation of untreated red-blooming horse chestnut *A.* × *carnea* was also determined.

In 2004, groups of *A. hippocastanum* were selected at the same locality for treatment. The first group of trees was sprayed with 0.2 mg/ml Dimilin on May 11; treatment with 0.1 mg/ml Calypso and 0.1 mg/ml Confidor was postponed to June 14 due to unfavourable weather. The same equipment was used as in the previous year. The presence of eggs and consecutive developmental stages of the leafminer was examined at the time of the first treatment (i.e. on May 11 and June 14) and then on three sequential terms (July 24, August 25 and September 22). As in the previous year the infestation of untreated *A.* × *carnea* was also determined.

Statistics. All sets of data were converted to a leaf area of 50 cm^2, and for each set of data and each variable (eggs, mines, pupae, imagoes) the basic statistical characteristics were calculated. Significance of differences between individual treatments was estimated by independent samples *t*-test for separate variances. Transformation $l_n(x + 1)$, where x stands for individual variables, was used when the requirement of normal distribution was not completed. Test of normality was based on skewness and kurtosis of the data treated. The program SYSTAT 5.0 was used for calculations.

***Parasitoids of** Cameraria ohridella*. The species diversity of parasitoids was determined during 2004. Leaves of *A. hippocastanum* and *A.* × *carnea* infested by *C. ohridella* were randomly collected at the New Jewish Cemetery, Prague, from the same branches (15 leaves from each group of treated and untreated trees) and on the same date when the efficacy of chemical treatments was scored. Ten leaves from each group were placed into paper bags attached to the necks of glass bottles and stored in laboratory conditions. Mines from the remaining five leaves from each examined group were dissected, the stages of larvae of *C. ohridella* were determined and larvae and pupae infested by parasitoids were placed with a small piece of leaf and a piece of moist cellulose into perforated cellophane bags and stored under laboratory conditions. After eclosion, parasitoids were determined and counted.

Monitoring the flight of** Cameraria ohridella **by pheromone traps. Two Delta traps BIOLATRAP® delta 155 (ZD Chelčice, Czech Republic) with sticky paper inserts 200 × 80 mm covered with Lonamelt PS 3199/05 glue (Vetox, Praha, Czech Republic) baited with dispensers containing 100 ng of sexual pheromone (8*E*,10*Z*)-tetradeca-8,10-dienal (Svatoš *et al.* 1999a) were used for monitoring the flight of *C. ohridella* during the 2004 season.

RESULTS

Experiments on horse chestnut seedlings

Dimilin was registered for control of *C. ohridella* in the Czech Republic in 2002 (Kupec 2002). Confidor and Calypso were used in our experiments because of their expected mode of action and recommendation for treatment of some fruit and ornamental trees. Results of the final scoring are presented in Table 1.

Two live larvae in large mines, seven mines with diapausing pupae and 53 mines (from which imagoes have eclosed) were found on seedlings sprayed only with water. It indicates that oviposition occurred just after infestation. The foliage was completely destroyed by the larvae of a single generation, some of which were unable to finish their development and there were no mines with younger larvae on untreated plants because of the lack of food.

Table 1. The efficacy of Dimilin, Confidor and Calypso against *Cameraria ohridella*, Prague-Zbraslav, 2003

Infestation in number	Size of mines	Untreated seedlings for control 43 days after spraying with water					
Mines	< 2	0					
	< 4	0					
Mines with	< 9	1					
live/dead + parasitised* larvae	> 9	1					
Nondiapausing pupae		0					
Diapausing pupae		7					
Eclosed imagoes		53					
		43 days		21 days		1 day	
		treated	untreated	treated	untreated	treated	untreated
Dimilin							
Mines	< 2	0	4	0	9	0	13
	< 4	0	2	0	6	0	10
Mines with	< 9	0/1	4	0	1	0	0
live/dead + parasitised* larvae	> 9	0/1	0/3 + 1*	0	1/2 + 5*	0	0/1
Nondiapausing pupae		0	1	0	1	0	0
Diapausing pupae		0	0	0	0	0	0
Eclosed imagoes		0	28	0	1	0	0
Confidor							
Mines	< 2	0	24	1	47	2	37
	< 4	0	1	0	19	0	13
Mines with	< 9	0	0/7	0	0/10	0	2/16
live/dead + parasitised* larvae	> 9	0	2/9 + 16*	0	0/82 + 34*	0	0/8 + 21*
Nondiapausing pupae		0	0	0	0	0	0
Diapausing pupae		0	1	0	17	0	0
Eclosed imagoes		0	8	0	32	0	4
Calypso							
Mines	< 2	17	35	18	64	6	39
	< 4	2	6	2	28	5	30
Mines with	< 9	0/1	8	0	1	0	0
live/dead + parasitised* larvae	> 9	0/1 + 1*	1/16 + 25*	0	2/12 + 39*	0	0/0 + 4*
Nondiapausing pupae		1	9	0	1	0	0
Diapausing pupae		0	19	0	53	0	4
Eclosed imagoes		0	24	5	98	0	17

Horse chestnut seedlings: upper parts of plants untreated, lower parts treated; infested with moths 1, 21 and 43 days after treatment, scored 54 days after infestation; *number of parasitised larvae

The high effectivity of Dimilin against *C. ohridella* was confirmed on treated parts of seedlings, no live larva, pupa or mine after eclosed imago was found. Only two dead larvae were found on treated leaves of seedlings infested 43 days after spraying. A few small mines occurred on untreated leaves of seedlings infested 1 day after spraying and several larger mines and 28 eclosed imagoes on untreated leaves of seedlings infested after 43 days delay.

Only three small mines were found on leaves infested 43 days after treatment with Confidor and not a single imago had developed. The dozens of small mines were present and 4 to 32 moths eclosed on the upper untreated leaves. A high mortality of penultimate and ultimate larval instars and their substantial parasitisation were recorded. With the exception of two, all ultimate larvae were found dead and 71 from 170 (41.8 %) were parasitised. Dozens of young larvae of the subsequent generation were observed on the still remaining green untreated leaves.

The efficacy of Calypso was less convincing as five imagoes eclosed and one pupa developed on the treated part of the plant. There were 139 eclosed imagoes and 87 pupae recorded on untreated upper leaves. Similarly, as in the experiments with Confidor, the majority of last instar larvae were found dead and almost 70% of them were parasitised. There were many small mines of the subsequent generation on untreated leaves, a lower number was found also on the treated area.

Experiments on full-grown trees

The results of Mospilan treatment of *A. hippocastanum* in 2003 are summarised in Table 2. The treatment did not prevent infestation by the pest and resulting damage to foliage, though neither was as high as on untreated trees where large confluent mines made an enumeration at the end of the season impossible (October 14). One diapausing pupa and nine eclosed imagoes of *C. ohridella*, evidential of completed development, were found on leaves of *A.* × *carnea*. Furthermore, high mortality (85.2%) and parasitisation (34.4%) of ultimate larvae was determined.

The infestations by *C. ohridella* on untreated *A.* × *carnea* and on insecticide treated and untreated *A. hippocastanum* were compared in the experiment of the season 2004. Differences in mean values of infestation with different developmental stages on 50 cm^2 of leaf area, referring to a particular treatment and day of examination, are presented in Table 3. A notable effect of chemical treatment of horse chestnut trees is evident from the two last examinations, e.g. on September 22 there were 8.6 imagoes per defined area (133 imagoes in the whole sample) found on untreated leaves, 0.2 (5)

Table 2. The efficacy of Mospilan against *Cameraria ohridella*, Prague, New Jewish Cemetery, 2003

Date of scoring	Infestation by *C. ohridella*						Pupae	Diapausing pupae	Eclosed imagoes
	size of mines				larvae from mines > 9				
	< 2	< 4	< 9	> 9	dead	with parasitoids			
Aesculus × *carnea* – untreated									
9. IX.	201	58	38	29	14	9	0	0	0
23. IX.	430	142	167	128	153	27	0	1	5
14. X.	243	77	107	93	46	50	0	0	4
Aesculus hippocastanum – untreated									
9. IX.	95	28	11	194*	5	3	0	43	120
23. IX.	69	29	13	171*	14	2	0	33	112
14. X.	19	13	6	**	5	5	0	13	71
Aesculus hippocastanum – treated									
9. IX.	37	19	15	83	7	6	2	24	15
23. IX.	13	13	35	112	11	8	2	24	40
14. X.	45	20	20	142	25	20	8	31	22

*approximated estimation of confluent mines; **many confluent mines on leaves treated by Dimilin, 0.5 (7) by Confidor ($P < 0.01$) and 0 treated by Calypso. The efficacy of chemical treatments and of unsuitability of *A.* × *carnea* as host for *C. ohridella* is evident from their number and the highly significant ($P < 0.01$) differences in mean number and size of mines during the season. The data of August 25 and October 22 showed significant (P < small (< 2 mm)) mines on untreated *A.* × *carnea,* and on Dimilin, Confidor and Calypso treated leaves in comparison with untreated leaves of *A. hippocastanum* (Tables 3 and 4). The efficacy of chemical treatments is also confirmed by the generally lower number of large mines (< 9 mm and > 9 mm) in comparison with untreated leaves. However, some data were insufficient (because of the pattern of data) for evaluation and some differences were not statistically significant.

The preference of *C. ohridella* females to lay eggs on leaves of *A. hippocastanum* (8.6 eggs per 50 cm^2 compared to only 0.05 eggs on *A.* × *carnea*) at the beginning of the season (May 11) is shown in Table 3. The tendency of the pest to take advantage of the remaining green leaves of *A.* × *carnea* and of treated *A. hippocastanum* for oviposition at the end of the season when foliage of untreated *A. hippocastanum* is heavily damaged is demonstrated by data of October 22. On untreated leaves of *A. hippocastanum* only 31.5 eggs per 50 cm^2 were found, while there were 175.6 eggs on *A.* × *carnea*, and 142.6 on Calypso and 107.9 eggs on Dimilin treated leaves of *A. hippocastanum.*

Parasitoids

A negligible number of parasitoids was found on *A.* × *carnea* as well as on Dimilin treated *A. hippocastanum* (four and two, respectively) during the 2004 season because of the very few ultimate larvae and pupae of *C. ohridella* present on collected leaves. More parasitoids were observed in ultimate larvae on Confidor and Calypso treated leaves and in diapausing pupae on untreated leaves.

Table 3. Mean values (mean/SD) of infestation by *Cameraria ohridella* on 50 cm^2 of leaf area, Prague, New Jewish Cemetery, 2004

Plant	Treatment	Date	Eggs	Mines				P	DP	EI
				< 2	< 4	< 9	> 9			
A. × *carnea*	untreated	11.V.	0.05/0.02	x	x	x	x	x	x	x
		14.VI.	3.31/0.33	11.81/1.11	0.26/0.10	0	0	0	0	0
		24.VI.	4.81/0.81	13.62/2.03	0.04/0.03	0	0	0	0	0
		25.VIII.	x	10.32/1.19	0.76/0.39	0.53/0.21	0.21/0.15	0	0	0
		22.IX.	175.6/15.5	53.10/5.53	0.49/0.19	0.05/0.04	0.09/0.06	0.22/0.11	0	0
A. hippocastanum	untreated	11.V.	8.62/1.13	x	x	x	x	x	x	x
		14.VI.	1.45/0.23	8.58/1.16	4.34/0.57	2.90/0.45	0.57/0.18	0	0	0
		24.VI.	2.44/0.50	4.40/0.67	2.06/0.38	2.86/0.41	6.37/0.71	0.26/0.09	0	0
		25.VIII.	x	2.27/0.81	0.15/0.15	0	8.85/0.62	1.36/0.31	0	4.11/0.60
		22.IX.	31.48/5.05	10.57/3.05	1.90/0.51	3.71/2.36	18.34/2.63	1.26/1.18	6.79/1.83	8.60/1.31
	Dimilin	24.VI.	14.91/2.17	0.45/0.14	0.29/0.09	0.04/0.02	0.02/0.02	0	0	0
		25.VIII.	110.5/17.6	40.57/15.24	0	1.04/0.43	0.65/041	0.04/0.04	0	0
		22.IX.	107.9/12.5	39.67/3.52	0.41/0.18	0.14/0.08	0.23/0.07	0	0	0.17/07
	Confidor	24.VI.	1.86/0.32	2.76/0.40	1.56/0.26	1.03/0.18	0.76/0.15	0.06/0.03	0	0
		25.VIII.	40.34/14.11	9.56/1.85	6.99/0.84	2.46 /0.44	10.05/1.45	0.16/0.12	0	0.81/0.32
		22.IX.	87.12/8.15	49.65/6.68	4.70/1.10	2.84/0.72	3.49/0.54	0.44/0.19	0.35/0.15	0.46/0.21
	Calypso	24.VI.	2.27/0.44	4.99/0.77	4.07/0.77	3.63/0.43	1.33/0.25	0.02/0.02	0	0
		25.VIII.	24.91/1.74	4.20/0.59	3.82/0.39	1.72/0.24	5.96/0.69	0.25/0.09	0.06/0.04	0.86/0.28
		22.IX.	142.55/22.56	33.78/4.31	6.17/1.25	2.95/0.47	3.72/1.49	0	0.54/0.27	0

SD – standard deviation of mean; P – nondiapausing pupae; DP – diapausing pupae; EI – emerged imagoes; x – not examined

Table 4. Significance of differences between treatments

Eggs

	A. carn.	*A. hippo.*			
	untreat.	untreat.	Dimilin	Confidor	Calypso
A. carn. untreat.		1st line: data of 24.VI. 2nd line: data of 25.VIII. 3rd line: data of 22.IX.			
A. hippo. untreat.	aa x cc				
A. hippo. Dimilin	aa x cc	aa x x			
A. hippo. Confidor	aa x cc	o x cc	aa bb o		
A. hippo. Calypso	aa x o	o x cc	aa bb o	o o o	

Mines < 2

	A. carn.	*A. hippo.*			
	untreat.	untreat.	Dimilin	Confidor	Calypso
A. carn. untreat.		1st line: data of 24.VI. 2nd line: data of 25.VIII. 3rd line: data of 22.IX.			
A. hippo. untreat.	aa bb cc				
A. hippo. Dimilin	x o c	x bb cc			
A. hippo. Confidor	aa o o	a bb cc	x o o		
A. hippo. Calypso	aa bb cc	o b cc	x bb o	a b o	

Mines < 4

	A. carn.	*A. hippo.*			
	untreat.	untreat.	Dimilin	Confidor	Calypso
A. carn. untreat.		1st line: data of 24.VI. 2nd line: data of 25.VIII. 3rd line: data of 22.IX.			
A. hippo. untreat.	x x c				
A. hippo. Dimilin	x x o	x x c			
A. hippo. Confidor	x x cc	o x c	x x cc		
A. hippo. Calypso	x x cc	a x cc	x x cc	a b o	

Mines < 9

	A. carn.	*A. hippo.*			
	untreat.	untreat.	Dimilin	Confidor	Calypso
A. carn. untreat.		1st line: data of 24.VI. 2nd line: data of 25.VIII. 3rd line: data of 22.IX.			
A. hippo. untreat.	x x x				
A. hippo. Dimilin	x o x	x x x			
A. hippo. Confidor	x bb x	aa x x	x b x		
A. hippo. Calypso	x bb x	a x x	x o x	aa o o	

Mines > 9

	A. carn.	*A. hippo.*			
	untreat.	untreat.	Dimilin	Confidor	Calypso
A. carn. untreat.		1st line: data of 24.VI. 2nd line: data of 25.VIII. 3rd line: data of 22.IX			
A. hippo. untreat.	x x x				
A. hippo. Dimilin	x x x	x x cc			
A. hippo. Confidor	x x x	aa o cc	x x cc		
A. hippo. Calypso	x x x	aa bb cc	x x cc	o b o	

Imagoes

	A. carn.	*A. hippo.*			
	untreat.	untreat.	Dimilin	Confidor	Calypso
A. carn. untreat.		2nd line: data of 25.VIII. 3rd line: data of 22.IX.			
A. hippo. untreat.	x x				
A. hippo. Dimilin	x x	x cc			
A. hippo. Confidor	x x	bb cc	x o		
A. hippo. Calypso	x x	bb x	x x	o x	

Parasitoids obtained by both methods from the leaves of *A. hippocastanum* and *A.* × *carnea* were determined (Table 5). Seven species of parasitic Hymenoptera were recorded, all belonged to the superfamily Chalcidoidea, specifically to the families Eulophidae and Mymaridae: *Cirrospilus* sp., *Clostericerus trifasciatus* Westwood, 1833, *Minotetrastichus frontalis* (Nees, 1834), *Pediobius saulius* (Walker, 1839), *Pnigalio pectinicornis* (Linnaeus, 1758), Tetrastichinae gen. sp., Mymaridae gen. sp. The most abundant was *Minotetrastichus frontalis*, representing nearly 50% of all reared parasitoids. The second most numerous was an undetermined species from the subfamily Tetrastichinae (family Eulophidae) which was predominantly reared from infested leaves of *A.* × *carnea*, its occurrence in samples collected on *A. hippocastanum* was low. The third most numerous species, *Pnigalio pectinicornis*, was found only on untreated leaves of *A. hippocastanum.* The remaining species were found in negligible numbers. The highest species diversity was recorded and 34% of all parasitoids were reared from samples of untreated leaves of *A. hippocastanum*. Three females of undetermined mymarids were recorded when reared from infested untreated and Dimilin and Calypso treated leaves of *A. hippocastanum*, infested by *C. ohridella*.

Flight patterns of *Cameraria ohridella* males

The flight of male moths during the 2004 season is illustrated in Figure 1. There were low catches of males during May, June and the first half of July in trap 1 hung on a Dimilin treated tree growing at a cleaned-up site. This contrasts with high catches in trap 2 on a tree at the untreated and poorly cleaned control area where the flight pattern of male moths show three apparent peaks (end of May, end of July and beginning of September); this indicates three generations at the untreated locality, while at the cleaned site and in the Dimilin treated tree the first generation of the pest was very low.

DISCUSSION

The only two dead larvae found on Dimilin treated leaves of seedlings infested 43 days after spraying was evidence for the long-lasting residual effect of diflubenzuron, ascertained also in our previous work (Najmanová *et al*. 2006). A few small mines occurred on untreated leaves of seedlings infested one day after spraying which was possibly caused by the contamination of imagoes with diflubenzuron on the treated part of plant and its consequent effect

Table 5. Parasitoids found on horse chestnut leaves infested by *Cameraria ohridella*, Prague, New Jewish Cemetery, 2004

Parasitoid	*A.* × *carnea* no treatment			*A. hippocastanum* no treatment			Dimilin		Confidor			Calypso		Occurence in	
	♀	♂	SND	♀	♂	SND	♀	♂	♀	♂	SND	♀	SND	N	%
Eulophidae															
Cirrospilus sp.				1		1								2	1.8
Clostericerus trifasciatus	1	1						1				1		4	3.7
Minotetrastichus frontalis	4	6		14	2	2	3	2	16	1	1		1	52	48.1
Pediobius saulius						4		1	1			1	1	8	7.4
Pnigalio pectinicornis				10	1							1		12	11.1
Eulophidae: Tetrastichinae	2	17	2			1			1	2	1		1	27	25
Mymaridae						1	1					1		3	2.8

SND – sex not determined

Explanation for Table 4

a, b, c – statistically significant at $P < 0.05$ level; aa, bb, cc – statistically significant at $P < 0.01$ level

o – difference of means is statistically not significant

x – can not be statistically treated because of insufficient data or not normal distribution

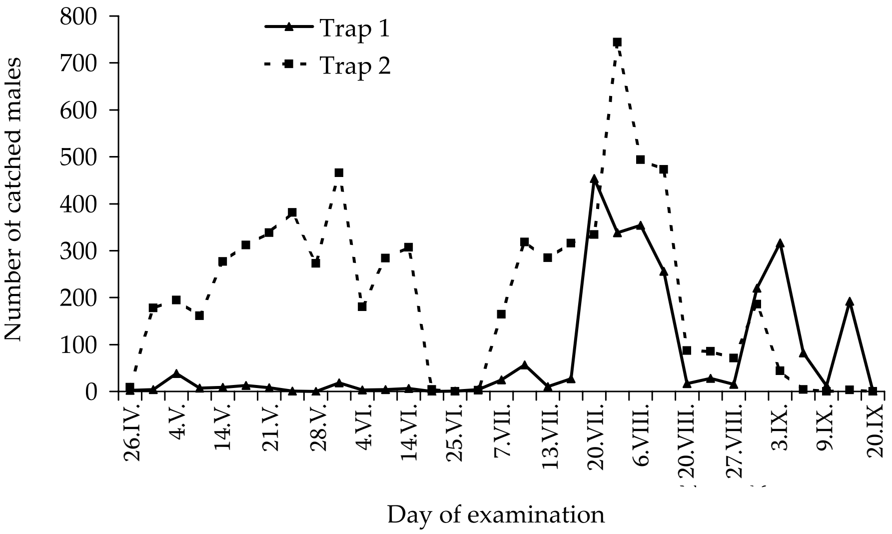

Figure 1. *Cameraria ohridella*, monitoring by pheromone traps, Prague, New Jewish Cemetery, 2004

on development of progeny. However, the finding of several larger mines and 28 eclosed imagoes on untreated leaves of seedlings infested after a 43 days delay showed that residual diflubenzuron on the treated part of plant is no longer sufficient for contamination of adults and a later effect on their progeny. It also showed that diflubenzuron did not translocate from the lower part of the plant to its upper level. Nevertheless, it does not preclude the penetration of the compound from the surface into the leaf tissue after spraying (Šefrová 2001; Najmanová *et al.* 2006).

Confidor was found to be also very effective in the experiment with seedlings, the residua of imidacloprid were still present on treated leaves to a sufficient extent at least 43 days after treatment. The observed small mines and several moths eclosed on the upper untreated leaves were obviously not enough to disapprove a possible translocation of imidacloprid within plant tissues. The tents of young larvae of the subsequent generation were present on upper untreated leaves that, in contrast to the control plants, still had enough green leaf area. It was difficult to determine if the observed high mortality of penultimate and ultimate larval instars was caused by the remaining residua of imidacloprid and/or by parasitisation. However, the recorded number of developmental stages (larvae, pupae) of parasitoids indicated surprisingly high effects of parasitisation on mortality of the pest.

In the experiment with Calypso a remarkable mortality and high percentage (almost 70%) of parasitised last instar larvae were also observed, as well as numerous small mines of the subsequent generation in green areas of both untreated and treated leaves. The recorded number of pupae and eclosed imagoes on untreated upper leaves indicated that thiacloprid probably does not translocate from the lower treated parts of the horse chestnut seedlings.

The Mospilan treatment of full-grown trees in 2003 was not completely effective, as many live pupae and mines with eclosed imagoes were observed on treated leaves. Unaffected leafminers on poorly treated and/or missed tops of trees were a reliable source for rebuilding of the pest population. Better control was achieved in 2004 with Dimilin, Confidor, and Calypso. The chemical treatments were efficient in spite of the fact that they were not perfectly timed due to bad weather (the difference between terms of treatment with Dimilin and with the other two insecticides was approximately one month; the efficacies of Confidor and Calypso are thus not fully comparable with that of Dimilin). Nevertheless, the visual survey at the term of final scoring (the end of September) was positive: the canopies of treated trees were still green in comparison with the brown canopies of untreated trees.

Contrary to the experiments with potted seedlings and the field survey in 2003, a negligible number of parasitoids was found on *A.* × *carnea* as well as on Dimilin treated grown trees of *A. hippocastanum* during the season 2004 because there were very few ultimate larvae and pupae present on the collected leaves. More parasitoids were observed in ultimate larvae on Confidor and Calypso treated leaves, and in diapausing pupae on untreated leaves.

Although the observed diversity of the parasitoid complex was quite low, we consider our results comparable with other studies carried out in Central Europe, where the number of recorded species varied from 3 to 16 (e.g. Moreth *et al.* 2000; Balász *et al.* 2002; Grabenweger 2002; Lethmayer 2002; Grabenweger *et al.* 2005, 2007; Lupi 2005; Volter & Kenis 2006). The spectrum of parasitoid species corresponds with previously published data except for one undetermined species of mymarid, which was recorded for the first time from horse chestnut leaves infested by larvae of *C. ohridella*. Generally, mymarids are egg parasitoids of other insects (Noyes 2005) and owing to the method of rearing it is possible that this species parasitised eggs of some other insect species occurring on horse chestnut leaves or eggs of some parasitoids of *C. ohridella*.

In accordance with the studies above mentioned, the most abundant species found was *Minotetrastichus frontalis,* which is a gregarious parasitoid of *C. ohridella* larvae and occasionally hyperparasitoid of other primary parasitoid chalcids (Grabenweger 2002; Lupi 2005). The remaining species of parasitoids were not so numerous and therefore might be considered as unimportant antagonists of *C. ohridella*.

In experiments with horse chestnut seedlings we recorded a surprisingly high number of parasitoids in mines of leaves in the upper untreated parts of plants whose lower parts had been treated with insecticides. Similarly, in 2003 we observed an apparently higher parasitisation in mines from leaves of *A. hippocastanum* treated by Mospilan as well as in mines on untreated leaves of *A.* × *carnea* in comparison to untreated leaves of *A. hippocastanum*. The shift to higher parasitisation after chemical treatment was also observed, though not so pronounced, in 2004. In our opinion, it could be caused by interference from insecticide residues in plant tissues with the normal developmental cycle of the pest, i.e. mortality and retardation of the leafminer life cycle. The disturbed homogeneity of the pest population may be advantageous for endemic parasitoids whose life cycles are not synchronous with the pest at "normal" conditions (Grabenberger 2004; Grabenberger *et al.* 2007). Chemical treatment can be considered (at those circumstances) as a positive impact on parasitisation of the horse chestnut leafminer. A similar effect could be attributed to unsuitable nutrition by *A.* × *carnea* on which the development of the leafminer is extremely extended and the majority of larvae is not able to pupate. Nevertheless, we recorded a few moths eclosed on *A.* × *carnea* at a locality with many red-blooming horse chestnut trees mixed up in alleys of *A. hippocastanum*.

As demonstrated in Figure 1, the pattern of flight activity of *C. ohridella* males at a site not treated by an insecticide was similar to the situation known from Central Europe. The males of the first generation are usually caught at the end of April, with a peak of flight in May, the second generation appears at the end of June, attaining its maximum at the end of July, and the third generation (if there is one) can be detected from the end of August till the middle of September (Kindl *et al.* 2002; Kuldová, Streinz, Hrdý in prep.). Yet it is also obvious that the horse chestnut leafminer has a high potential to rebuild a quite strong second and relatively populous third generation from a very low overwintering generation on a Dimilin treated tree, augmented by immigration of moths from heavily infested trees in the near vicinity.

Acknowledgements. We thank Ing. E. Dušková for useful help during the field work. We gratefully acknowledge Ing. I. Kopřiva and Ing. L. Ondrušková from Uniroyal Chemical Export, Prague, for supplying the Dimilin sample and Ing. M. Kroulík from Bayer Crop Science, Prague, for supplying samples of Confidor and Calypso. We also wish to thank Ing. K. Vaculová from the Division of City Greenery, Municipality of Prague, and Ing. I. Berkovič from the management of the New Jewish Cemetery, for their kind support. We thank Dr. J. Hrdá for help with the statistical evaluation of data.

References

Balász K., Thuróczy Cs., Ripka G. (2002): Parasitoids of horse chestnut leaf miner *Cameraria ohridella* Deschka et Dimić, 1986. In: Melika G., Thuróczy C. (eds): Parasitic Wasps: Evolution, Systematics, Biodiversity and Biological Control. International Symposium Parasitic Hymenoptera: Taxonomy and Biological Control. 14–17 May 2001, Köszeg, Hungary: 405–412.

Buchberger W. (1997): Wirkungen und Nebenwirkungen von Dimilin. Forstschutz Aktuell, **21**: 19–20.

Freise J., Heitland W., Tosevski I. (2001): Parasitism of the horse-chestnut leaf miner, *Cameraria ohridella* Deschka & Dimić (Lep., Gracillaridae), in Serbia and Macedonia. Journal of Pest Sciences, **75**: 152–157.

Grabenweger G. (2002): Primary and secondary parasitism in the *Cameraria ohridella* complex (Lepidoptera: Gracillariidae). In: Melika G., Thuróczy C. (eds):

Parasitic Wasps: Evolution, Systematics, Biodiversity and Biological Control. International Symposium Parasitic Hymenoptera: Taxonomy and Biological Control. 14–17 May 2001, Köszeg, Hungary: 396–399.

Grabenweger G. (2004): Poor control of the horse chestnut leafminer, *Cameraria ohridella* (Lepidoptera: Gracillariidae), by native European parasitoids: a synchronisation problem. European Journal of Entomology, **101**: 189–192.

Grabenweger G., Avtzis N., Girardoz S., Hrasovec B., Tomov R., Kenis M. (2005): Parasitism of *Cameraria ohridella* (Lepidoptera, Gracillariidae) in natural and artificial horse-chestnut stands in the Balkans. Agricultural and Forest Entomology, **7**: 291–296.

Grabenweger G., Hopp H., Jäckel B., Balder H., Koch T., Dchmolling S. (2007): Impact of poor host-parasitoid synchronization on the parasitism of *Cameraria ohridella* (Lepidoptera: Gracillariidae). European Journal of Entomology, **104**: 153–158.

Guichard S., Augustin S. (2002): Acute spread in France of an invasive pest, the horse chestnut leafminer *Cameraria ohridella* Deschka & Dimić (Lep., Gracillariidae). Journal of Pest Sciences, **75**: 145–149.

Kalinová B., Svatoš A., Kindl J., Hovorka O., Hrdý I., Kuldová J., Hoskovec M. (2003): Sex pheromone of horse-chestnut leafminer *Cameraria ohridella* and its use in pheromone-based monitoring system. Journal of Chemical Ecology, **29**: 384–404.

Kindl J., Kalinová B., Freise J., Heitland W., Augustin S., Guichard S., Avtzis N., Svatoš A. (2002): Monitoring the population dynamics of the horse chestnut leafminer *Cameraria ohridella* with synthetic pheromone in Europe. Plant Protection Science, **38**: 131–138.

Kupec V. (2002): List of the Registered Plant Protection Products. State Phytosanitary Administration, Brno.

Laštůvka Z., Liška J., Vávra J., Elsner V., Laštůvka A., Marek J., Dufek T., Dvořák M., Kopeček F., Petrů M., Skyva J., Vítek P. (1994): Faunistic records from the Czech Republic – 18. Lepidoptera. Klapalekiana, **30**: 197–206.

Lethmayer C. (2002): The parasitism of the horse chestnut leafminer moth (*Cameraria ohridella*) in Austria. In: Melika G., Thuróczy C. (eds): Parasitic Wasps: Evolution, Systematics, Biodiversity and Biological Control. International Symposium Parasitic Hymenoptera: Taxonomy and Biological Control. 14–17 May 2001, Köszeg, Hungary: 400–404.

Lupi D. (2005): A 3 year field survey of the natural enemies of the horse-chestnut leaf miner *Cameraria ohridella* in Lombardy, Italy. BioControl, **50**: 113–126.

Moreth L., Baur H., Schönitzer K., Diller E. (2000): Zum Parasitoid-Komplex der Roßkastanien-Miniermotte in Bayern (*Cameraria ohridella*, Gracillariidae, Lithocolletinae). Mitteilungen der Deutschen Gesellschaft für Allgemeine und Angewandte Entomologie, **12**: 489–492.

Najmanová J., Cvačka J., Hrdý I., Kuldová J., Mertelík J., Muck A. Jr. Nešněrová P., Svatoš A. (2006): Residues of diflubenzuron on horse chestnut (*Aesculus hippocastanum*) leaves and their efficacy against the horse leafminer, *Cameraria ohridella*. Pest Management Science, **62**: 274–278.

Noyes J.S. (2005): Universal Chalcidoidea Database. The Natural History Museum London. Available at http://www.nhm.ac.uk/entomology/chalcidoids, accessed October 6, 2005.

Svatoš A., Kalinová B., Hoskovec M., Hovorka O., Hrdý I. (1999a): 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.

Svatoš A., Kalinová B., Hoskovec M., Kindl J., Hrdý I. (1999b): Chemical communication in horse-chestnut leafminer *Cameraria ohridella* Deschka & Dimić. Plant Protection Science, **35**: 10–13.

Šefrová H. (2001): Control possibility and additional information on the horse-chestnut leafminer *Cameraria ohridella* Deschka & Dimić (Lepidoptera, Gracillariidae). Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, **49**(5): 121–127.

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

Volter L., Kenis M. (2006): Parasitoid complex and parasitism rates of the horse chestnut leafminer, *Cameraria ohridella* (Lepidoptera: Gracillariidae), in the Czech Republic, Slovakia and Slovenia. European Journal of Entomology, **103**: 365–370.

Received for publication March 8, 2007
Accepted after corrections April 12, 2007

Corresponding author:

Doc. RNDr. Ivan Hrdý, DrSc., Ústav organické chemie a biochemie, AV ČR, v.v.i.,
Flemingovo nám. 2, 166 10 Praha 6, Česká republika
tel.: + 420 220 183 295, fax: + 420 220 183 578, e-mail: hrdy@uochb.cas.cz