Monitoring the Regional Spread of the Invasive Leafminer *Cameraria ohridella* (Lepidoptera: Gracillariidae) by Damage Assessment and Pheromone Trapping

S. AUGUSTIN,[1] S. GUICHARD,[1] A. SVATOŠ,[2, 3] AND M. GILBERT[4, 5]

ABSTRACT The leafminer *Cameraria ohridella* Deschka & Dimič is an invasive pest of horse chestnut, *Aesculus hippocastanum* L., trees that has spread rapidly across Europe over the past 19 yr. It was recently reported in the United Kingdom, Denmark, and the Ukraine, and this spread is expected to continue through the Scandinavian countries until the northern limit of the host tree distribution is reached. The presence of the species is generally reported first in the cities, either as consequence of human-related transportation or because of the higher number of host trees in these areas. As a consequence, detailed studies of the spread of this pest through rural areas have not yet been carried out. We have monitored the spread of the moth at the fringe of its known distribution in eastern France during the period 2001–2003. The population was estimated by observing the damage caused by the pest and by establishing a network of pheromone traps. Pheromone traps were set up to measure two generations in 2001 and 2002, whereas the spatial pattern of the spread of the species measured by damage assessment was followed for each generation between 2001 and 2003 (nine generations). Spatial and temporal patterns in the population estimates made using these two methods were compared. We found that estimates made from damage assessment correlated with log-transformed estimates from pheromone trap catches, suggesting that both techniques can be used to monitor the spread of this pest. Over the period 2001–2003, the spread rate ranged from 17.0 to 37.9 km/yr, depending on the population threshold and method used.

KEY WORDS *Aesculus hippocastanum*, *Cameraria ohridella*, biological invasion, spatial dynamics, pheromone trapping

AN INVASIVE LEAFMINER OF unknown origin, *Cameraria ohridella* Deschka and Dimič (Lepidoptera: Gracillariidae) (Grabenweger and Grill 2000), was recorded for the first time near Lake Ohrid in Macedonia in 1985 (Simova-Timosic and Filev 1985). Over the past 19 yr, it has spread across most of central and western Europe (Šefrová and Laštuvka 2001, Guichard and Augustin 2002, Karsholt and Kristensen, 2003). It was recently reported in the United Kingdom (The United Kingdom Parliament 2002), Denmark (Karsholt and Kristensen 2003), and the Ukraine (Akimov et al. 2003). This monophagous species almost exclusively

develops on white-flowered horse chestnut, *Aesculus hippocastanum* L., trees and is the only serious lepidopteran pest that targets this tree species. Because the parasitism rates are very low (usually <10%; Grabenweger and Lethmayer 1999, Freise and Heitland 2001, Freise et al. 2002) but the growth rate of the leafminer very high (mean of 10 offspring per generation with an average of three generations per year; Tomiczek and Krehan 1998), populations are limited by the amount of leaves available for the development of mines. This pest can cause extensive damage, resulting in premature defoliation in the summer and the total loss of foliage of heavily infested trees by midsummer (Pschorn-Walcher 1994). Horse chestnut trees were introduced into central and western Europe from southeastern Europe, where only a few natural stands remain. In central and western Europe, this species has been widely used as an ornamental tree in parks, gardens, and streets in towns and villages. Because this pest has only a minimal effect on host tree vigor (Salleo et al. 2003, Thalmann et al. 2003), the real impact of the leaf damage caused by *C. ohridella* is mostly esthetic. First-stage larvae hatch from 20 to 40

[1] Institut National de la Recherche Agronomique, Unité de Zoologie Forestière, BP 20619 Ardon, 45166 Olivet Cedex, France.
[2] Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, 16610 Praha, Czech Republic.
[3] Max Planck Institute of Chemical Ecology, Hans-Knoll Str. 8, 07745 Jena, Germany.
[4] Biological control and spatial ecology, Université Libre de Bruxelles, CP 160/12, 1050 Brussels, Belgium.
[5] Fonds National de la Recherche Scientifique, 1000 Brussels, Belgium.
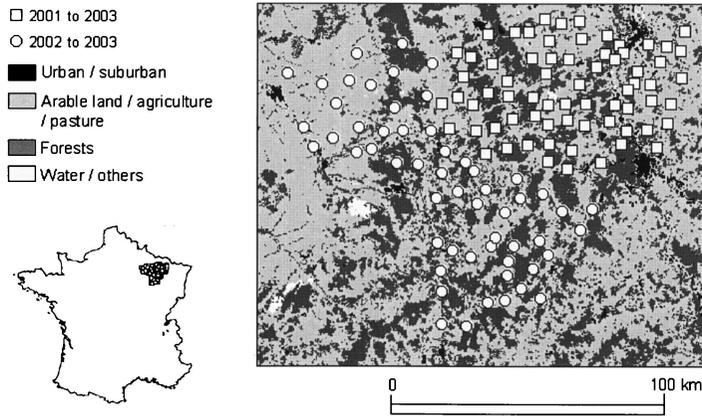
Fig. 1. Distribution of the sampling points. Leaf damage was analyzed at the sites indicated in 2001, 2002, and 2003. Pheromone traps were set up at these sites in 2001 and 2002 only. The land use is indicated, and the study area is situated within France.

eggs laid by females on the upper surface of leaves (Freise and Heitland 2001). These larvae drill into the leaves and pass through five instars, feeding first on sap and then on the palisade parenchyma. Larvae pupate in the mines in a small cocoon and then emerge from the leaves. Males locate females by using a sex pheromone (Svatoš et al. 1999). The number of pupae entering diapause in each generation depends on population density (a higher proportion enter diapause when the number of mines per leaf is high) and photoperiod (Freise 2001).

On the basis of the date of the first-recorded sighting, the large-scale spread rate for *C. ohridella* was estimated at 60 km/yr for European countries (Šefrová and Laštuvka 2001). This value is much higher than those calculated for other invasive Lepidoptera [e.g., *Lymantria dispar* (L.) has a spread rate of 9.45–20.78 km/yr; Liebhold et al. 1992; and invading Lepidoptera in the United Kingdom have a spread rate of 0.06–10.3 km/yr; Agassiz 1996)], but is similar to the spread rates calculated for other invasive leafminers (Agassiz 2002). At the country scale, the spread of *C. ohridella* has a stratified dispersal pattern with a positive association between the establishment of distant foci and human population density (Gilbert et al. 2004). The authors attributed these observations to two nonexclusive mechanisms: long-distance dispersal by wind with a higher probability of establishment in cities because of the higher density of the host plant; and passive long-distance transportation of *C. ohridella* between highly populated areas by vehicles. The short-distance component of the stratified dispersal rate of *C. ohridella* was estimated at ≈10 km/yr, but combined with long-distance jumps, the overall spread rate was estimated at ≈30 km/yr (Gilbert et al. 2004). Within cities, the damage inflicted on trees growing alongside streets is limited, because the leaves are generally removed from the ground surrounding these trees during winter. In contrast, removing leaves from the ground in green areas and private gardens is usually impractical because of

the ground vegetation. As a consequence, a source–sink type of relationship has been established for *C. ohridella* population gradients, with trees located in green areas supplying pests that then migrate toward trees located in the city streets (Gilbert et al. 2003). These gradients suggested that within cities, the rate of spread could be a few hundred meters per generation, a value that seems contradictory to the spread rates measured on the country scale.

The objective of this study was to measure the spread rate of *C. ohridella* in a rural area, on an intermediate spatial scale (100–150 km), and with a higher temporal resolution than that used in previous studies (one population estimate per generation instead of one estimate per year). We also compared the population estimates obtained using two different methods— one based on a measure of visual damage and the other on pheromone trap catches—with the aim of evaluating the suitability of these methods for use in future monitoring programs.

Materials and Methods

Data. A network of observation and pheromone trap sites was established in 2001 in a rural area of eastern France. This area was located at the fringe of the known distribution of *C. ohridella* (Fig. 1). In 2001, this network was made up of 69 sites, most of which had already been invaded by *C. ohridella.* As a result, the network was extended to contain 56 additional locations in 2002 and was therefore composed of 125 sites in total. Each experimental site contained at least one white-flowered horse chestnut tree. The population density of *C. ohridella* was estimated from observations of foliage damage and pheromone trap catch data. For the observational data, a damage score (cf. Gilbert and Grégoire 2003) was derived from visual assessment of the overall proportion of leaves damaged by the moth. Damage (%) was assessed on a scale of 0 to 7 as follows: 0, 0%; 1, 0–2%; 2, 2–5%; 3, 5–10%; 4, 10–25%; 5, 25–50%; 6, 50–75%; and 7, 75–100%).

These estimates (referred to the damage score throughout this article) are linearly related to log-transformed [$\log10(X + 1)$] mine numbers at leaf level for leaf damage caused by the first generation (Gilbert and Grégoire 2003). However, these damage scores should be interpreted with caution. First, the scale used for the damage scores was originally developed to provide a proxy estimate of pest population density at the leaf level. However, because leaf damage is not always homogeneously distributed throughout the tree, the density in the area of the tree where leaves are assessed may bias the general estimation. Leaf damage caused by the first generation of *C. ohridella* is occasionally more concentrated in the lower canopy of the tree; subsequent generations then tend to colonize the rest of the tree. Because the lower branches are the most visible, one can assume that visual damage scores for the first generation are more likely to be overestimated compared with those for the second and the third generations. Second, because leaf damage is cumulative, the relationship between the damage score and the actual population present in the leaf is different for each generation. Despite these two limitations, no other method for measuring the abundance of *C. ohridella* over a wide range of population levels has yet been developed. Population density also can be measured using pheromone traps; however, studies on other Lepidoptera suggest that the data generated using this method are not necessarily always highly correlated with actual damage (Liebhold et al. 1995, Hillier et al. 2004). In this study, three evaluations of damage were made per year in 2001, 2002, and 2003. These evaluations took place at the end of June, August, and September–October, periods corresponding to the stage of highest leaf damage for each generation. The damage observed in June was a consequence of the first generation, that in August was an accumulation of damage by the first and the second generations, and damage in September–October was due to all three generations.

Pheromone traps also were set up at each sampling location at the beginning of the second and third flight in 2001 and 2002. One delta trap baited with a rubber septa (catalog no. 1780-J07, Thomas Scientific, Swedesboro, NJ) treated with 500 ng of (8*E*,10*Z*)-tetradeca-8,10-dienal (Svatoš et al. 1999, Kalinová et al. 2003) was set up in each sampling location. The trap was hung on the lower branches of a horse chestnut tree so that it was positioned 2–4 m above the ground. Sticky inserts were replaced after each flight period, and the pheromone dispenser was changed at the beginning of the third flight. Males were caught on the sticky trap insert, and the number of adults caught was counted at the end of each flight period.

Analysis. Spatial patterns in the damage score and log-transformed pheromone trap catches [$\log10(X + 1)$] were analyzed using estimates of the spatial correlogram function $\rho_{(h)}$ (Rossi et al. 1992), at lag intervals of 5 km. This interval was chosen so that all estimates were based on data obtained from a mini-

mum of 30 pairs of sampling points. Estimates were made using the VARIOWIN software for spatial analysis (Pannatier 1996) and the following equation:

$$\rho_{(h)} = \frac{\frac{1}{N_h} \sum_{(i,j)|h_{i,j}=h} V_i \cdot V_j - M_{ih} \cdot M_{jh}}{S_{ih} \cdot S_{jh}}$$

where v_i and v_j are observation values at the head and tail of a vector with separation distance h, and M and S are the respective mean and standard deviations of the heads and tails of the h separation distance. Lag separation was 5 km with a 2.5-km tolerance limit and the lag vectors were omnidirectional. The inverted correlogram $1 - \rho_{(h)}$ was modeled using a spherical model as follows:

$$1 - \rho_{(h)} = \begin{cases} (C_0 + C[(3h/2A) - (h^3/2A^3)]), & h \leq A \\ C_0 + C, & h > A \end{cases}$$

where Co, C, and A are the inverted correlogram nugget, scale, and range, respectively. Parameters for the model were obtained using nonlinear regression analysis with bootstrapped estimates of the standard errors (SPSS 12.0; SPSS Inc., Chicago, IL). Spatial correlograms for 2001 were calculated using the 69 initial sampling sites, whereas those from 2002 and 2003 were estimated from data obtained from the 125 sampling points (some data points where excluded if the pheromone traps had been damaged). Damage scores were interpolated by ordinary kriging (Isaaks and Srivastava 1989) by using the best-fit spherical model. Ordinary kriging was performed using the software Surfer 8.0 (Golden Software Inc., Golden, CO). Interpolated surfaces of damage scores were transformed into population measures by using the relationship identified by Gilbert and Grégoire (2003): $\text{Log10}(\text{mines per leaf} + 1) = 0.3301$ damage score. This relationship allows the accumulated number of mines per leaf at each generation (for example, Cpop1, Cpop2, and Cpop3 for 2001) to be calculated. These values were then used to determine the absolute population estimate for each generation as follows: Apop1 = Cpop1; Apop2 = (Cpop2 − Cpop1); Apop3 = (Cpop3 − Cpop2). The same method was used to estimate absolute population per leaf from the interpolated damage scores for 2002 and 2003.

Two methods were used to estimate the rate of spread. The first method, termed radial distance versus time, consisted of regrouping the interpolated population measurements into eight classes on the basis of the number of mines per leaf (0, 0–1, 1–2, 2–5, 5–10, 10–15, 15–20, and >20 mines per leaf), measuring the area occupied by each population class within a defined quadrant surrounding the presumed site of the initial focus (Fig. 2), and determining the radial distance of each area by calculating the square root $((\text{area} * 360)/(\pi * 76))$. The increase in radial distance was plotted for each population class as a function of generation, and the spread rate was estimated from the slope of the regression line for this increase.
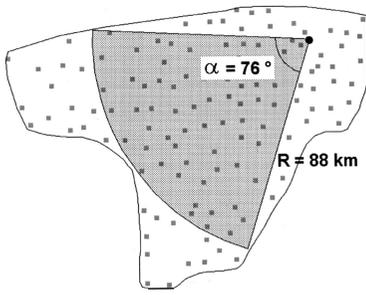
Fig. 2. Subset area used to estimate the radial spread rate (shaded gray area). The assumed initial focus of invasion is indicated by the black dot.

Fig. 3. Change in damage score over time for 2001 (generations 1–3), 2002 (generations 4–6), and 2003 (generations 7–9). Values are expressed as the log-transformation of the number of mines per leaf or as the log-transformation $(\log(Ca + 1))$ of the pheromone trap catch data. Damage is cumulative and the damage score at the end of generation 3, for example, reflects the accumulated damage caused by the populations of the first, second, and third generations. Absolute population densities, expressed as the number of mines per leaf, were thus estimated by converting the damage scores into population data and then subtracting the estimate for the previous generation.

All points from the first nonzero radial distance to the first maximum radial distance (distance where all the potential expansion area is occupied, i.e., a radial distance of 88 km) were included in this calculation. The second method, termed average distance versus time, consisted of converting the raw data from damage score observations into absolute population measures for each generation. These absolute population measurements were then regrouped into the eight classes defined above. The spread rate was estimated for each population class from the slope of the regression line for sampling points at all distances from the presumed site of the initial focus (Fig. 2) as a function of time (Liebhold et al. 1992).

Results

Our analysis of the changes in population estimates as a function of time (Fig. 3) indicated that although the level of damage increased within each year, the estimated absolute population (i.e., noncumulative estimates) did not increase within each year (although the variability was very high). Most of the population increases occurred from year to year. These results are in contrast to those expected on the basis of data recorded in the literature. Population levels estimated from the damage scores were consistent with those estimated from pheromone trap catches. The strength of the correlation between the damage scores for each generation tended to decrease as a function of time (Table 1). However, this effect is weak because damage score observations separated by eight generations were still significantly correlated. The correlations between the damage scores and the log-transformed pheromone trap catch data were significant for all generations. For the pheromone trap data, there was no trend toward a reduction in the strength of the correlation between observations separated by increasing time lags. For all damage score estimates, the correlation between the trap catch data and damage score was weakest for the fifth generation.

No large differences in spatial patterns of the damage scores were found within or between years (Fig. 4), and estimates of the range of spatial dependence and the nugget ranged from 59 to 73 km and from 0.42
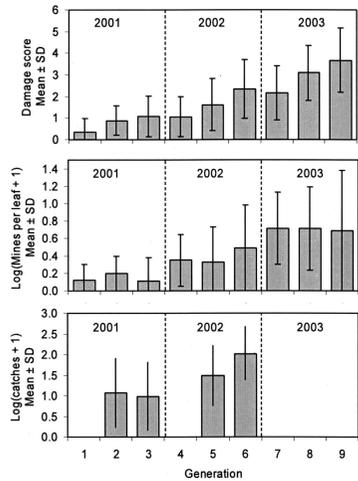
to 0.67, respectively (Table 2). In contrast, the spatial dependence patterns for the log-transformed pheromone trap catches did differ between one generation and the next (Fig. 4b; Table 2). In particular, the range covered by the spatial correlogram of the pheromone trap catch data for generation 5 was much lower than that covered by the spatial correlograms of the other generations.

The spatial distribution of population estimates from the damage score and that from the number of pheromone trap catches (Fig. 5) illustrates that most of the 2001 study area was already infested by *C. ohridella* before the start of our study. The expanded study area set up in 2002 was quickly overtaken by the spread of the leafminer. Most of this spread occurred from one year to the next (e.g., from generations 1–4 and 4–7), with no spread, or even a decrease (particularly in 2001) in the distribution area, taking place within years. In addition to this trend, a population focus occurred in generation 4 (2002) in the southwest of the study area. This population was spatially distinct from the main population front in the northeast of the study area. These two population foci had started to merge by the end of 2003. Despite the significant correlations between the damage scores and the pheromone trap data (Table 1), the extent of the similarity between the interpolated population estimates from damage scores and those from the pheromone trap catch data varied depending on the generation. For generations 3 and 6, and to some extent generation 2,

Table 1. Pearson r correlation between the damage scores of the nine observation periods (DS1–9) and the log-transformed [Log10(X + 1)] data for the pheromone trap catches (LGCA2, LGCA3, LGCA5, and LGCA6)

	DS2	DS3	DS4	DS5	DS6	DS7	DS8	DS9	LGCA2	LGCA3	LGCA5	LGCA6
DS1	0.731***	0.771***	0.782***	0.729***	0.698**	0.684***	0.647**	0.681**	0.716***	0.726**	0.562**	0.594*
DS2		0.854***	0.761***	0.704***	0.723**	0.659**	0.608**	0.635**	0.713***	0.670*	0.555*	0.658**
DS3			0.856***	0.812***	0.771***	0.718***	0.664**	0.714***	0.694***	0.712**	0.626**	0.661**
DS4				0.861***	0.786***	0.764***	0.686***	0.727***	0.711***	0.729**	0.623***	0.651***
DS5					0.886***	0.815***	0.766***	0.802***	0.772***	0.784***	0.572***	0.641***
DS6						0.845***	0.775***	0.817***	0.737***	0.772***	0.550***	0.681***
DS7							0.886***	0.896***	0.761***	0.712**	0.596***	0.662***
DS8								0.939***	0.671***	0.674**	0.551***	0.675***
DS9									0.743***	0.685***	0.549***	0.672***
LGCA2										0.806***	0.587***	0.641**
LGCA3											0.574*	0.696*
LGCA5												0.718***
LGCA6												

Significance levels are measured using the modified *t*-test of Dutilleul (1993) to account for spatial autocorrelation.

there is a reasonable degree of similarity between the two distributions. In contrast, there is very little similarity between the two distributions for generation 5. The short-range spatial autocorrelation model used to interpolate the trap catch data for generation 5 resulted in the occurrence of much smaller population aggregates, a pattern that clearly does not fit with the distribution obtained from the damage scores.

The spread rates calculated from the increases in radial distance over time (Fig. 6a and b) ranged from 7.9 ± 3.3 to 12.6 ± 4.0 km per generation, whereas the spread rates calculated using the average distances from the initial focus ranged from 5.7 ± 2.4 to 9.2 ± 1.7 km per generation (Table 3). We found no statistical differences between the slopes of the regression lines for the two methods (two-tailed *t*-tests). Data collected in 2001 were obtained from a smaller study area than those collected in 2002 and 2003. The distribution of interpolated population estimates (Fig. 5) suggests

that this difference in the size of the study area only affected areas with only 1–2 mines per leaf because areas with higher populations (e.g., 2–5 miners per leaf) were located either within the 2001 study area, or absent from the 2001 interpolated distribution (population classes >5 mines per leaf). Therefore, the spread rates estimated for the population class with 1–2 mines per leaf should be interpreted with caution, regardless of the method used, because the measurements of radial distance and average distance from the initial focus for the year 2001 may be underestimated. For both methods, the population classes with 5–10 and 1–2 mines per leaf had the lowest standard error of the slope estimates, with spread rates estimated at 10.5 ± 1.4 and 8.9 ± 1.1 km per generation, respectively. Because the spread rate estimate for the population class with 1–2 mines may be biased by the smaller study area in 2001, the best spread rate estimated using the second method was that for the 5–10 mines per leaf population class, which had a spread rate of 9.2 ± 1.7 km per generation.
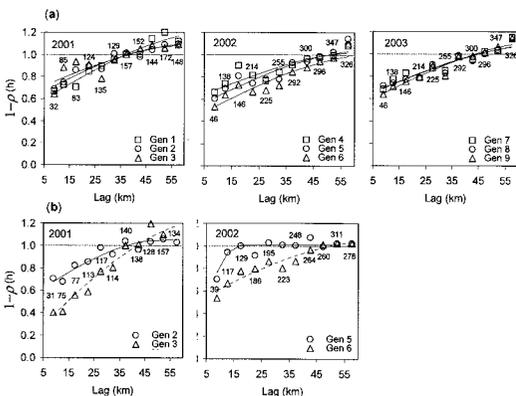

Fig. 4. (a) Spatial correlograms of *C. ohridella* damage scores recorded for each generation from 2001 to 2003. (b) Spatial correlograms of log-transformed pheromone trap catch data for the second and third generations of 2001 and 2002.

Table 2. Estimated values (±SE) for the Nugget, Scale, and Range of the best-fit spherical models of the spatial correlograms for the *C. ohridella* damage scores (DS1–9) and log-transformed [Log10(X + 1)] pheromone trap catches (LGCA2, LGCA3, LGCA5, and LGCA6)

|	Nugget	Scale	Range (km)	r^2
DS1	0.52 ± 0.044	0.69 ± 0.067	73.9 ± 6.2	0.934
DS2	0.61 ± 0.051	0.47 ± 0.044	59.0 ± 10.9	0.695
DS3	0.67 ± 0.105	0.44 ± 0.182	67.2 ± 12.1	0.654
DS4	0.59 ± 0.067	0.43 ± 0.089	64.0 ± 11.1	0.779
DS5	0.51 ± 0.049	0.53 ± 0.077	66.4 ± 13.2	0.864
DS6	0.42 ± 0.050	0.58 ± 0.084	68.6 ± 14.3	0.868
DS7	0.61 ± 0.042	0.47 ± 0.069	70.1 ± 8.6	0.896
DS8	0.60 ± 0.042	0.49 ± 0.065	72.2 ± 8.7	0.886
DS9	0.54 ± 0.047	0.58 ± 0.072	72.9 ± 8.9	0.940
LGCA2	0.55 ± 0.063	0.50 ± 0.061	52.0 ± 7.2	0.658
LGCA3	0.18 ± 0.044	1.05 ± 0.001	70.8 ± 4.5	0.855
LGCA5	0.12 ± 0.059	0.88 ± 0.054	16.6 ± 1.2	0.858
LGCA6	0.49 ± 0.065	0.54 ± 0.058	64.1 ± 9.5	0.922
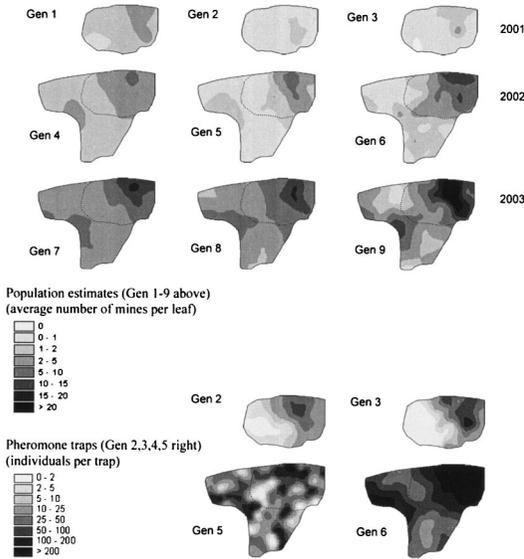
Fig. 5. Distribution of *C. ohridella* population estimates from 2001 to 2003. Estimates were obtained by back-transforming interpolated damage scores into average numbers of mines per leaf (generation 1–9 top) and back-transforming interpolated log-transformed pheromone trap catch data into the number of individuals per trap (generations 2, 3, 5, and 6, bottom). The boundaries of the 2001 study area are indicated on the 2002 and 2003 maps by a dashed line.

Fig. 6. Data used to estimate the rate of spread using the two methods. (a) Radial distance as a function of generation for population classes with different density thresholds (left) and their linear regression line (right). (b) Average distance of sites from the initial focus (black dot in Fig. 2) as a function of generation for population classes with different density thresholds (left) and linear regression lines of the raw distance data (right, for clarity, only average distances are presented in the plot on the left).

Discussion

There were two potential sources of error concerning the estimates of spread rate: measuring the local *C. ohridella* population density and determining the spread rates from the population data. The problem of estimating *C. ohridella* population densities has been addressed previously (Gilbert and Grégoire 2003). In addition, Gilbert et al. 2003 found that the visual damage assessment method provided population density estimates that correlated strongly with those made from local pheromone trap catches (the linear regression value for the correlation between the first generation damage score and the first generation log-transformed pheromone trap catch data had an $R^2 = 0.71$, $n = 46$, $P < 0.001$). However, only the damage score of the first generation was considered in both studies; therefore, we cannot assess how well this method can be applied to studies of the accumulated damage caused by the second and third generations. Also, we are unable to assess whether the method we used to calculate absolute population estimates takes into account insects that may emerge from leaves. It has been reported that the proportion of second and third generation pupae entering diapause is positively correlated with mine density at the leaf level (Freise 2001). The number of adults emerging from a given damage area may therefore be higher in the first generation, when population density is still low and a low number of individuals enter diapause, than in the
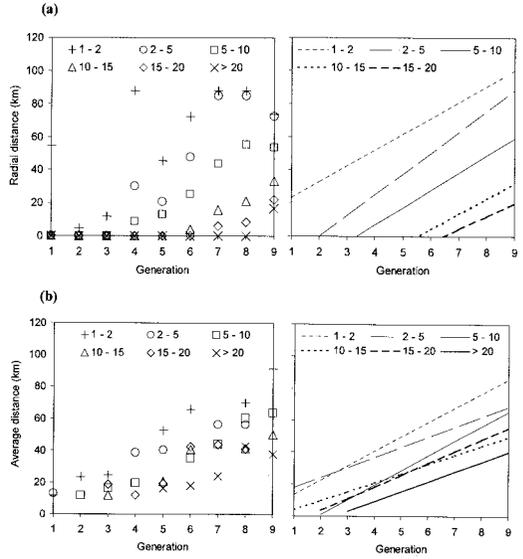
second generation when the density of mines is higher and therefore a higher proportion of individuals enter diapause. As a consequence, the relationship between damage area and the number of emerging pupae may not be consistent between generations and the

Table 3. Spread rate estimates for the different pop classes

	n	r^2	P	Spread rate ($\pm$ SE)
Radial distance vs. time relationship				
1–2 mines/leaf	7	0.371	0.146	9.6 ± 5.6 km/gen.
2–5 mines/leaf	6	0.712	0.035	12.6 ± 4.0 km/gen.
5–10 mines/leaf	6	0.967	0.002	10.5 ± 1.4 km/gen.
10–15 mines/leaf	4	0.966	0.011	9.3 ± 1.0 km/gen.
15–20 mines/leaf	3	0.851	0.251	7.9 ± 3.3 km/gen.
>20 mines/leaf	1		N.S.	
Observation distance vs time relationship				
1–2 mines/leaf	103	0.375	<0.001	8.9 ± 1.1 km/gen.
2–5 mines/leaf	66	0.099	0.010	6.3 ± 2.4 km/gen.
5–10 mines/leaf	102	0.217	<0.001	9.2 ± 1.7 km/gen.
10–15 mines/leaf	46	0.11	0.024	5.7 ± 2.4 km/gen.
15–20 mines/leaf	27	0.12	0.077	7.3 ± 4.0 km/gen.
> 20 mines/leaf	67	0.059	0.047	6.1 ± 3.0 km/gen.

Estimates are calculated from the linear regression plots of distance as a function of generation (gen.), by using the radial distance corresponding to areas of similar pop density (Fig 5b), or by using the actual distances from the initial invasion focus of observation sites with similar pop densities (Fig. 5c).

method used to calculate absolute population numbers may have led to the values for the second and third generations being overestimated. In addition, the use of damage classes may have led to the same damage score being assigned to two consecutive generations even if, within the limits of the class, the leaf damage actually increased. If such an increase occurred between the first and second generation, for example, this would provide a misleading population estimate of zero mines per leaf. If a high number of sampling units were used, this problem would not necessarily lead to errors in the estimates of the overall population level as in such large studies, the number of cases for which the damage estimate leads to a change to a higher class should prevent the relatively small number of cases in which the damage score is unchanged from significantly influencing the overall population estimate. However, in this study, each sampling point provided information about the size of the population in a given location. This information was then used to determine the population class boundaries. Thus, in our study, overestimating the number of sites with populations equal to zero may have affected the population spatial distribution estimates. Finally, as mentioned previously, damage score estimates may overestimate first generation populations because of the spatial heterogeneity of the tree damage, with lower leaves being more frequently infested than upper ones. The above-mentioned limitations, however, only apply to the quantitative comparison of within-year generations and generally do not apply to the estimates of first generation damage spanning years (generation 1, 4, and 7). We assume that these estimates have provided an accurate measure of population abundance, as found in other studies (Gilbert and Grégoire 2003, Gilbert et al. 2003).

Apart from the pheromone trap catches from generation 5, there was a good correlation between log-transformed pheromone trap catches and damage score estimates (Table 1). There was also a good level of similarity between the interpolated distributions generated from the two sets of data (Fig. 5). We have no definitive explanation for the distinct spatial pattern obtained for generation 5, or for the lower correlation of the damage score and trap catch data for this generation. The region experienced heavy rains during this infestation period, which may have affected long-distance dispersal and resulted in a shorter autocorrelation range. However, this explanation is purely speculative, and we have no quantitative data to support it. The correlation between trap catch data and damage score estimates found in our study was weaker than that expected on the basis of results from previous studies. This can be attributed to the use of delta traps and sticky inserts in our study, which tended to become fully covered with adults when the population density was high and thus did not allow new catches unless renewed frequently. Other trap models (for example, the one used by Gilbert et al. 2003) are capable of trapping large numbers of individuals (up to 5,000) and thus would have been more suitable for this type of monitoring. In contrast, the sensitivity of visual estimates at low population levels was higher than estimates from pheromone trap catches data, because we found that the frequency of cases with zero catches and nonzero damage scores was higher than that of cases with zero damage scores and positive catches. These results indicate that fine monitoring of within-year population dynamics would be better evaluated by the use of a network of pheromone traps with trap models that permit a high number of catches per trap. Conversely, it would be more cost efficient to use damage-scale observations for analyses of population dynamics spanning years and large-scale networks, because the only costs associated with this method are those incurred by travel.

There was no significant difference between the spread rates estimated using the two methods for the 5–10 mines per leaf population class (two-tailed t-tests, N.S.). The average spread rate was ≈10 km per generation (Table 3). We found that the average distance versus time method was more suitable for assessing the spread rate: first, because it was easier to implement, and second, because we do not know precisely to what extent spatial interpolation of the damage score influenced the results of the radial distance versus time method. However, the relative merits of these two methods would be better evaluated by comparison to "true" spread rates. A simulation study, comparing the results obtained by several methods with a known simulated spread rate, would probably be the best way of assessing the accuracy of these types of techniques.

Our results are consistent with those obtained from the national-scale study of *C. ohridella* spread in Germany (Gilbert et al. 2004). For example, we found that the range of spatial autocorrelation varied between 59 and 73 km; this range is similar to that presented in the previous report by using presence/absence data (Gilbert et al. 2004 estimated ranges of 104, 68, 75, and 69 km in 1996, 1997, 1998, and 1999, respectively). Although the range estimates for the study in Germany seem slightly higher than those obtained in our study, this minor discrepancy may be due to differences in host abundance and landscape characteristics, or to the smaller study area used in our investigation (the correlograms were generated using distances no >55 km to reduce the edge effects introduced at larger distances). Also, we observed a second infestation in the southeast of the study area in 2002, very close to the city of Saint Dizier (Fig. 1). This pattern of small foci occurring ahead of the main population fringe, particularly near areas of high human population density, was typical of the pattern of *C. ohridella* spread in Germany (Gilbert et al. 2004). Finally, the overall radial increase reported in this study is consistent with that reported by Gilbert et al. (2004), where estimates of 21 km and 36 km/yr, depending on the stage of the invasion, were reported.

Acknowledgments

References Cited

Agassiz, D.J.L. 1996. Invasion of Lepidoptera into the British Isles, pp. 9–36. *In* A. M. Emmet [ed.], The moths and butterflies of Great Britain and Ireland, Harley Books, Colchester, United-Kingdom.

Agassiz, D.J.L. 2002. The invasion and spread of Lepidoptera in Britain, pp. 171–178. *In* M. Peeters and J. L. Van Goethem [eds.], Proceedings, Symposium: Belgian fauna and alien species, 14 December 2001, Bulletin of the Royal Belgian Institute of Natural Sciences, Biology, Suppl. 72: 171–178.

Akimov, I. A., M. D. Zerova, Z. S. Gershenson, N. B. Narolsky, O. M. Kochanez, and S. V. Sviridov. 2003. The first record of horse-chestnut leaf-miner *Cameraria ohridella (Lepidoptera: Gracillariidae) on Aesculus hippocastanum* (Hippocastanaceae) in Ukraine. Vestnik. Zoologii 1: 3–12.

Dutilleul, P. 1993. 1993. Spatial heterogeneity and the design of ecological field experiments. Ecology 74: 1646–1658.

Freise, J. 2001. Untersuchungen zur Biologie und Ökologie der Roßkastanien-Miniermotte (*Cameraria ohridella* Desch. & Dim. (1986) (Lepidoptera: Gracillariidae). Ph.D. dissertation, Technical University of Munich, Munich, Germany.

Freise, J., and W. Heitland. 2001. Aspects of the biology and ecology of the horse-chestnut leaf miner, *Cameraria ohridella* Deschka and Dimic (1986), a new pest on *Aesculus hippocastanum.* Mitt. Dtsch. Ges. Allg. Angew. Entomol. 13: 135–139.

Freise, J., W. Heitland, and I. Tosevski. 2002. Parasitism of the horse-chestnut leaf miner, *Cameraria ohridella* Deschka and Dimic (Lep., Gracillariidae), in Serbia and Macedonia. J. Pest Sci. 75: 152–157.

Gilbert, M., and J.-C. Grégoire. 2003. Visual, semi-quantitative assessments allow accurate estimates of leafminer population densities: an example comparing image processing and visual evaluation of damage by the horse-chestnut leafminer *Cameraria ohridella.* J. Appl. Entomol. 127: 354–359.

Gilbert, M., A. Svatoš, M. Lehmann, and S. Bacher. 2003. Spatial patterns and infestation processes in the horse chestnut leafminer *Cameraria ohridella* Deschka and Dimic (Lepidoptera: Gracillariidae): a tale of two cities. Entomol. Exp. Appl. 107: 25–37.

Gilbert, M., J.-C. Grégoire, J. Freise, and W. Heitland. 2004. Long-distance dispersal and human population density allow the prediction of invasive patterns in the horse-chestnut leafminer *Cameraria ohridella.* J. Anim. Ecol. 73: 459–468.

Grabenweger, G., and C. Lethmayer. 1999. Occurence and phenology of parasitic Chalcidoidea on the horse chestnut leafminer, *Cameraria ohridella* Deschka and Dimic (Lep., Gracillaridae). J. Appl. Entomol. 123: 257–260.

Grabenweger, G., and R. Grill. 2002. On the place of origin of *Cameraria ohridella* DESCHKA and DIMIC (Lepidoptera: Gracillariidae). Beiträge. zur. Entomofaunistik. 1: 9–17.

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

Hillier, N. K., P. L. Dixon, and D. J. Larson. 2004. Trap captures of male *Grapholita libertina* (Lepidoptera: Tortricidae) moths: relationship to larval numbers and damage in wild lingonberry. Environ. Entomol. 33: 405–417.

Isaaks, E. H., and R. M. Srivastava. 1989. An introduction to applied geostatistics. Oxford University Press, Oxford, United Kingdom.

Kalinová, B., A. Svatoš, J. Kindl, O. Hovorka, I. Hrdý, J. Kuldova, and M. Hoskovec. 2003. Sex pheromone of horse-chestnut leafminer *Cameraria ohridella* and its use in a pheromone-based monitoring system. J. Chem. Ecol. 29: 387–404.

Karsholt, O., and N. P. Kristensen. 2003. Kastaniemøllet: et smukt nyt skadedyr i Danmark. Dyr. I. Nat. Mus. 1: 9–11.

Liebhold, A. M., J. A. Halverson, and G. A. Elmes. 1992. Gypsy moth invasion in North America: a quantitative analysis. J. Biogeogr. 19: 513–520.

Liebhold, A. M., J. S. Elkington, G. Zhou, M. E. Hohn, R. E. Rossi, G. H. Boettner, C. Burnham, and M. L. McManus. 1995. Regional correlation of gypsy moth (Lepidoptera: Lymantriidae) defoliation with count of egg masses, pupae and male moths. Environ. Entomol. 24: 193–203.

Pannatier, Y. 1996. VARIOWIN: software for spatial data analysis in 2D. Springer, New York.

Pschorn-Walcher, H. 1994. Freiland-Biologie der eingeschleppten Roßkastanien-Miniermotte *Cameraria ohridella* Deschka et Dimic (Lep., Gracillaridae) im Wienerwald. Linzer. Biol. Beitr. 26: 633–642.

Rossi, R. E., D. J. Mulla, A. G. Journel, and E. H. Franz. 1992. Geostatistical tools for modeling and interpreting ecological spatial dependence. Ecol. Monogr. 62: 277–314.

Salleo, S., A. Nardini, F. Raimondo, M. A. Lo Gullo, F. Pace, and P. Giacomich. 2003. Effects of defoliation caused by the leaf miner *Cameraria ohridella* on wood production and efficiency in *Aesculus hippocastanum* growing in the north-eastern Italy. Trees-Struct. Funct. 17: 367–375.

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

Simova-Timosic, D., and S. Filev. 1985. Contribution to the horse chestnut miner. Zastita Bilja 36: 235–239.

Svatoš, A., B. Kalinová, M. Hoskovec, O. Hovorka, and I. Hrdý. 1999. Identification of a new lepidopteran sex pheromone in picogram quantities using an antennal biodetector: (8E,10Z)-tetradeca-8,10-dienal from *Cameraria ohridella.* Tetrahedron Lett. 40: 7011–7014.

Thalmann, C., J. Freise, W. Heitland, and S. Bacher. 2003. Effects of defoliation by horse chestnut leafminer (*Cameraria ohridella*) on reproduction in *Aesculus hippocastanum.* Trees-Struct. Funct. 17: 383–388.

Tomiczek, C., and H. Krehan. 1998. The horsechestnut leaf-mining moth (*Cameraria ohridella*): a new pest in central Europe. J. Arboric. 24: 144–148.

The United Kingdom Parliament. 2002. Commons Hansard Written Answers text for Tuesday 15 Oct 2002, Vol. No. 390, Part 189, Column 579W, http://www.parliament.the-stationery-office.co.uk/pa/cm200102/cmhansrd/cm021015/text/21015w15.htm.