

# 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* (Lep., Gracillariidae)

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**Abstract:** Qualitative or semi-quantitative visual assessments are most often used for estimating population size of herbivorous insects. The precision of these estimates, however, is often difficult to establish. A 'simulation game' with the horse chestnut leafminer, *Cameraria ohridella* Deschka & Dimic (Lep., Gracillariidae) shows that visual, semi-quantitative assessments can provide accurate information. Damaged areas of 411 horse chestnut leaves collected in 100 sites were closely related to mine numbers despite some variability in mine and leaf size ( $R^2 = 0.915$ ;  $n = 411$ ;  $P < 0.001$ ). On the basis of this relationship, two methods of population assessment are compared: (i) digital image processing of leaf damage and (ii) visual assessment using a damage key reflecting the relative infested area on each leaf (0, 0%; 1, 0–2%; 2, 2–5%; 3, 5–10%; 4, 10–25%; 5, 25–50%; 6, 50–75%; 7, 75–100%). Both methods used to estimate damage presented a similar, close relationship to the 'real' numbers of mines ( $R^2 = 0.858$ ;  $n = 777$ ;  $P < 0.001$  for image processing and  $R^2 = 0.905$ ;  $n = 777$ ;  $P < 0.001$  for visual assessment). The potential of using visual assessments as an accurate and fast method *in situ* at the tree scale is discussed.

**Key words:** *Aesculus hippocastanum*, *Cameraria ohridella*, damage key, image processing, leafminer, visual assessment

## 1 Introduction

Assessing the damage caused by herbivorous insects is a difficult process, especially when large areas are concerned. Precise estimates require quantitative measurements, which are extremely time-consuming. On the contrary, qualitative or semi-quantitative observations are faster, but their accuracy may be questioned. The trade-off between accuracy and feasibility is obviously difficult to establish, but is of major importance when sampling plans are to be designed. Results from a 'simulation game' involving the horse chestnut leafminer, *Cameraria ohridella* Deschka & Dimic are presented, which suggest that semi-quantitative visual assessments can provide results almost as accurate as the more time-consuming quantitative techniques.

*Cameraria ohridella* is an invasive species which has gradually invaded western and central Europe over the past 15 years (SEFROVA and LASTUVKA, 2001 and references therein). Damage caused by this leafminer to its main host plant *Aesculus hippocastanum* is particularly spectacular in cities where horse chestnut trees are abundant and where the aesthetical impact of trees yellowing and browning in streets, parks and gardens in June raises significant public demand for research on

the pest's ecology (SVATO et al., 1999; FREISE and HEITLAND, 2001), natural enemies (GRABENWEGER and LETHMAYER, 1999), monitoring (CLABASSI, 2000; HEITLAND et al., 2000; M. GILBERT and A. SVATOS, unpublished data) and control methods (FEEMERS, 1997; KREHAN, 1997). In many cases, the effect of new control methods has been tested by comparing population levels in treated vs. untreated sites (KREHAN, 1997; MARX, 1997). Population measurements were also used to carry out surveys to monitor population extension or spatial patterns (HEITLAND et al., 2000), or to target locations most suitable for control. Leaf dissections and individual insect counts cannot be realistically used for assessing population densities; most methods proposed so far are therefore based on visual interpretation of mine damage size on the attacked leaves (HEITLAND et al., 2000). However, no attempt has been made so far, to assess the quality and accuracy of these visual methods. Moreover, the distribution of infestations at the leaf scale was unknown. In this study, the infestation patterns at the leaf scale are established by counting mines and, in parallel, damage estimates are also made using image processing. The results of these two processes are compared to visual assessments made by volunteers with the help of a semi-quantitative damage scale.

## 2 Materials and methods

Leaves were collected at the end of June 2001 at a time when the second cycle flights were about to start and when the damage to the leaves was the consequence of the insect's first flight cycle. This sampling time was chosen because all leaf damage results from a single moth generation (no confusion with older infestations), and also because at this time, damage by the pathogen *Guinardia aesculi* is very low (therefore there is no confusion between very close symptoms). Four to five leaves per site were collected from the bottom branches of trees located in 100 sites in Brussels (M. GILBERT and A. SVATOS, unpublished data). Leaves from each site were disposed on a 700 × 700 mm white panel and photographed using a digital camera (Olympus Camedia C2000 Zoom, Olympus Optical Co., Europa GmbH, Hamburg, Germany) at a resolution of  $2.1 \times 10^6$  pixels. At low density, i.e. when mines were clearly distinct on leaves, the insect population density per leaf was established by counting the number of mines made visible through the leaves by back lighting. At higher densities, population was estimated by counting the number of larvae or pupae visible by back lighting through the leaves. No distinction was made between living and dead developmental stages of *C. ohridella*.

Digital images were cropped to the size of the white panel and resampled to 700 × 700 pixels so that each pixel corresponded to 1 mm<sup>2</sup>. Each leaf was extracted from the resampled image and treated separately (411 leaves in total). Image processing was carried out by classifying pixels respectively as 'background' (white) or 'undamaged' (green) using a tool which allows the selection of a group of pixels on the basis of their colour closeness (Magic Wand, PaintShopPro v. 7.0, Jasc Software Inc., Eden Prairie, MN, USA). The classification was supervised, i.e. subjected to the user's choice to incorporate a given area as undamaged or not. The rule used throughout this process was to incorporate all pixels thought to be unrelated to *C. ohridella* infestation (including browning because of the pathogen *G. aesculi*) in the 'undamaged' category (green). The number of pixels associated with each category was then calculated using a software program developed in VisualBasic, and the damaged areas were estimated as the total numbers of pixels minus those classified as white or green. The relative infested areas were estimated as: damaged area/(damaged area + undamaged area). The surface area of individual mines was estimated per leaf by

dividing the leaf damaged area by the number of individuals counted.

On the basis of the image database, a key of eight standardized levels or classes of leaf damage was designed (fig. 1). The use of a damage key is frequent in the assessment of damage caused by insect pests (ROGERS et al., 1994), or plant pathogens (FORBES and KORVA, 1994). However, these keys are damage-specific and their efficiency relies on their specific design which is fitted to leaf shape and damage patterns (DUVEILLER, 1994). Twenty-five images (4–5 leaves/image) were randomly extracted from the image database and presented sequentially to eight volunteers. With the help of the damage key, they were asked to make a general estimate of the relative damage in each whole image, and to estimate the relative damage on each individual leaf (97 in total). They were asked not to refine their global estimates per image after having assessed individual leaf damage. Among the eight participants, four were researchers working on *C. ohridella* and four were not familiar with the species.

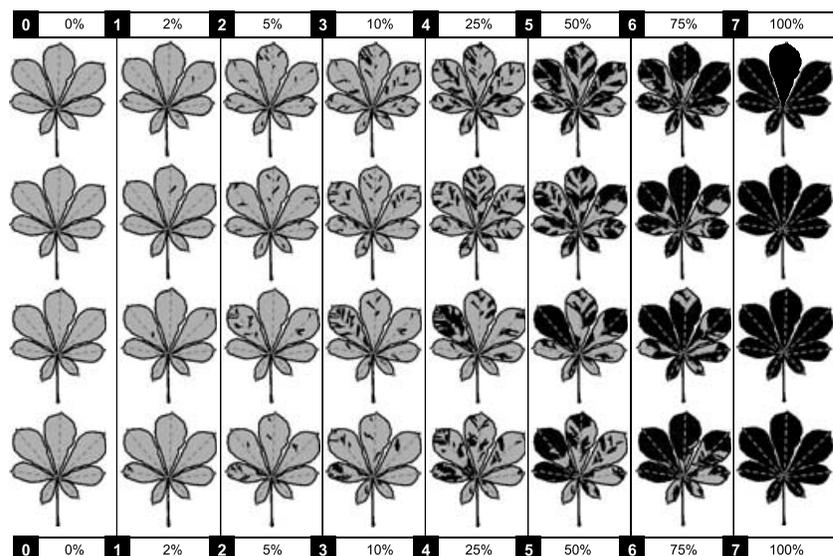
## 3 Results

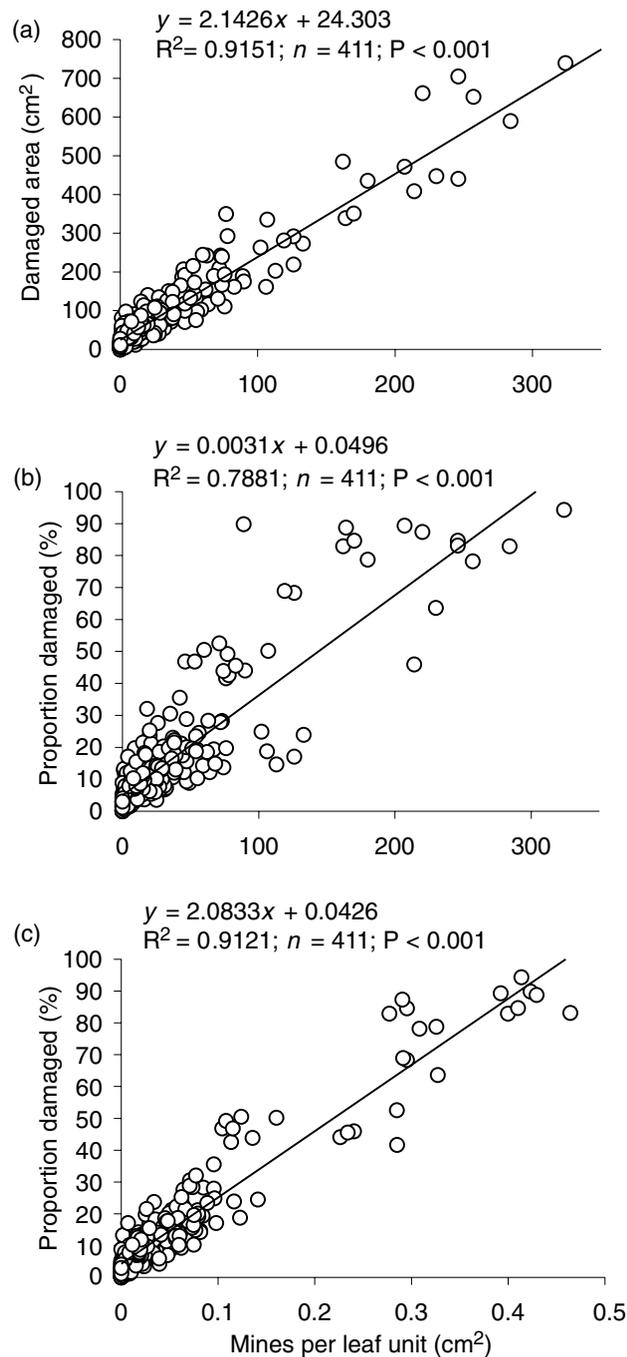
The relationship between the numbers of mines counted and damage surface estimated by image analysis is strong and linear (fig. 2a). However, fig. 2b shows that the link between damage expressed as leaf proportion and mine counts on individual leaves is weaker. This is mainly because of the variability in leaf size as shown in fig. 2c, where the numbers of mines are standardized with respect to leaf size.

Similar relationships are observed at the site level (which gives a closer reflection of *in situ* estimates on whole trees), [damaged area vs. number of mines ( $R^2 = 0.936$ ;  $n = 100$ ;  $P < 0.001$ ), proportion damaged vs. number of mines ( $R^2 = 0.856$ ;  $n = 100$ ;  $P < 0.001$ ) and proportion damaged vs. mines per leaf area unit ( $R^2 = 0.939$ ;  $n = 100$ ;  $P < 0.001$ )].

Mines sizes also present a very high variability, especially at low population density as shown in fig. 3 in which the size of the mines is plotted against the proportion of leaf damaged. The mine size tends to a minimum of 2.33 cm<sup>2</sup> when damage intensity is at its highest.

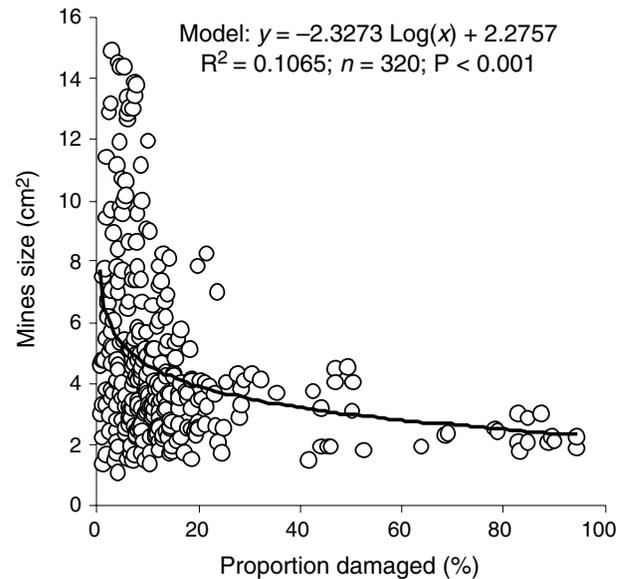
**Fig. 1.** Score sheet used to estimate infestation density by *Cameraria ohridella*, either as damage code (in the black squares: 0, 0%; 1, 0–2%; 2, 2–5%; 3, 5–10%; 4, 10–25%; 5, 25–50%; 6, 50–75%; 7, 75–100%), or as a help to estimate proportion of damage





**Fig. 2.** Relationships between mine numbers counted and damaged areas estimated by image analysis: (a) number of mines per leaf vs. the damaged area showing a strong linear relationship, (b) number of mines vs. proportion of the leaves being damaged showing that the relationship is less predictable and (c) same relationship as (b) but the number of mines per leaf has been standardized by leaf area showing that the relationship is then closer to (a)

Estimates made by visual interpretation of pictures with the help of damage key were strongly correlated to those made by image processing (fig. 4) either measured as relative leaf damage proportion or as damage code. Furthermore, a similar relationship was observed at the 'site' level (per image, i.e. measured over the 4–5 leaves present in each image) [proportion



**Fig. 3.** Relationship between *Cameraria ohridella* mine size (estimated by dividing damaged area by the number of mines per leaf) and relative size of damaged leaf area showing that mine size, and size variability decrease as a function of leaf damage

of damage (visual estimate) vs. proportion of damage (image processing):  $R^2 = 0.915$ ;  $n = 200$ ;  $P < 0.001$  and damage code (visual estimate) vs. damage code (image processing):  $R^2 = 0.828$ ;  $n = 200$ ;  $P < 0.001$ ].

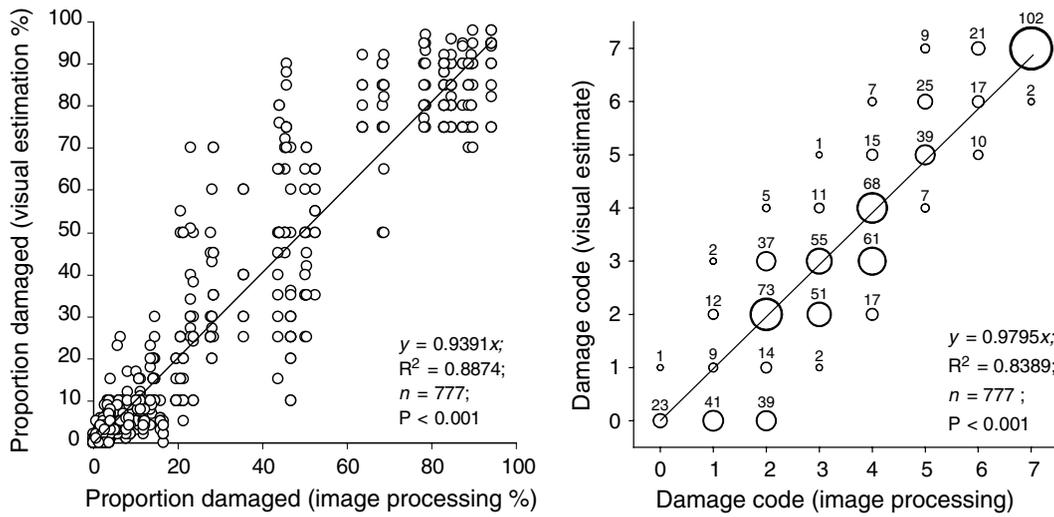
Damage code estimated by both methods were shown to be strongly related to the log-transformed number of mines (fig. 5). Interestingly, measures made visually provided a better  $R^2$  value than those measured by image processing. Again, similar relationships were observed at the site level [damage code (visual estimate) vs.  $\log(\text{number of mines} + 1)$ :  $R^2 = 0.855$ ;  $n = 200$ ;  $P < 0.001$  and damage code (image processing) vs.  $\log(\text{number of mines} + 1)$ :  $R^2 = 0.900$ ;  $n = 200$ ;  $P < 0.001$ ] although here, image processing provided a better  $R^2$  value.

There was no significant difference between the results [as measured by the slope of damage code vs.  $\log(\text{number of mines} + 1)$  relationship shown in fig. 5] provided by users familiar with *C. ohridella*'s ecology and the others.

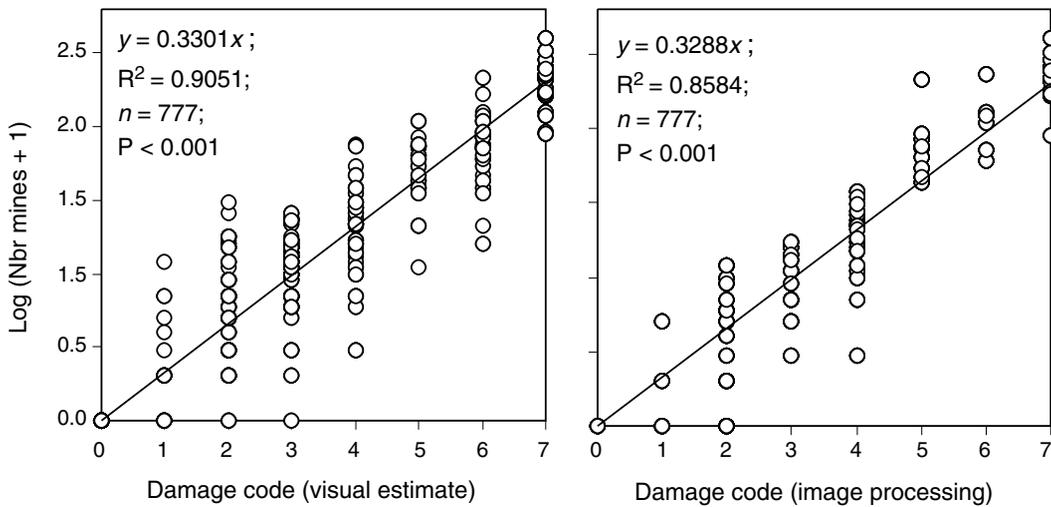
## 4 Discussion

Foliage damage is closely related to population density in *C. ohridella* and can be used efficiently to provide accurate population estimates. Although some variability in leaf size or mine size may alter this relationship, the quantification of damage allows to capture a large proportion of the variability in mine density.

Mine size tends to decrease as a function of infestation density per leaf and this is clearly the effect of competition. If one assumes that the average mine size in the absence of competition (near 1% of leaf damage) is a measure of the amount of food required to develop (7–8 cm<sup>2</sup>; fig. 3 left part of the model), the reduction of this food resource to 2.3 cm<sup>2</sup> (fig. 3 right



**Fig. 4.** Relationships between estimates made by image processing and by visual assessment using the damage pattern score sheet (a) as leaf damaged proportion and (b) converted into damage scores (the circles' radii are proportional to the numbers of points indicated beside)



**Fig. 5.** Relationship between damage code (measured visually or by image processing) and log-transformed mine numbers [ $\log_{10}(\text{number of mines} + 1)$ ]. Data on mine counts were log-transformed in order to eliminate heteroscedasticity observed in the raw data model's residuals

part of the model) may have a strong impact on mortality and fertility which would be interesting to estimate. The variability caused by leaf size could easily be reduced either by selecting leaves with similar size, or by standardizing results by leaf size (provided it is measured or estimated).

Quantification of leaf damage can be carried out either through image processing or by visual assessment. Despite the fact that it provides an accurate estimate and allows to account for the variability in leaf size, image processing presents several drawbacks.

First, as it can be observed in fig. 2a–c, it tends to overestimate the damaged surface (as shown by the y-axis intercept). This is probably related to the classification method. By default, all pixels not classified by the user as 'white' (background) or 'green' (undamaged), are considered as damaged by the moth. Even when this supervised classification process is performed cautiously, several causes may contribute to

this error: (i) pixels such as those located on the leaf edges (i.e. in contrasted areas between the leaf and the background); (ii) shadows which are difficult to eliminate; (iii) leaf stalk or veins not properly classified as 'green'. This could probably be improved by carrying out the classification for 'damaged' pixels separately. However, this might be rather difficult as areas damaged by *C. ohridella* may present highly contrasted colours depending on the age of damage (from light green to brown).

Secondly, image processing is time consuming. It was estimated in this study, that each leaf required 4–5 min to be processed (picture taking, transfer and process). Knowing that sampling a site to estimate local population density may require several samples, the added value of such a method in comparison with directly counting the mines is questionable. Processing time could certainly be improved by using equipments specifically designed to scan and analyse

such type of leaf samples in a row. For example, Delta-T Devices Ltd (Cambridge, UK) claim that their WINDIAS devices is able to treat up to 800 leaves per hour from small leaf samples with an accuracy of 4% of object area. The accuracy and cost-benefit of such a method with chestnut tree leaves infested by *C. ohridella* still remains to be tested. Finally, this method requires to reach the leaves physically at any level in the trees.

In comparison with image processing, visual assessments present a complementary source of variability because of the users' interpretation of damaged areas as shown by the relationship between image processing and visual damage assessments (fig. 4). Such level of variability in the visual assessment cannot be avoided and is frequently observed in similar studies (OBRIEN and VANBRUGGEN, 1992; ROGERS et al., 1994). However, these two sources of variability (damaged areas vs. mine counts and visual assessments vs. image processing estimates) are not additive as shown in fig. 5, where visual assessments and image processing estimates present similar relationships to the mine counts. In other words, given the variability of the relationship between damaged areas and mine numbers, image processing presents no added value compared with visual assessments for estimating population density at the leaf level. The same results are obtained at the 'site' level (i.e. when estimating several leaves collectively), although here image processing provides slightly better results. This indicates that the use of visual damage assessments with the help of a damage key allows accurate population density estimates on several leaves assessed together. Furthermore, these results do not depend on the users' experience of the pest. In addition to its inherent advantages (it can be performed in less than 10 s per leaf with a bit of training and it allows damage estimates directly in the field even where leaves are not within reach), these results demonstrate quantitatively the validity of visual damage assessment methods to estimate *C. ohridella* population density. However, infestation by *C. ohridella* at the tree level is not homogeneous and may exhibit some gradients. Indeed, a pattern commonly observed is that leaves from the bottom branches tend to be more infested at the first cycle than those located higher up in the canopy (TOMICZEK and KREHAN, 1998). Selecting leaves from the lower branches for damage assessment may thus overestimate the damage at the tree scale. This problem is difficult to take into account because this pattern is highly variable (from trees where damage is homogeneously distributed in trees where the upper leaves are almost untouched) and very difficult to quantify. Again, given this additional source of variability, the cost/benefit value of more accurate damage measurements such as image processing is questionable. Visual assessment of damage by an experienced user with the help of a damage scale is probably the easiest measure of infestation density at the tree level. At least, this results show that the use of eight infestation classes is realistic and positively related to measured population densities,

and that estimating several leaves globally provides similar results.

In the present case of *C. ohridella*, the trade-off between accuracy and feasibility goes clearly in favour of visual assessment methods and this can largely be attributed to the variability in mine size which degrades the relationship between accurately measured damage area and actual population. Despite the fact that the quality of visual assessment may vary according to the type of damage pattern and leaf shape, similar results may be expected in damage assessment of other herbivorous insects where the relationship between damage and population presents similar level of variability.

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