

Spatial epidemiology of the horse chestnut leafminer *Cameraria ohridella* at the scale of a city

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Abstract

The spatial pattern of the horse chestnut leafminer *Cameraria ohridella* population density was analysed in Brussels in order to explore the population spatial dynamic. 60 pheromone traps were distributed in Brussels and collected two times per population cycle. A survey was carried out to assess foliage infestation level at 262 locations in the city in relation to local characteristics. These two types of population density measures are analysed in relation to the amount of overwintering leaves left on the ground. A direct quantitative relationship is found between local population density (measured by pheromone traps and direct observations) and the amount of overwintering leaves left on the ground. Two other factors are related to observed infestation levels: the proportion of green areas within 100 m and the amount of other chestnut trees within 200 m.

The dynamic of pheromone trap catches in locations where all fallen leaves had been removed suggests that dispersal may be carried out predominantly by females after mating. The pattern of infestation levels along three street transects coming from green areas confirms that *C. ohridella* re-invade areas where the leaves have been properly removed from refuges areas with an estimated spread rate of 2068 m/cycle.

The dispersal behaviour of the species and the applied perspectives of the results are discussed.

Keywords: *Cameraria ohridella*, biological invasion, urban ecology, spatial dynamic, pheromone trapping, dispersal.

Introduction

The horse chestnut leafminer *Cameraria ohridella* is an invasive species, which rapidly spreads through Central and Western Europe over the last 15 years (SEFROVA & LASTUVKA 2001). The damages caused by the mine larvae feeding in the leaves are particularly spectacular in cities where the main host species *Aesculus hippocastanum* is abundant and where the aesthetical impact of trees yellowing and browning in streets, parks and gardens in June raise significant public concern.

The mines overwinter in the dead leaves fallen on the ground and the destruction of these leaves is currently the only advisable control method in an urban context where the environmental impact of the use of chemicals is to be minimised. This method does however not allow controlling of the population at an acceptable level. Firstly because removing all leaves is technically impossible in green areas and secondly because *C. ohridella* typically presents an "r" strategy allowing the population to re-

generate annually from a very low proportion of surviving individuals [3 cycles are usually observed per year (TOMICZEK & KREHAN 1998) and the population increase factor is estimated approximately to 1:10 by cycle (SEFROVA & LASTUVKA 2001)]. No parasite seems to have evolved to feed on *C. ohridella* such as to exert a significant pressure on populations even in areas where the pest has been present for more than 10 years (GRABENWEGER & LETHMAYER 1999) and the perspective of finding more efficient exotic natural enemies is still hypothetical as the true origin of the leafminer is still unknown (HOLZSCHUH 1997).

The dispersal behaviour of the species as well as its population spatial dynamic remains mostly unexplored. At a large scale, interpretation of colonisation patterns throughout Europe suggests that long-distance dispersal is carried out by air current transport (SEFROVA & LASTUVKA 2001) or passively by the mean of people and goods transportation. At the scale of a city, *C. ohridella* colonises most available chestnut trees within 1-2 years following the first observation, which suggests a very high dispersal ability. It is however unclear as to whether this is the effect of active flight or passive transportation by wind or other means. Despite this widespread distribution, population density presents spatial variations and the analysis of these spatial patterns at the scale of the city may provide insight into the underlying ecological processes having produced them. For example, if dispersal is predominantly influenced by wind, population density spatial pattern may present more continuity along main wind directions as it was observed with the horse chestnut scale *Pulvinaria regalis* spatial distribution in Oxford (SPEIGHT *et al.* 1998).

This study analysed the spatial patterns of *C. ohridella* population density at the scale of Brussels (Belgium) in order to explore how much can be inferred about the pest dispersal behaviour and the causality factors producing variations. The population density was evaluated using pheromone trapping and field survey observations. Spatial variations of population density measured by these proxies was then analysed in relation to causality factors such as the amount of overwintering leaves and site characteristics. The hypothesis that green areas constitute

population refuges that allow annual re-colonisation of streets was also tested.

Material and methods

Data

Pheromone traps were distributed in 60 locations throughout Brussels during the two first population cycles (fig. 2). For purely logistical reasons, it was not possible to collect the traps at the frequency required by the Delta traps. Traps allowing insect accumulation and less frequent visits were therefore designed (fig. 1).

A set of 10 Delta traps baited with the same pheromone and observed daily were used to assess the temporal dynamic of males emergences. All traps were hung at bottom branches of white horse chestnut trees (*Aesculus hippocastanum*). The 60 traps were disposed on the day the first catches were observed in the Delta traps and renewed 15 days later for a second trapping period within the first population cycle. The first trapping period was thus from 25.04.2001 to 09.05.2001. The second trapping period took place from 09.05.2001 to 23.05.2001. On 09.05.2001 and 23.05.2001, respectively 36 % and 96 % of the cumulated catches were made in the Delta traps during the first cycle.

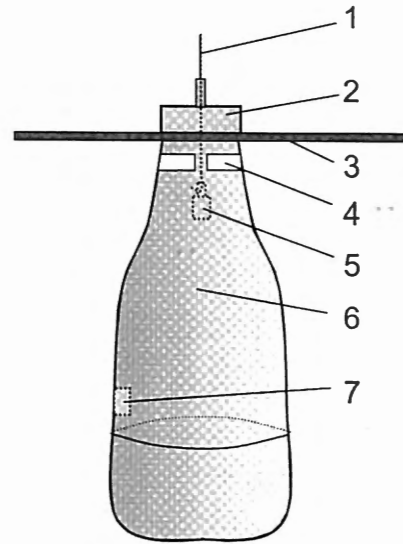


Fig. 1 — The milk-bottle trap is made of recycled PET milk bottle container (6) cut in two parts: the bottom part where dead insects will accumulate and the top part where a Vapona strip (7) is stapled. The milk bottle cap (2) is used to maintain a square hood (3) designed to protect the trap from the rain and to direct the males into the holes (4). The hanging wire (1) is fixed through the cap and holds the pheromone dispenser (5).

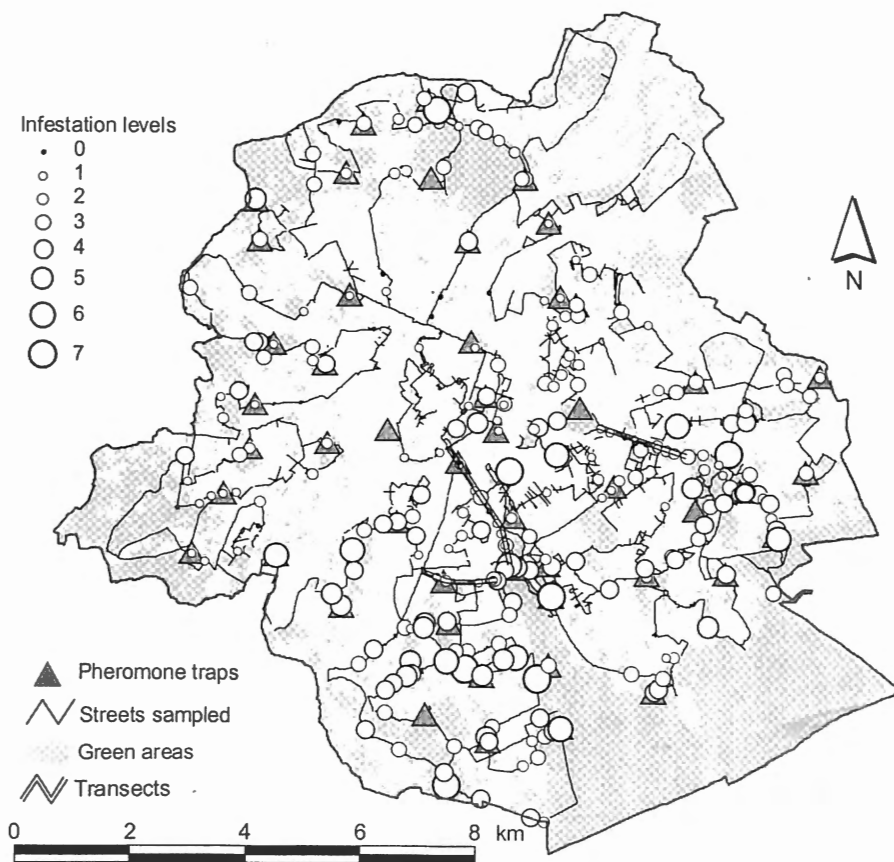


Fig. 2 — Map of the distribution of pheromone traps, survey routes, survey infestation observations, street transects and green areas in the city of Brussels.

Following the first trapping campaign results, it was decided to renew and collect the traps every three weeks during the second cycle to better adjust to the expected median of the temporal dynamic. The first trapping period took place from 29.06.2001 to 20.07.2001 and the second trapping period from 20.07.2001 to 10.08.2001. On 20.07.2001 and 10.08.2001, respectively 52 % and 99 % of the cumulated catches were made in the Delta traps during the second cycle.

Infestations of *C. ohridella* on trees were surveyed during the first two weeks of July 2001 at a period when flights of the second cycle were occurring and when damages were mostly the consequence of the first cycle flights. Infestation levels were estimated visually with the help of pictures of foliage for which percentage of damaged foliage had been estimated using image processing (GILBERT 2001). This technique allowed us to differentiate 8 classes of percentage of damaged foliage: 0 %; 0-2 %; 2-5 %; 5-10 %; 10-25 %; 25-50 %; 50-75 % and 75-100 %.

Several variables were assessed during the survey (table 1) whereas two additional types of variables were extracted using a Geographical Information System. The proportion of green area in a given neighbourhood was estimated at each sample points for 30 different distance radius (from 100 to 3000 m by 100 m step) using a GIS data layer of green areas census provided by the IBGE ("Institut Bruxellois pour la Gestion de l'Environnement") which estimates the proportion of green area in each city block of houses. The number of chestnut trees was estimated for the same distance neighbourhood using a GIS layer of chestnut trees location based on several

sources of information: a database identifying all chestnut trees located in the streets administered by the Brussels Capital Region, a database of all chestnut trees located in the streets administered by the 19 city councils, a database of the number of chestnut trees found within green areas administered by IBGE, and a database of all chestnut trees that we have been observing during our field survey.

The survey covered all 19 Brussels city councils (fig. 2). Only *A. hippocastanum*, known to be the favoured host for, *C. ohridella* was included in the survey. The sampling was carried out by driving randomly along streets in the city (fig. 2) and stopping when a chestnut tree was observed. A sampling point was taken every 200 m in case of chestnut trees alignments. Green areas located along the sampling route were also surveyed by entering them to search for chestnut trees.

Analysis

Insects count data are known to present strong proportionality between local mean and variance, a highly left-skewed frequency distribution, and tend to be far from normal (TAYLOR 1961). This was the case with pheromone trap catches data and they were therefore log-transformed [$\log_{10}(x+1)$] to reduce the proportionality between mean and variance and to normalise the frequency distributions of catches. Log-transformed pheromone catches were analysed in relation to OLI using linear regressions.

Stepwise multiple regression analyses were carried out to analyse the relationship between infestation levels classes (0-7), OLI and other factors described in table 1.

Table 1 — Explanatory variables tested in the linear model. (1) Overwintering Leaves Index (OLI): 0: absent (no leaves); 1: rare refuges (it is possible to find at least one intact leaf after a thorough search); 2: refuges (intact leaves can be seen from under the tree without having to search); 3: abundant (leaves are abundant on the ground but the litter is not intact); 4: intact litter (the litter does not seem to have been modified). (2) Tree height was estimated visually in three classes 1: < 5 m; 2 : 5 to 10 m; 3: above 10 m. (3) Presence/absence of the pathogen *Guignardia aesculi* (PECK) assessed visually. (4) The proportion of green area within a given distance radius was assessed using a Geographical Information System and the geographical database layer of green areas provided by the IBGE. (5) The number of chestnut trees within a given distance was estimated using a GIS and a database combining all sources of information available on the location of chestnut trees in Brussels.

Category	Measurement type	Code
Location	GPS XY coordinatie	XY
Overwintering leaves Index (1)	Score from 0 to 4	OLI
Tree Height (2)	Score from 0 to 3	TH
Presence of <i>Guignardia aesculi</i> (3)	Yes or no	GUI
Is located in a Street	Yes or no	STR
Is located in a private garden	Yes or no	GRD
Is located in a park	Yes or no	PRK
Evidence of ground vegetation	Yes or no	VEG
Proportion of Green Area within X m distance (X ranging from 100 to 3000 m by 100 m steps) (4)	Absolute	AVGAX
Estimated number of chestnut trees within X m (X ranging from 100 to 3000 m by 100 m steps) (5)	Absolute	TNAEX

Spatial distribution of population density was analysed using geostatistical tools allowing quantification of the statistical spatial dependence of sampling points replicated through space (ISAACS & SRIVASTAVA 1989). Statistical spatial dependence is important to analysis of spatial dataset firstly for the insight it provides on the variable under study when subject to interpretation (ROSSI *et al.* 1992) and secondly because it represents a bias to the assumption of independence among samples that needs to be taken into account in parametric statistical analyses such as linear models (LENNON 2000). Spatial dependence in log-transformed [$\log_{10}(x+1)$] results of pheromone catches, in infestation code (0 to 7) and in residuals of infestation code multiple regressions was thus estimated by the standardized semi-variogram:

$$\hat{\gamma}_s(h) = \frac{\sum_{i=1}^{N(h)} (x_i - x_{i+h})^2}{2N(h) \cdot \sigma_{-h} \cdot \sigma_{+h}}$$

where $N(h)$ is the number of data points separated by a distance lag h (h can be a scalar or a vector), x_i is an observation at location i , x_{i+h} an observation at lag h , σ_{-h} the variance of the h vectors tail values and σ_{+h} the variance of the h vectors head values (PANNATIER 1996). This estimator was chosen because it allows comparison of semi-variance among data sets of different overall variance. In the presence of spatial dependence, the semi-variogram is typically small at small lags, and increases when the lag increases. In most cases, there is a lag value beyond which the semi-variogram estimator no longer increases (spatial dependence is no longer perceptible): this lag value is called the range, and the corresponding semi-variogram plateau is known as the sill.

Finally, the decrease in infestation level as a function of distance from green areas was modelled by pooling observation data from three transects avenues (fig. 2) where all leaves had been removed during the winter (OLI=0). The modelling function used in the model is similar to a normal distribution function. This choice stemmed from the assumption that insect movement can be described by random Brownian motion which follows the simple diffusion model:

$$\frac{\partial N}{\partial t} = D \frac{\partial^2 N}{\partial x^2}$$

where N is population density at spatial coordinates x and D is the diffusion coefficient that measures dispersal rate (HOLMES *et al.* 1994). When it is combined with exponential local population growth and when the population is assumed to start from a single point source, this differential equation translates into a normal function (SKELLAM 1951):

$$N(x,t) = \frac{N_{(0,0)}}{4\pi Dt} \exp\left(10\left(rt - \frac{x^2}{4Dt}\right)\right) \text{ and the rate of spread is}$$

measured by $\sqrt{4rD}$

where r is the rate of population increase, x is the distance from the point of origin and t is the time. r was estimated using the ratio of total pheromone traps catches between the first and the second cycles and t was set to 1 (one time unit being equivalent to one population cycle). Using the parameters providing the best fit to the observed distribution, it was possible to estimate $N_{(0,0)}$ (which is of little interest as we measured infestation classes and not real population estimates), D , and the rate of spread in the streets from the green areas.

Results

Pheromone trapping of both population cycles showed a clear positive relationship between catches and the amount of overwintering leaves (fig. 3a, b and c). In the first cycle (fig. 3a), the two trapping periods issued similar results showing a strong relationship between catches and OLI and a very low amount of catches in places where no leaves were present. The second period of the first cycle had however more catches than the first. This increase is simply due to the fact that the first trapping period covered 36 % of the emergence catches made by the Delta traps whereas the second trapping period covered 60 % of the catches (including the peak).

In the second cycle (fig. 3b), both trapping periods were also very close, but the relationship differed markedly from the first cycle. Firstly the slope of the linear model was lower, secondly, the intercept was about 10 times higher and finally the relationship as measured by both R2 was much weaker. In addition, the average number of log-transformed catches per trap increased by a much higher factor (5.17) in locations where no overwintering leaves were observed than in other locations (fig. 3c).

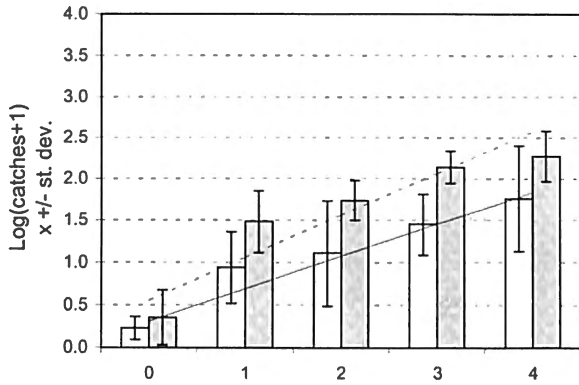
The semi-variograms of all trapping periods were flat showing that catches were spatially independent (fig. 4a). Only values from the first lag differed slightly from this pattern but one cannot conclude to spatial dependence from these points because of the lower number of pairs of points they are based on.

In comparison, infestation levels showed evidence of strong spatial dependence up to a distance of 2000-2500 m. However, the standardized semi-variogram of the multiple regression residuals (see below) showed no evidence of statistical dependence indicating that most of the spatial dependence observed in infestation levels was caused by spatial autocorrelation of explanatory variables [Moran's effect (KOENIG 1999)].

Three parameters were found significantly related to infestation levels in the multiple regression analysis: the OLI, the average proportion of green areas within 100 m, and the total number of chestnut trees found in a 2 km neighbourhood (table 2). No spatial dependence was found in the linear model's residuals and it was thus not necessary to adjust it to take account of spatial autocorrelation.

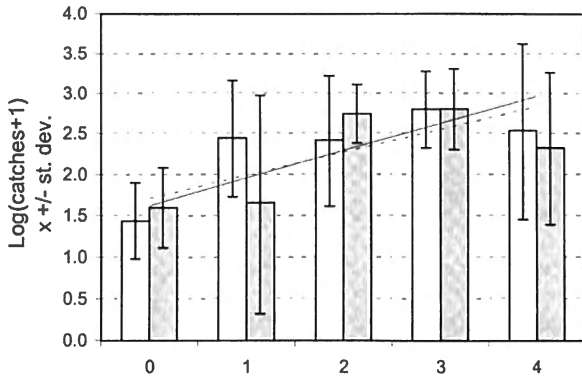
(a) First Cycle

First Period (white / cont. line)
 $y = 0.3811x + 0.3121$
 $R^2 = 0.6278$; $p < 0.001$; $n = 43$
 Second Period (grey / dotted line)
 $y = 0.5034x + 0.5539$
 $R^2 = 0.7105$; $p < 0.001$; $n = 47$



(b) Second Cycle

First Period (white / cont. line)
 $y = 0.336x + 1.6172$
 $R^2 = 0.2569$; $p < 0.001$; $n = 41$
 Second Period (grey / dotted line)
 $y = 0.2821x + 1.7056$
 $R^2 = 0.2165$; $p < 0.003$; $n = 37$



(c) 2nd cycle / 1st cycle

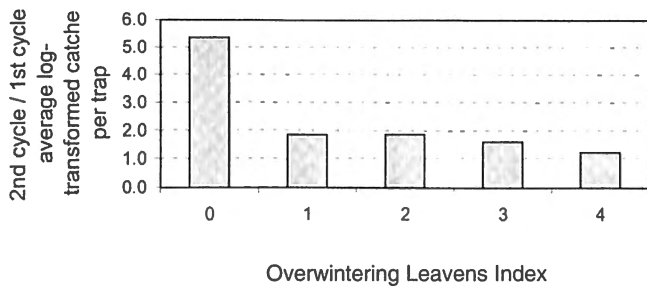


Fig. 3 — Relationship between log-transformed pheromone trap catches and Overwintering Leaves Index (OLI) in Brussels: first population cycle (a), second population cycle (b) and ratio between the two population cycles (c). Results of linear regression of log-transformed catches vs OLI are presented beside each graph.

The relationship between pheromone catches carried out at the first cycle and subsequent infestation observed visually during the survey was found to be fairly strong ($R^2 = 0.71$; $p < 0.001$; $n = 46$).

Finally, using the parameters providing the best fit between Skellam's model and the observed decrease in infestation level (fig. 5) and an estimated rate of increase of 8.557 per cycle (Brussels' average catches per pheromone traps in 2nd population cycle / average catches per trap in 1st cycle), the diffusion coefficient was estimated to 125,000 m²/cycle which converts into a rate of population expansion of 2068 m/cycle.

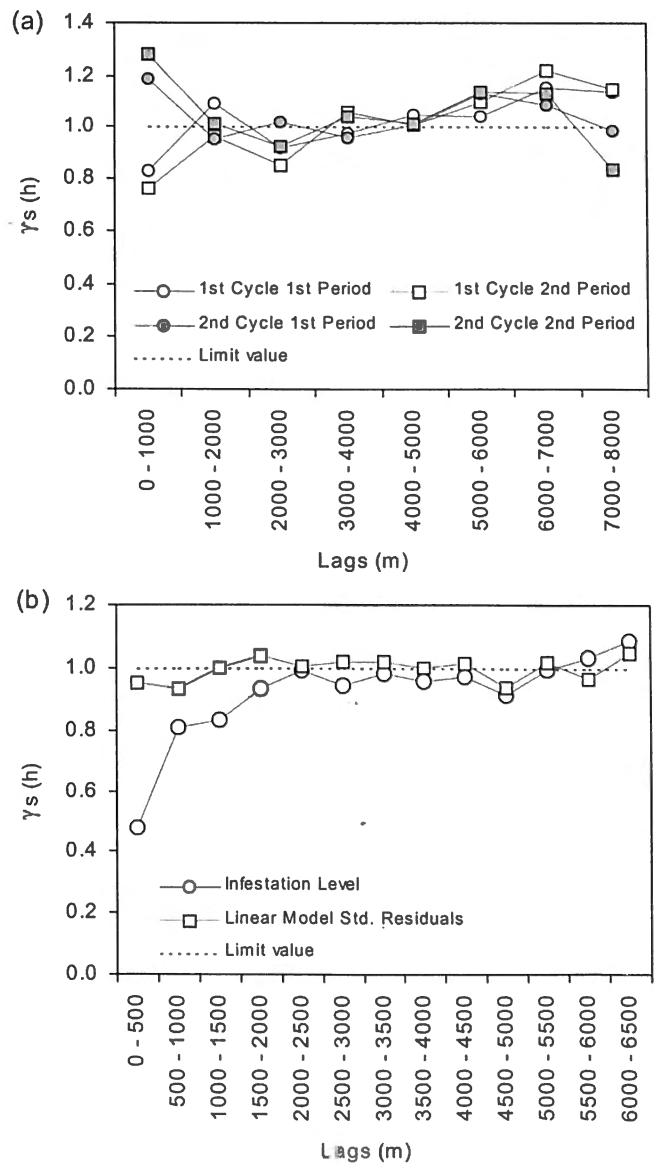


Fig. 4 — Standardized semi-variograms of log-transformed pheromone catches from the 4 trapping periods showing no statistical spatial dependence (a), standardized semi-variogram of infestation levels showing a strong spatial dependence up to a distance of 2500 m (b), and of the multiple regression residuals showing no evidence of spatial dependence (b).

Table 2 — Linear model of observed infestation levels as a function of overwintering leaves index (OLI), the proportion of green areas in a 100 m neighbourhood (AVGA100) and the total number of white horse chestnut trees in a 2 km neighbourhood (TNAE2000).

n	Constant (SE) t; P	OLI (SE) t; P	AVGA100 (SE) t; P	TNAE2000 (SE) t; P	SE of estimate	R ²	F	P
257	0.389 (0.204) 1.883; 0.061	0.975 (0.059) 16.414; <0.001	0.0170 (0.003) 5.586; <0.001	0.267 (0.130) 2.046; 0.042	1.01	0.618	137.32	<0.001

Discussion

Our results clearly establish quantitatively that the amount of overwintering leaves is the major factor explaining population density variations at the scale of a city. The pheromone trap catches are related to OLI at all trapping period (although additional variability is observed at the second cycle), the infestation level is strongly related to OLI, and the relationship between infestation levels and the proportion of green areas within a 100 m neighbourhood may be interpreted by the fact that green areas may potentially include more refuges where leaves are difficult to remove than in the dense

urban habitat (these more distant leaves are not taken into account in the OLI which concentrates on the leaves found under the sampled tree). Looking closer at these different relationships with OLI reveals interesting features on *C. ohridella* dispersal behaviour.

Firstly, it was observed that there was much more population increase between the 1st and 2nd population cycle in locations where the overwintering leaves had been removed during the winter. This is clearly the effect of dispersal of insects coming from areas with numerous refuges toward areas where leaves are absent. This interpretation is confirmed by the results of the observations along street transects showing a clear gradient of decreasing infestation level from the green areas where refuges are abundant toward trees located in the street where the ground was properly cleaned during the winter. This effect of dispersal can also be the interpretation of the relationship found between infestation level and the amount of chestnut trees found in a 2000 m neighbourhood. Indeed, in presence of dispersal, a tree where OLI=0 is more likely to be re-colonised if it has many other chestnut trees in the neighbourhood than if it is isolated from any other tree by dense urban habitat. Furthermore, the neighbourhood radius of 2 km fits well with the result of the diffusion model along street transects (estimated spread rate: 2068 m/cycle). Having the same hypothesis supported by results of two separate experiments (pheromone trapping and survey) is a strong argument to estimate that *C. ohridella* actively disperses in the city and that 2 km/cycle is a realistic figure for the spread rate within Brussels.

Secondly, the linear relationship between catches and OLI has a lower slope at the second population cycle reflecting lower differences in pheromone traps catches between locations having different OLI status. In addition to the dispersal, which increases population growth where OLI was equal to 0, intraspecific competition may have decreased the population growth in locations where these leaves were particularly abundant (OLI=3 or 4) and explains why the relationship tends to flatten. It is thus assumed that results of trapping carried out in the third

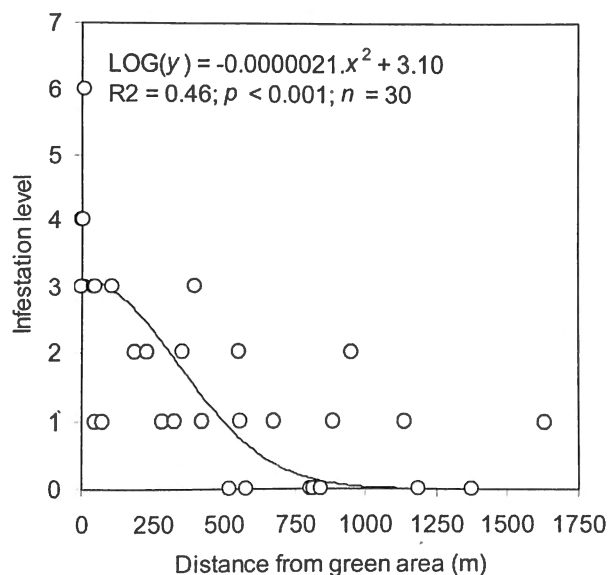


Fig. 5 — Best-fit model of the decrease in infestation density as a function of the distance from green areas along street transects.

cycle would have shown even lower differences in population density in relation to OLI status.

Thirdly, it is interesting to observe that there was no such change in slope between the trapping periods within the cycles indicating that most of the dispersal occurs from cycle to cycle. If both males and females were dispersing into locations where $OLI=0$, the second period relationship would be expected to be flatter than the first and the contrary is observed. Similarly, no change is observed during the second cycle between the first and the second trapping periods. Thus most of the change occurs between the cycles and not between the trapping periods. If only females are dispersing, it is normal to not observe change between the trapping periods because we trap only males, and the effect of females dispersing is observed only through their male progeny caught at the second cycle. Our hypothesis is thus that males and females mate on the site of emergence and that females disperse after mating.

Dispersal by fertilised females would be an interesting strategy for an invasive species such as *C. ohridella* because each single female dispersed with the help of wind or other mean would not require to find a mate to establish a new population. Such strategy, observed in other invasive species such as the great European bark beetle *Dendroctonus micans* (KUG.) (GRÉGOIRE 1988), may explain the speed at which *C. ohridella* colonised Europe over the last 10 years. Furthermore, it presents the advantages of allowing the insect to move more quickly away from natural enemies showing some degrees of specificity (although no specific parasitoid has yet been observed on *C. ohridella* in Europe) that can hardly follow their prey over long distances and prey-free landscape. Such evolutionary advantage may largely compensate for the genetic depletion associated with this strategy. Moreover, this drawback may be eluded by latter genetic exchanges occurring when early established colonies eventually coalesce and form the new population front following a stratified dispersal pattern (HENGEVELD 1989).

If this hypothesis is confirmed by further experimental results, a very low number of early colonisers would be enough to explain the colonisation of a whole city. Helped with a very high spread rate (estimated here approximately to 2 km/cycle) these early colonisers would be able to cover a whole city within 3 cycles only (for example, Brussels has a radius of approximately 8 km, thus spatially covering the city within a year when 3 cycles are present would at the extreme limit require only 5-10 initial individuals). The following year, the population would grow and touches virtually all trees. This scenario fits well with what is observed at the scale in the city: the insect is firstly observed at a low density in scarce locations and is found nearly everywhere the following year.

Although it has not been proved by a temporal analysis of its dynamic, the hypothesis that green areas act as population reservoir from which street trees are gradually re-invaded every year is supported by the results of our

diffusion model along street transects. Such annual dynamic offers an interesting field-scale experimental set-up to study host-parasitoid systems: it provides a location where the host population is kept stable and abundant in connection through dispersal with locations where the host population is set to zero every year. Furthermore, the fact that the spatial dimension from the reservoir toward the streets is limited to 1 dimension would greatly facilitate the analysis of such dynamic and allow confrontation of the observed dynamic with spatio-temporal models developed in theoretical studies (COMINS *et al.* 1992).

Three additional conclusions can be drawn from our results in an applied perspective.

Firstly, pheromone traps respond quantitatively well to local population as shown by the relationship with OLI at the first cycle and allow to predict subsequent infestation levels better than the single measure of OLI. They can therefore be used efficiently in spatial monitoring and the spacing that was used in the present study prevents from spatial autocorrelation of their catches.

Secondly, the quantitative relationship established between infestation levels and OLI allows predicting the reduction in infestation one can expect from foliage removal and could therefore be helpful to managers willing to estimate the cost/benefit of such practices.

Thirdly, if the hypothesis that dispersal is mostly achieved by fertilized females is confirmed, control methods affecting mating (mating disruption by sexual pheromones, males mass trapping, or chemicals applied to trunks where females are thought to mate and mature their eggs) are useless in locations where the insect has to disperse in, i.e. locations where the local population is set to zero by careful leaves removal.

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