Monitoring the Population Dynamics of the Horse Chestnut Leafminer *Cameraria ohridella* with a Synthetic Pheromone in Europe

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Abstract

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A monitoring system for *Cameraria ohridella* males based on a synthetic sex pheromone was tested in the Czech Republic, Germany, France and Greece. From the obtained data on the insect phenology we concluded that in Central Europe *C. ohridella* typically has three generations per year. The pheromone monitoring can be used to detect the pest when it appears in uninfested areas and is also suitable to determine population densities.

Keywords: horse chestnut leafminer; *Cameraria ohridella*; *Lepidoptera*; *Gracillariidae*; female sex pheromone traps; monitoring; population density

The horse chestnut leafminer *Cameraria ohridella* Deschka & Dimić (*Lepidoptera*, *Gracillariidae*, *Lithocolletinae*), a species found and described first from Macedonia (SIMOVA-TOŠIĆ & FILEV 1985**;** DESCHKA & DIMIĆ 1986), has spread since over almost all of Central Europe and is currently invading its western parts (PUCHBERGER 1990; HOLSZCHUH & KRBHAN 1992; BUTIN & FÜHRER 1994; HEITLAND *et al.* 1999; SKUHRAVÝ 1999; DE PRINS & PUPLESIENE 2000; AUGUSTIN & REYNAUD 2000; ŠEFROVÁ & LAŠTŮVKA 2001). This tiny leafminer infests almost exclusively horse chestnut trees *Aesculus hippocastanum* L. (*Hippocastanaceae*). Heavily infested leaves abscise prematurely and consequently the trees are defoliated already in the middle of the vegetative season in some areas (HEITLAND *et al.* 1999). Females of *C. ohridella* produce a sex pheromone to attract males for mating (SVATOŠ *et al.* 1999a). The sex pheromone was recently identified as (8*E*,10*Z*)-tetradeca-8,10-dienal (8E10Z-14:Al) (SVATOŠ *et al.* 1999b**).**

Here we describe a pheromone-based monitoring system for this species, and its use to determine the number of generations per year from the catches of males in pheromone traps. We also investigated whether the monitoring system was able to detect the spread of *C. ohridella* in several locations in Europe with different infestation levels.

MATERIALS AND METHODS

Pheromone trapping experiments, using standard methods (CARDÉ & ELKINTON 1984), were performed in the Czech Republic, Germany, France and Greece during the

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Table 1. Test sites, number and age of horse chestnut trees, and elevation above sea level

Country	Location	Number of trees	Age	Elevation
Czech Republic	Čimice (Prague 9)	2	30	280
	Sv. Matěj (Prague 6)	30	40	250
Germany	Freising	3–5	40	420
Greece	Edhessa	15	40	200
	Pillon (Volos)	2	30	570
France	Amiens	20	20	24
	Beaune	20	40	220
	Charleville-Mézière	20	20	153
	Grenoble	30	50	219
	Lille	15	80	24
	Nogent-sur-Vernisson	3	80	122
	Reims	15	80	98
	Annecy	12	80	469
	Besançon	4	80	334
	Colmar	30	40	186
	Metz	9	80	164
	Nancy	20	20	199
	Strasbourg	18	20	139

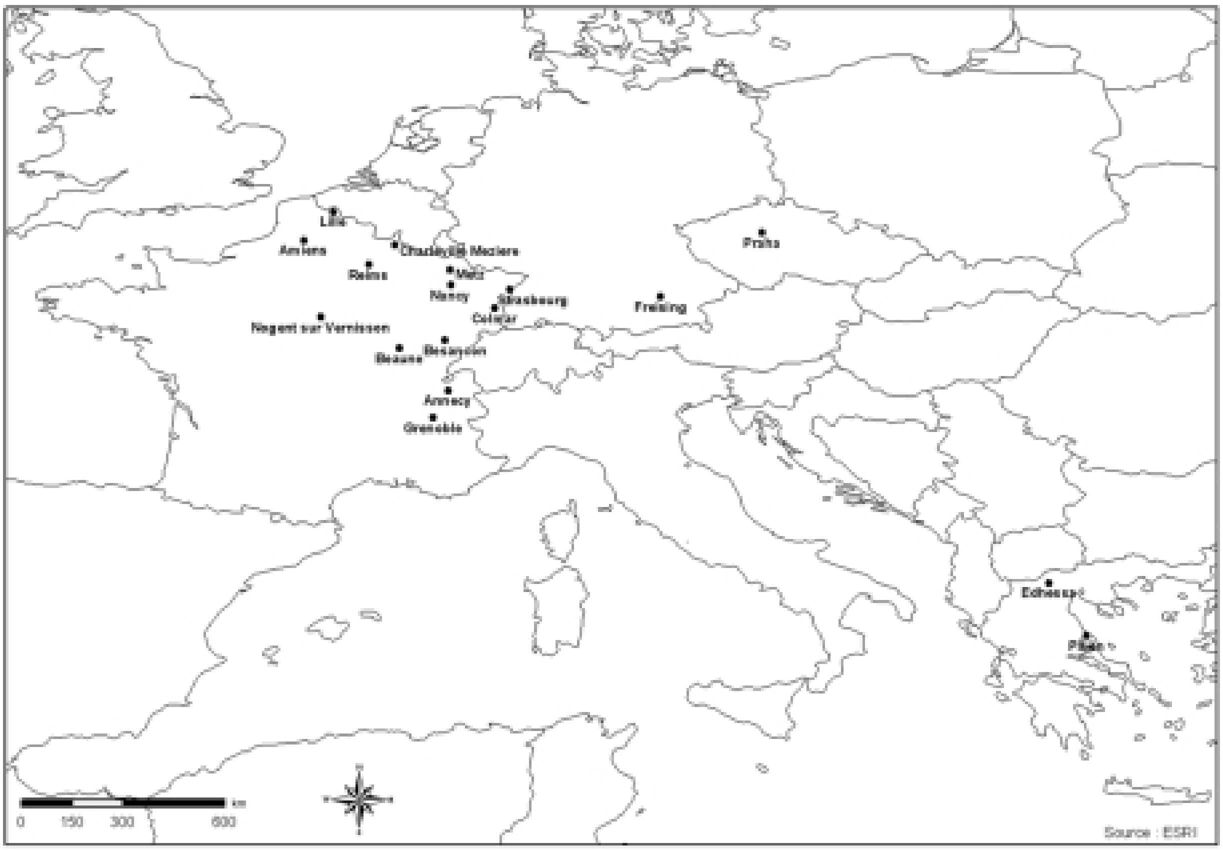

Fig. 1. Monitoring sites in Europe

summer seasons of 2000 and 2001 (Table 1 and Fig. 1). All horse chestnut trees used for the monitoring had been artificially planted as ornamental trees both isolated and in groups or in lines along streets. The fallen leaves had been either partially removed or not removed at all. 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), were used in the experiments. At every testing site one trap was placed in law branches of a horse chestnut tree at a height of 1.5–2 m above the ground. Rubber septa (THOMAS SCIENTIFIC®, Swedesboro, NJ, USA, Cat. No.1780-J07) impregnated with the *C. ohridella* sex pheromone (8E10Z-14:Al, 100 ng) were used as pheromone dispensers; they were replaced every 30 d. The sticky inserts were replaced when the insects were counted, which occurred twice a week at Prague, Freising and Strasbourg. In the western part of France and in Greece the inserts were replaced when they were more or less covered with males or when the glue was dusty and no longer effective, typically after 2–4 weeks. Dates of the beginning and end of the monitoring experiments are evident from the *x*-axis in Figs 2–5. The insects caught on the sticky inserts were identified by the colour patterns on their wings in comparison with known males of the species, and counted under a stereo-microscope.

At Freising (Germany) every week during the season of 2000 five complete leaves were randomly sampled from the lower branches of a tree. The leaves were dissected under a stereo-microscope and the number of living eggs were recorded. Infestation was determined using a method described elsewhere (HEITLAND *et al*. 2000).

RESULTS

Monitoring was performed in areas of Central Europe with a high population density of *C. ohridella* (Czech Republic and Germany) and also in areas with very low density (eastern France and Greece; Fig. 1). In France (northern and western parts), no males were trapped in places with visually non-observable damages. In eastern France, with very low densities, only a few males were trapped (Table 2). In areas of high densities of *C. ohridella,* its males were nearly the only identified species (of about 400 trapped adults that were dissected and sexed only one was female). Other insects, usually spiders, wasps or micro-lepidoptera, were occasionally found in the traps placed in uninfested areas when the sticky inserts were replaced after one month.

The pattern of flight activity of *C. ohridella* based on males trapped in pheromone traps in Central Europe was quite similar and showed several peaks (Figs 2–5). The first males (from overwintering pupae) were usually trapped in April, then their number increased gradually, reached a maximum in May, and decreased to zero at the end of May. The period without any males trapped lasted

Table 2. Detection of *Cameraria ohridella* by pheromone Delta traps and visual observation of mines on trees in Greece and France during 2000

Location	Geographic position		Pheromone traps		Visual observation % infestation
	latDMS	longDMS	date	No. of males	
Greece					
Edhessa	40°48′N	22°03′E	03.8.–23.9.	0	0
Pilion (Volos)	39°24′N	23°02′E	09.8.–18.9.	16	
France					
Amiens	49°54′N	2°18′E	16.6.–13.10.	0	0
Beaune	47°02′N	4°50′E	08.6.–13.10.	0	0
Charleville-Mézière	49°46′N	4°43′E	15.6.–13.10.	0	0
Grenoble	45°11′N	5°43′E	09.6.–13.10.	0	0
Lille	50°39′N	3°05′E	16.6.–13.10.	0	0
Nogent-sur-Vernisson	47°51′N	2°45′E	19.5.–13.10.	0	0
Reims	49°15′N	4°02′E	15.6.–13.10.	0	0
Annecy	45°54′N	6°07′E	09.6.– 13.10.	0	0–5
Besançon	47°14′N	6°02′E	08.6.–13.10.	1	0–5
Colmar	48°05′N	7°21′E	25.5.–13.10.	3	0–5
Metz	49°07′N	6°11′E	01.6.–01.9.	3	0–5
Nancy	48°42′N	6°12′E	01.6.–13.10.	1	0–5
Strasbourg	48°35′N	7°45′E	15.5.–13.10.	4037	75–100

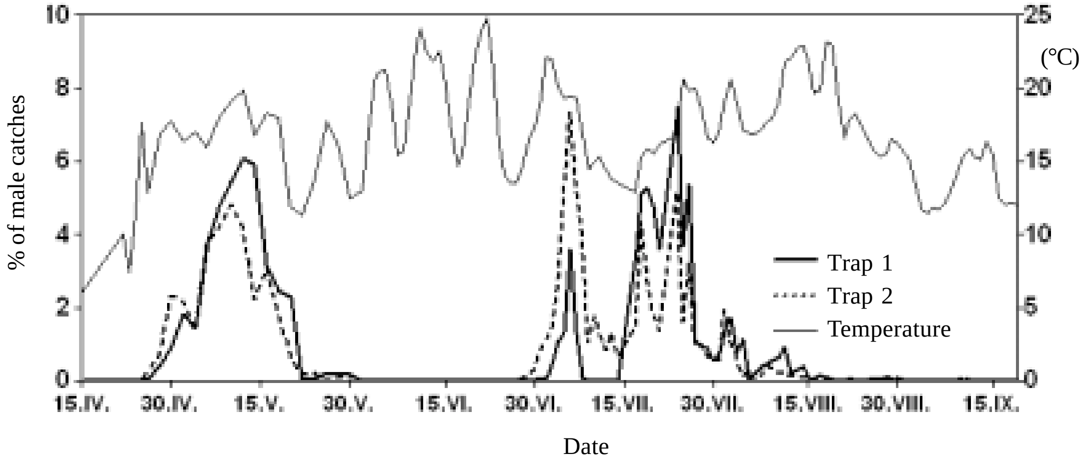

Fig. 2. Results of the monitoring of *Cameraria ohridella* males in pheromone traps at two locations at Freising, Germany, and average day temperatures from this area during the season 2000. Catches in two independent traps are expressed as percentiles of the number of total trapped males ($n1$ = 8570 and $n2$ = 9918)

approximately 4 weeks. The second generation of *C. ohridella* appeared in June, catches again increased gradually, attained one to several maxima during July, and then slowly decreased during August. In some areas and years (Figs 2, 5 and 6), a third generation was detected during late August to early September. In Central Europe most males caught belonged to the second generation, while the numbers of those belonging to the first and third generation was comparably lower (Tables 3 and 4).

The number of males in traps obviously depended on environmental factors, e.g. temperature. The decline in June (Fig. 2) could be attributed to a decrease in the average day temperature below 15°C. When it increased again above 15°C the number trapped rose again (Fig. 2). The catches of males also corresponded with the infestation level (estimated by the number of laid eggs). The maxima of laid eggs was reached about 1 week after the flight maxima of males (Fig. 3). The presence of eggs on horse chestnut leaves confirmed the existence of a third generation (Fig. 3), as also observed in traps (Fig. 2).

Slight differences in the flight patterns of *C. ohridella* males were observed depending on areas and seasons. For instance in 2000 in the Czech Republic (Fig. 4), the first generation started flying on 24th April, peaked around 5th May, stopped after 26th May and then there was no flight for 3 weeks. Males of the second generation appeared on 13th June and had two maxima (24th June and 4th July). After that the number of trapped males gradually reached zero at the beginning of August. A third generation could not be detected, while in Germany the third generation was present in 2000. In contrast, during 2001 the third generation was detected both in the Czech Republic (Fig. 5) and Germany (not shown). In the Czech Republic during 2001 (Fig. 5) the second generation was about 1 month later and the third about 2 weeks later than in the 2000 season at the same locations, presumably due to the colder spring.

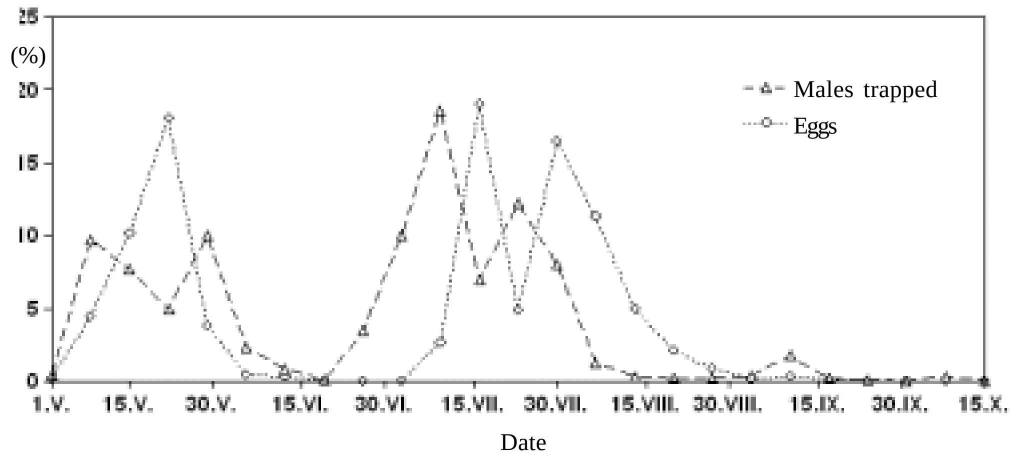

Fig. 3. Comparisons of population dynamics of eggs (open triangles) detected on infested leaves of horse chestnut trees and of *Cameraria ohridella* males (open circles) in pheromone traps at Freising, Germany, during the season 2000. Number of counted living eggs (5905) and trapped males (18 488) are expressed as percentiles on the same scale

Table 3. Population densities and percentile distribution between three generations determined by monitoring. *Cameraria ohridella* males in pheromone traps at several sites during 2000

Generation	Percentile of trapped males			
	Čimice I[a]	Čimice II[b]	Freising I[c]	Freising II[d]
1st	21.5	41.1	39.2	32.2
2nd	77.5	58.8	50.4	60.5
3rd	1.0[e]	0.1[e]	10.4[f]	7.3[f]

Sum of males caught: [a]N = 4648, [b]N = 3462, [c]N = 8570, [d]N = 9918; [e]heavily infested after the second generation, foliage completely brown; [f]moderately infested after the second generation

Table 4. Population densities and percentile distribution between three generations determined by monitoring *Cameraria ohridella* males in pheromone traps at several sites during 2001

Generation	Percentile of trapped males			
	Čimice I[a]	Čimice II[b]	Sv. Matěj I[c]	Sv. Matěj II[d]
1st	18.4	12.6	16.6	12.7
2nd	67.6	68.1	60.4	61.7
3rd	14.0[e]	19.3[e]	23.0[e]	25.6[e]

Sum of males caught: [a]N = 4946, [b]N = 5127, [c]N = 3885, [d]N = 3923; [e]trees moderately infested after the second generation

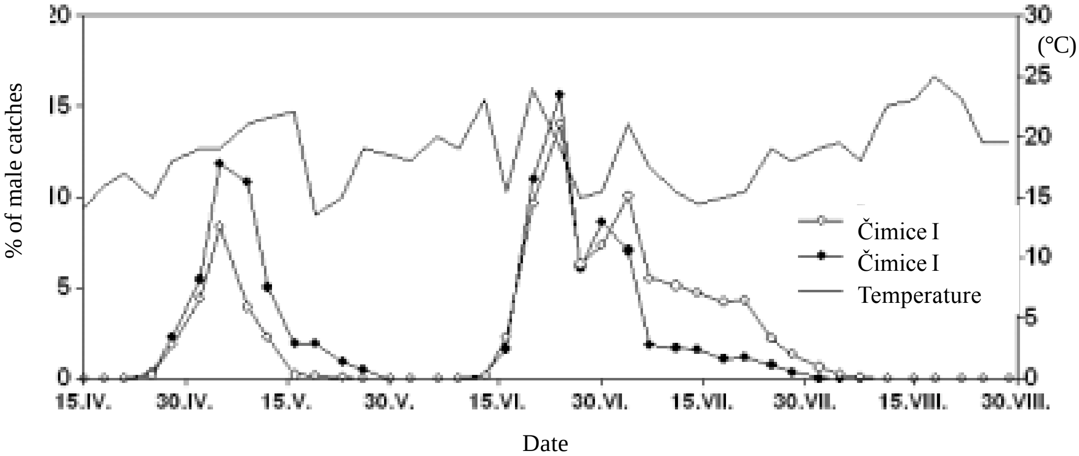

Fig. 4. Results of the monitoring of *Cameraria ohridella* males in pheromone traps in two horse chestnut trees at Čimice, Prague, Czech Republic (open and full circles), and average day temperatures in this area during the season 2000. Catches in two independent traps are expressed as percentiles of the number of total trapped males ($n1$ = 4648 and $n2$ = 3462)

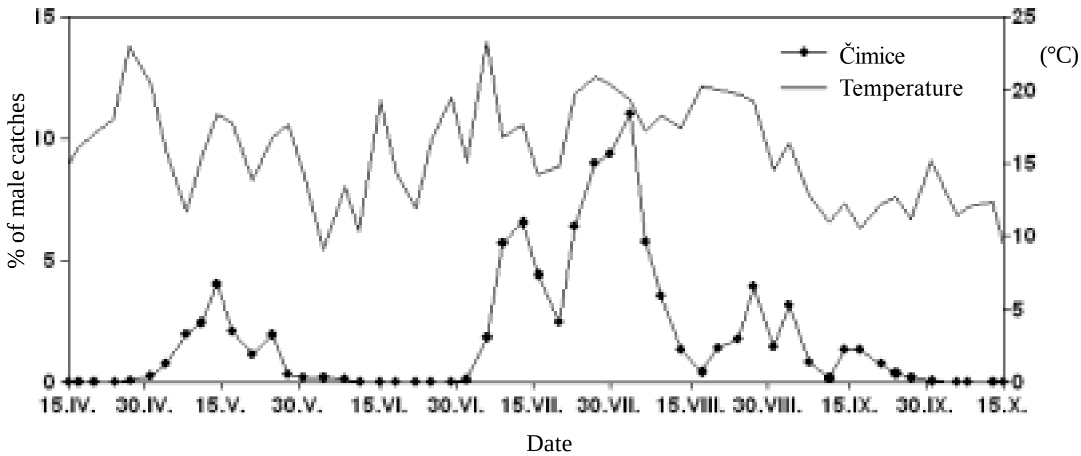

Fig. 5. Results of the monitoring of *Cameraria ohridella* males in pheromone traps; averaged values on two horse chestnut trees at Čimice, Prague, Czech Republic (open circles), and average day temperatures from this area during the season 2001. Catches are expressed as percentiles of the number of total trapped males (n = 4946)

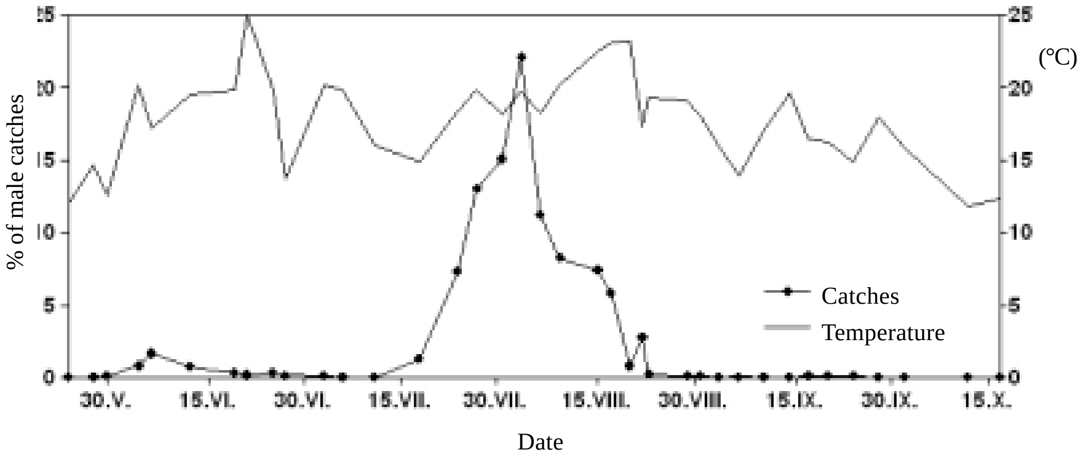

Fig. 6. Results of the monitoring of *Cameraria ohridella* males in three pheromone traps in Strasbourg, France, and average day temperatures from this area during the season 2000. Catches in three traps are expressed as percentiles of the number of total trapped males (n = 4037)

From the data gained in Germany and the Czech Republic in 2000 and 2001 (Figs 2, 4 and 5) it can be concluded that in Central Europe *C. ohridella* usually has three generations per year, with about 1.5–2 months of developing time between generations. The third generation can be virtually absent if the damage to foliage is severe after the second generation (Fig. 4).

At Strasbourg (Fig. 6), where lower population densities prevailed, there was one period of low and one of high flight activity around 19th June and 15th August 2000, respectively. Unfortunately, for technical reasons the monitoring started only at the end of May and we cannot report data for the overwintered first generation. Assuming higher average temperatures at Strasbourg at the beginning of spring we could attribute the observed peaks to the second and the third generations. At this location the abundance of *C. ohridella* increased gradually during the whole season, and in 2000 the third generation was the strongest. It is noteworthy that infestation of horse chestnuts by *C. ohridella* at Strasbourg had first been observed in spring of 2000. Thus, the insect had arrived only recently and consequently the population density of the first and second generation in 2000 was relatively low and had the potential to increase during the third. In 2001 the population densities were lower (data not shown). The first generation started to emerge on 24th April, the second generation came later than in 2000, and the third generation seemed again to be the strongest. An additional flight period was detected in October, but only a few males were caught due to the high ratio of pupae of the previous generation entering diapause.

DISCUSSION

Different patterns of pheromone entrapment observed in Central and Western Europe in the 2000 and 2001 seasons seem to be related to population density (Table 3) and damage ratio. If the first generation was weak, the increase of the second and eventually of the third generation was significant. If on the other hand the first generation was strong and trees were severely damaged already at the beginning of the vegetative season, the increase of *C. ohridella* abundance during the second generation was lower and the third generation was even less pronounced or missing. One factor that could account for this effect is food availability. A significant increase in mortality of *C. ohridella* larvae was reported when mines on a leaf start to coalesce (HEITLAND *et al.* 1999). Also, the portion of pupae entering diapause (to guarantee the species' survival) increases during each generation when the infestation level is high (FREISE & HEITLAND 2001). Next spring a relatively small number of adults will be able to re-infest large areas at a rate of about 2 km/generation in cities or 20 km on a regional scale (GILBERT *et al*. unpublished). The pest can thus utilize the ecological niche efficiently without any risk of being weakened or eliminated due to a food shortage when the last generation develops.

We observed that the phenology of populations of *C. ohridella* within Europe is similar even though local climatic conditions might vary considerably (compare DESCHKA & DIMIĆ 1986; PSCHORN-WALCHER 1994**;** DE PRINS & PUPLESIENE 2000). Only SKUHRAVÝ (1998) reported four to five overlapping generations from the Czech Republic, which is rather unlikely considering the time of development of eggs, larvae and pupae (HEITLAND *et al.* 2000).

The pheromone-based monitoring of population densities of *C. ohridella* described here seems to be robust and effective under different environmental conditions. It can be used to detect this pest at low population densities as well as to observe the variations of individual generations. The used sex pheromone lures are credibly selective for *C. ohridella* males. However, further improvements of the formulation of the pheromone, to dispensers and traps are required and are proceeding at our laboratories.

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Souhrn

KINDL J., KALINOVÁ B., FREISE J., HEITLAND W., AUGUSTIN S., GUICHARD S., AVTZIS N., SVATOŠ A. (2002): **Monitorování populační dynamiky klíněnky jírovcové,** *Cameraria ohridella,* **v Evropě pomocí syntetického feromonu**. Plant Protect. Sci., **38**: 131–138.

Montorovací systém pro samce klíněnky jírovcové založený na syntetickém feromonu byl testován v České republice, Německu, Francii a v Řecku. Ze získaných dat vyplývá, že ve střední Evropě má *C. ohridella* ročně tři generace. Montorovací systém lze použít k detekci škůdce v oblastech, které leží na rozhraní jeho rozšíření a je vhodný i k určení jeho populačních hustot.

Klíčová slova: klíněnka jírovcová; *Cameraria ohridella; Lepidoptera; Gracillariidae;* samičí sexuální feromon; monitorování; populační hustota

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