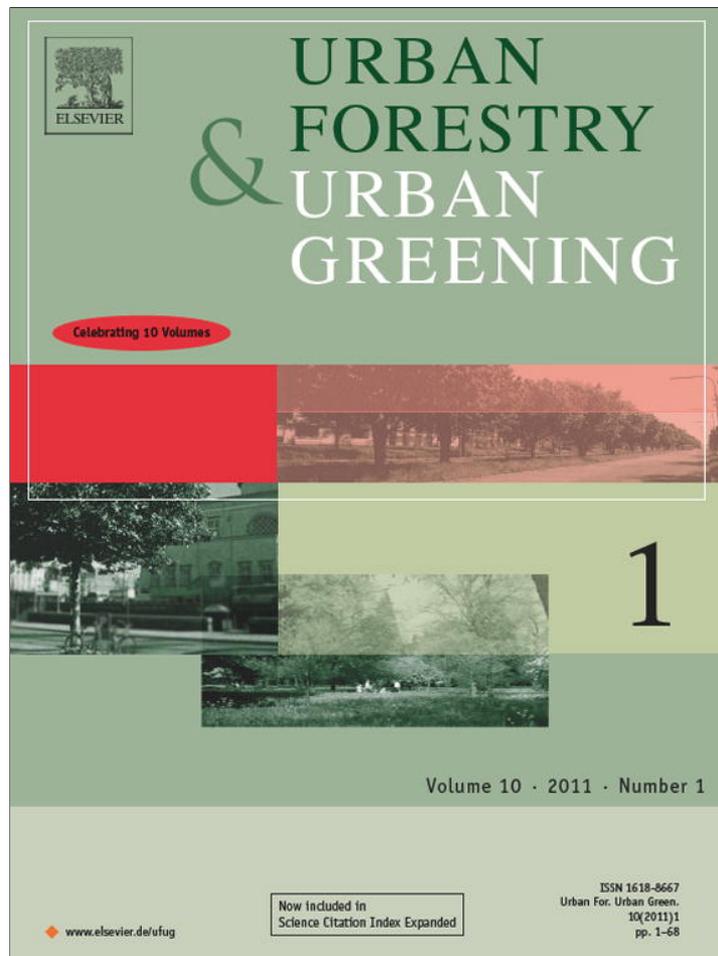


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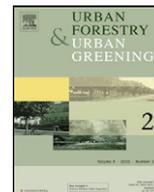
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## The impact of horse chestnut leaf miner (*Cameraria ohridella* Deschka and Dimic; HCLM) on vitality, growth and reproduction of *Aesculus hippocastanum* L.

Glynn C. Percival<sup>a,\*</sup>, I. Barrow<sup>b</sup>, K. Noviss<sup>a</sup>, I. Keary<sup>c</sup>, P. Pennington<sup>d</sup>

<sup>a</sup> R.A. Bartlett Tree Research Laboratory, John Harborne Building, School of Biological Sciences, The University of Reading, Reading, Berkshire RG6 6AS, UK

<sup>b</sup> Bartlett Consultancy, Shenley Lodge Farm, Rectory Lane, Shenley, Radlett, Hertshire WD7 9BG, UK

<sup>c</sup> Department of Entomology, John Harborne Building, School of Biological Sciences, The University of Reading, Reading RG6 6AS, UK

<sup>d</sup> Boxmoor Trust, Station Moor off London Road, Boxmoor, Hemel Hempstead, Hertshire, UK

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## ABSTRACT

The aim of this study was to evaluate the impact of horse chestnut leaf miner (*Cameraria ohridella* Deschka and Dimic; HCLM) on whole-tree energy loss, growth and reproduction on mature horse chestnut trees *Aesculus hippocastanum* L. Electron transport (ET<sub>o</sub>) flux per cross section (CS) of photosystem II (ET<sub>o</sub>/CS) was measured on HCLM infested and insecticide treated trees located in the South of England, UK. Comparison of insecticide treated trees where no HCLM activity was recorded compared with non-insecticide controls where severe HCLM activity was recorded indicated a total energy loss of 37.2% over a growing season. The relationship between phenology, leaf mining activity and subsequent loss in leaf energy indicated that up until petal fall/initial formation of seed (April–late June) energy loss ranged from 0% to 15%. However, this energy loss was not statistically significant from controls. From late June to late September energy loss significantly declined from 16% to 98%. A positive impact of controlling HCLM activity was recorded on growth, storage and reproduction. Average seed weight, seed germination and relative growth rates of germinated seedlings were 90.5, 47.6 and 35.2% higher respectively compared to trees where HCLM activity was not controlled. In addition, stem extension, root carbohydrate concentration and twig starch content values were 85.0, 33.3 and 1719.0% higher in trees where no leaf miner activity was recorded. Results indicate that while mature horse chestnuts produce sufficient energy via photosynthetic activity early in the growing season to provide energy for bud break, leaf flush and expansion and therefore maybe able to cope with repeated HCLM infestations in the long term, the impact on reproduction is of particular concern. Reduced seed weight, germination rates and seedling vigour could detrimentally impact on the long term persistence of *A. hippocastanum* in the UK.

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## Introduction

Since its introduction to Britain in the 1600s, the common white flowering horse chestnut tree (*Aesculus hippocastanum* L.), represents one of the most commonest ornamental trees found within UK towns, cities, parks and woodlands. Over the past 7–8 years however, this particular tree species has suffered heavily from attack by a leaf mining insect known as *Cameraria ohridella* Deschka and Dimic, the horse chestnut leaf miner (HCLM; Tilbury and Evans, 2003). Foliar damage occurs when larva tunnels into the leaves of the tree, causing physical destruction of leaf tissue and subsequent stunting of future growth. Infected leaves are covered initially in small, brown patches which spread rapidly across the entire tree, giving an autumnal appearance by late July–early August. Eventu-

ally leaves die and fall prematurely. If new leaves are formed they can be re-infested (Kehrli and Bacher, 2003; Raimondo et al., 2003; Salleo et al., 2003; Thalmann et al., 2003).

The full impact of repeated HCLM infestations on long term tree vitality and growth is still not fully understood. Based on the fact that trees have re-flushed on an annual basis following heavy HCLM infestation with no significant crown die-back or other symptoms of decline, many researchers consider HCLM a cosmetic pest (Salleo et al., 2003; Nardini et al., 2004; Straw and Bellet-Travers, 2004). Contrary to this, Thalmann et al. (2003) studying the repeated effects of HCLM defoliation on horse chestnut reproduction, concluded the allocation of photo-synthates to seeds was strongly reduced in infested trees with respect to controls and that reduced seed weight may severely impair future growth and survival of horse chestnut seedlings that, in turn, may endanger the long term persistence of this species throughout South-East Europe. Mathematical model calculations of HCLM infested trees in Northern Italy have showed that photosynthetic net primary production loss of infested trees was about 30% on an annual basis (Nardini

\* Corresponding author.

E-mail addresses: [gpercival@bartlettuk.com](mailto:gpercival@bartlettuk.com) (G.C. Percival), [ibarrow@bartlettuk.com](mailto:ibarrow@bartlettuk.com) (I. Barrow), [knoviss@bartlettuk.com](mailto:knoviss@bartlettuk.com) (K. Noviss).

et al., 2004). Due to the hotter drier climate present in Northern Italy the authors also stated that trees close down their photosynthetic machinery relatively early in year whereas in cooler, more humid climates such as the UK carbon assimilation may occur later into the season. Consequently the second and third moth generation may have a greater impact on photosynthetic net primary production on horse chestnut trees located in the UK compared to Northern Italy.

Chlorophyll fluorescence measures changes in chlorophyll *a* fluorescence due to altered photosystem II (PSII) activity, caused directly or indirectly by stress effects on leaf tissue (Strasser et al., 1995; Maxwell and Johnson, 2000). Consequently, measurement of chlorophyll fluorescence provides a quantifiable link between the internal metabolism of a tree and the urban environment (Percival et al., 2006). When leaves are briefly exposed to a saturating light level plastoquinone A a molecule responsible for electron transfer in PSII is reduced and a maximum yield of fluorescence ( $F_m$ ) is observed that in turn is used as a measure of oxidation/reduction reactions in the plastoquinone pool and provides a fluorescence variable directly correlated with the physiological efficiency of the photosynthetic machinery (Demmig and Björkman, 1987). In addition some fluorimeters are capable of calculating light absorption (ABS), trapping (TRo), electron transport (ETo) and dissipation (Dio) flux per cross section (CS) of photosystem II within leaf tissue (Strasser and Strasser, 1995). Light energy absorbed by the leaf pigments are referred to as absorbed flux (ABS). Part of this light energy is dissipated (Dio) as, for example heat, that can be used as a measure of non-photochemical work, but most of it is transferred as trapping flux (TRo) to the reaction centres. Reaction centres are pairs of chlorophyll *a* molecules located within photosystem II that promote electron transfer. The reaction centre within photosystem II is known as P680. In the reaction centres the trapped energy flux is converted to redox energy by reducing the plastoquinone pool (specialized molecules found in photosystem II that are involved in electron transfer between photosystems I and II) which are then re-oxidised leading to an electron transport flux (ETo) which maintains the metabolic reactions of the photosynthetic apparatus (Strasser and Strasser, 1995; Clark et al., 2000; Hermans et al., 2003). Consequently leaf energy fluxes can now be calculated and either quantified numerically as ETo/CS fluxes or expressed diagrammatically as energy pipeline models using the Biolyzer software package from data derived from leaf chlorophyll fluorescence emissions. The use of chlorophyll fluorescence to quantify electron transport flux (ETo/CS) as a measure of photosynthetic metabolism and subsequent efficiency of the photosynthetic machinery in response to HCLM infestation remains unknown. From a practical point of view chlorophyll fluorescence measurements are non-invasive or destructive to leaf tissue and readings can be obtained in less than one second allowing for rapid evaluation of many trees within a day. In addition, the fluorimeter is a small portable piece of equipment ideally suited for arborists to use within the tree crown (Percival, 2004).

Aims of this study were to quantify the impact of HCLM infestation on leaf photosynthetic energy fluxes (ETo/CS) over a growing season based on chlorophyll fluorescence emissions and record subsequent effects on growth and reproduction.

## Materials and methods

### Field site and experimental trees

The trial site consisted of an avenue of mature horse chestnut (*Aesculus hippocastanum* L.) trees located at the Box Moor Trust, Station Moor, Box Moor, Hemel Hempstead (51°74' N, 0.49' W),

UK. Ten trees selected for experimental purposes had an average height of  $20 \pm 2.0$  m with mean trunk diameters of  $120 \pm 12$  cm at 1.3 m above the soil level. Historically the horse chestnut trees suffered from HCLM infestation on an annual basis. Prior to the trial commencing in 2008 trees were inspected in September 2007 and 10 trees with >70% of leaves affected with HCLM infestation showing concomitant severe defoliation were included in the trial. No weed control, watering or fertilisation was applied during the 2008 trial. Average climatic conditions during the 2008 growing season (April–September) were as follows: max temperature 22.9 °C, min temperature 12.7 °C, sunshine hours 603.4, total rainfall 221 mm respectively.

### Insecticide treatments and timing

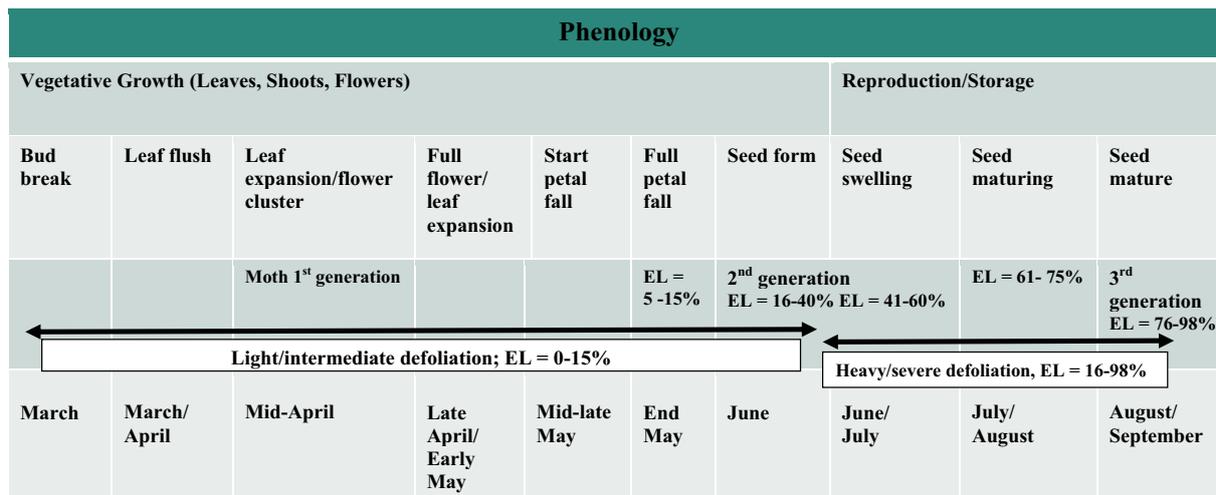
To quantify energy loss between HCLM infested trees and non-HCLM infested trees the soil drench insecticide Admire (imidacloprid) was applied using a Tom Wanner Soil Injection System based on an  $1 \times 1$  m spacing to an area three times the diameter of the trunk of five trees. In line with manufacturers recommended dosage and pesticide safety directorate guidelines (Anon., 2008), imidacloprid was applied at 1.4 g product per 2.5 cm diameter at breast height in November 2007, six months prior to the experiment commencing to permit absorption and root uptake of the insecticide. A maximum of 250 ml of imidacloprid plus dilutant was injected per hole to a depth of 20–25 cm at a pressure of 30 psi. This was split into a minimum of four equal applications around the base of the tree (North, South, East, West). The imidacloprid and water (control) treatments were applied as a randomized complete block with a single tree as the experimental unit, giving a total of 10 observations per response variable. Trees located either side and to the front of each imidacloprid treated trees within the avenue acted as guard trees to reduce any possibility of cross contamination between treated and control trees.

### Phenology and quantification of leaf energy fluxes

Tree phenology (bud break, leaf flush, leaf expansion, etc.) of all experimental trees was closely monitored and recorded according to criteria stipulated by Pallardy (2008). Evaluation of HCLM infestation on reductions in leaf electron transport flux (ETo/CS) as a measure of photosynthetic metabolism and subsequent energy loss was conducted at monthly intervals commencing in April to the cessation of the growing season (late September) 2008 by recording ETo/CS on 45 fully expanded leaves at random according to the height of the tree (15 leaves in the lower canopy, 15 in the middle canopy, 15 in the high canopy) using chlorophyll fluorescence measurements at ambient temperature with a *Plant Efficiency Analyser* chlorophyll fluorometer (Hansatech Instruments, King's Lynn, Norfolk, UK). During the measurement, the leaf sample was shielded from ambient light by a clip system to reach a dark adapted state (30 min adaptation to the dark) and illuminated with 660 nm light from an LED source built into the fluorimeter sensor. Continuous light excitation (at  $2500 \mu\text{mol}/\text{m}^2 \text{ s}$ ) was provided by an array of six light-emitting diodes focused on the leaf surface to provide homogeneous irradiation over a 4 mm diameter leaf surface. ETo/CS were calculated automatically by the *Plant Efficiency Analyser* and where required expressed diagrammatically as energy pipeline models (Fig. 1) using the Biolyzer *Plant Efficiency Analyser* software package.

### Quantification of leaf mining activity

Following leaf chlorophyll fluorescence readings the same leaf was detached by hand via a trained and certified professional climber. Excised leaves were placed in a darkened cool box and



**Fig. 1.** The relationship between phenology, leaf mining activity and subsequent loss in leaf energy (EL) as recorded by electron transport (ETo) flux per cross section (CS) of photosystem II over the 2008 growing season. Trees were located at the Box Moor Trust, Station Moor, Box Moor Hemel Hempstead (51°74' N, 0.49° W), UK.

returned to the laboratory within 2 h of collection. Leaf mining activity per leaf was quantified using an area metre and contrast scanner (Delta-T, Cambridge, UK) to quantify and distinguish the un-mined green leaf photosynthetic area from the translucent mined leaf area.

*Growth, storage and reproduction*

Stem extension was recorded by measuring the distance from the tip of 15 stems per tree (5 in the lower, middle and high canopy respectively) to the preceding years' growth using a hand ruler at the cessation of the growing season. Each stem starch concentration was then quantified using the high performance liquid chromatography system of Wilson et al. (1995). At the same time 40 seeds per tree were collected from underneath the canopy of each insecticide treated and non-insecticide control tree. Twenty seeds per tree were dried for 4 days at 110 °C, cooled down in a desiccator and immediately weighed to determine their dry weight (Thalmann et al., 2003). The remaining 20 seeds per tree were soaked in sterile distilled water for 24 h to enhance germination and then sown into 10 cm pots containing an general purpose seed compost (loamy texture, with 23% clay, 46% silt, 31% sand, 3.1% organic carbon, pH 6.6). All pots were then laid out in a complete randomised block design that was re-randomised every 10 days under glasshouse conditions i.e. 22C ± 2C (72F ± 36F) supplemented with 400 W high pressure sodium lamps (SON/T) providing a photoperiod of 16 h light/8 h dark and minimum 250 μmol m<sup>-2</sup> s<sup>-1</sup> Photosynthetically Active Radiation (PAR) at the seed compost surface. Percent germination was then assessed at day 30 after sowing. Of the seeds that had germinated the relative growth rate (RGR) between days 30 and 50 was assessed.

$$RGR = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}$$

where W<sub>2</sub> and W<sub>1</sub> are total dry weight at times t<sub>1</sub> and t<sub>2</sub> respectively.

The carbohydrate (glucose + fructose + sucrose) and starch concentration within root tissue of insecticide and non-insecticide treated trees was quantified using HPLC (Wilson et al., 1995). Fine roots were collected by using a root density corer at the cessation of the growing season (late September 2008). Four 35 cm deep, 7 cm diameter cores were taken (North, South, East, West) 1.5 m from the base of each tree and stored at 4 °C until processing. Soil was

washed from the roots, and horse chestnut roots were separated from debris and any grass roots by hand.

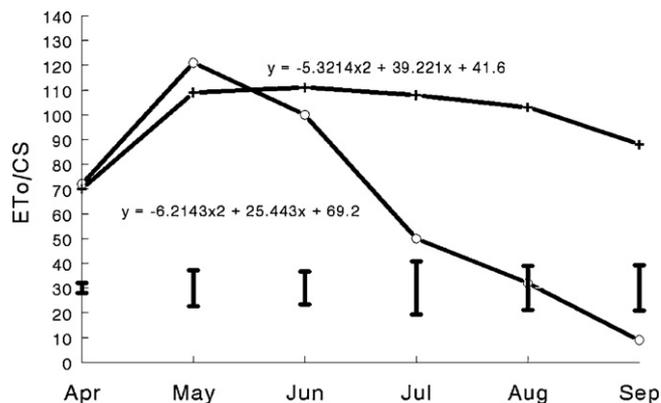
*Statistical analysis*

Data (stem extension, seed DW, root carbohydrate content, RGR) were analyzed by one way ANOVA using the Genstat V program for Windows. Prior to the analysis data were examined for normality and homogeneity of variance (Levene, 1960) and data were transformed [log(y + 0.5)] when necessary. Significant differences between means were separated by LSD test (P < 0.05). As ETo/CS were measured over time and were obtained from the same tree the relationship between HCLM infestation and ETo/CS were determined using the curve fitting feature of SlideWrite using quadratic polynomial regression analysis. As the line of best fit produced a classic curve shape the total difference in energy fluxes over a growing season between insecticide and non-insecticide control trees could then be quantified as area under a curve using the equation Area = ∫<sub>a</sub><sup>b</sup> f(x) dx. The binary data obtained (0 for no germination and 1 for germination) was subjected to survival analysis statistics using the Wilcoxon–Gehan method.

**Results**

*Phenology and quantification of leaf energy fluxes*

The relationship between phenology, leaf mining activity and subsequent loss in leaf energy as recorded by ETo/CS of photosystem II is shown in Fig. 1. For the first month following bud break and subsequent leaf flush and prior to the observation of HCLM (early April to early May) ETo/CS of photosystem II increased by 37–42% in both control and insecticide treated trees. ETo/CS at these months were also not significantly (P < 0.05) different between control and insecticide treated trees (Fig. 2). Such a response indicates that the insecticide applied (imidacloprid) to control HCLM has no significant influence on tree vitality during this period. This is supported by measurement of light absorption (ABS), trapping (TRo) and electron transport (ETo) flux per cross section (CS) of photosystem II shown diagrammatically for non-insecticide and insecticide treated trees where values did not significantly differ (Fig. 3). From May to August ETo/CS in horse chestnut trees treated with insecticide remained relatively constant with ETo/CS values



**Fig. 2.** The impact of HCLM activity on whole-tree energy loss based on electron transport (ETo) flux per cross section (CS) of photosystem II as measured by leaf chlorophyll fluorescence emissions of mature horse chestnut tree *Aesculus hippocastanum* L. over the 2008 growing season based on quadratic regression analysis. All values mean of five trees, 45 leaves per tree. Error bars represent LSD significance at  $P < 0.05$  + = insecticide treated tree, o = non-insecticide control tree.

ranging between 100 and 110. Lowering ETo/CS values from August (ETo/CS = 105) to September (ETo/CS = 92) in insecticide treated trees were indicative of the onset of senescence. For this reason no readings were taken in October where energy loss due to leaf senescence would have confounded results. During the growing season (April to September) no HCLM activity was observed in insecticide treated trees (Fig. 4). From May to September ETo/CS in horse chestnut trees not treated with insecticide and where leaf miner activity was observed declined continually from May (ETo/CS = 121) to September (ETo/CS = 9) (Fig. 2). Greater HCLM defoliation was observed in non-insecticide treated trees with time over the growing season (Fig. 4). ETo/CS became significantly ( $P < 0.05$ ) reduced compared to trees where no HCLM activity was observed by early June (Fig. 2). Such a result is supported by measurement of light absorption (ABS), trapping (TRo) and electron transport (ETo) flux per cross section (CS) of photosystem II shown diagrammatically for non-insecticide and insecticide treated trees where ETo/CS were substantially lower compared to trees where no HCLM activity was observed (Fig. 3). Increased dissipation fluxes per cross section of photosystem II in HCLM infested trees indicated a switch from photochemical (light energy absorbed used to provide the chemical energy for CO<sub>2</sub> fixation) to non-photochemical work (heat dissipation); a response indicative of breakdown or impairment of the leaf photosynthetic system (Fig. 3). Greatest reductions ( $P < 0.05$ ) in light absorption (ABS; 81%), trapping (TRo; 58%) and electron transport (ETo; 96%) flux per cross section (CS) of photosystem II were found between HCLM infested and non-HCLM infested trees in September. Quantification of energy loss based on chlorophyll fluorescence ETo/CS as determined by the equation for the area under a curve indicated the total energy loss caused by HCLM defoliation and infestation equated to 37.2% over the growing season (Fig. 5).

#### Quantification of leaf mining activity

In insecticide treated trees no leaf mining activity was recorded in any of the leaves sampled over the growing season. For reasons of clarity, in Fig. 4 a leaf mining activity value of 2 was given. In non-insecticide treated trees leaf mining activity was initially recorded in May (5%) with a steady increase in activity recorded throughout the growing season. By September leaf mining accounted for 98% loss of leaf photosynthetic area. By early June leaf mining activity was significantly ( $P < 0.05$ ) greater than insecticide treated trees (Fig. 4).

**Table 1**

The influence of HCLM activity on reproduction and seedling vigour of horse chestnut trees at the cessation of the 2008 growing season.

	Average seed DW (g)	Germination (%)	RGR
HCLM	4.2	42	0.102
No HCLM	8.0 <sup>*</sup>	62 <sup>*</sup>	0.138 <sup>*</sup>

RGR values mean of germinating seeds from an initial number of 100.

Germination analysis statistics using the Wilcoxon–Gehan method RGR = relative growth rates.

<sup>\*</sup> Significant at  $P < 0.05$  based on LSD.

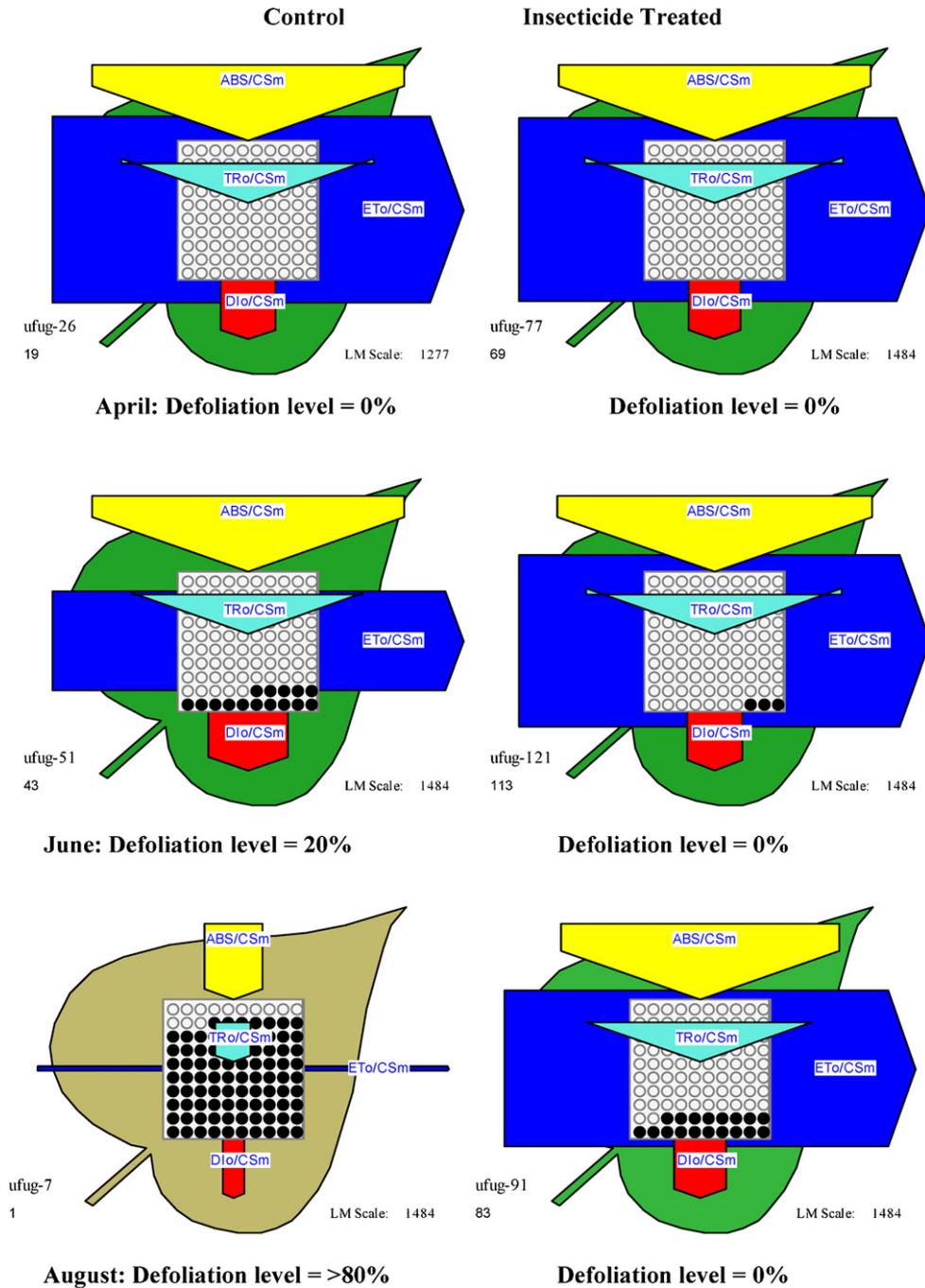
#### Growth, storage and reproduction

A marked positive impact of controlling HCLM activity was recorded on average seed weight, seed germination and relative growth rates where values were 90.5, 47.6 and 35.2% higher respectively compared to trees where HCLM activity was not controlled (Table 1). Similar responses were recorded with respect to stem extension, root carbohydrate concentration and twig starch content of mature trees. In this instance values were 85.0, 33.3 and 1719.0% higher in trees where no leaf miner activity was recorded (Table 2).

#### Discussion

From May until June a marked increase in ETo/CS was recorded in both control and insecticide treated trees. This can be accounted for by the fact that following leaf flush most plants require 21–28 days before the internal leaf photosynthetic machinery is fully functional and leaves show maximum photosynthetic performance (Kitao et al., 1998). ETo/CS measurements of healthy, non-stressed deciduous trees are associated with values ranging from 90 to 110 (Clark et al., 2000; Hermans et al., 2003; Percival, 2004). ETo/CS values of all trees tested in this study were within this range by May indicating all trees tested in this investigation were healthy at the onset of the experiment and not suffering from any prior stresses.

Studies of whole-tree loss of net primary productivity (NPP) between horse chestnut trees infested with HCLM compared to trees chemically treated to prevent HCLM were calculated to equate to 30% over the growing season (Nardini et al., 2004). Loss of NPP was deduced by monitoring of leaf gas exchange and leaf area of horse chestnut trees located in Northern Italy and integrating data into a complex mathematical model. Results of this study indicate an energy loss of 37.2% over the 2008 growing season of horse chestnut trees located in the South of England where a milder more temperate climate prevails. In our study energy loss was based on leaf ETo/CS derived from chlorophyll fluorescence emissions as a measure of photosystem II activity to record photosynthetic metabolism (Strasser and Strasser, 1995; Clark et al., 2000; Percival and Fraser, 2001; Hermans et al., 2003). Differences in energy loss values between this and the study of Nardini et al. (2004) may result from the fact that in a hotter drier climate, typical of Northern Italy trees tend to close down their photosynthetic machinery relatively early in year. In cooler temperate climates such as the UK, photosynthate assimilation may occur later into the season (Nardini et al., 2004). Consequently, the second and third moth generation may have a greater impact on whole-tree energy loss in the UK compared to Northern Italy. Results of this investigation support this hypothesis. However, it is important to emphasize that the 37.2% energy loss recorded in this study was expressed over the whole of the growing season. In reality, energy loss based on leaf ETo/CS did not become significantly different between trees with and without leaf miner activity until late June/early July. Comparison of energy loss with stages of phenology indicates that up until petal fall/initial formation of seed



**Fig. 3.** Energy pipeline models of horse chestnut trees with and without HCLM activity at three sampling dates during the 2008 growing season. The relative magnitude of absorbance (ABS), trapping (TRo), electron transport (ETo) and dissipation (Dlo) flux per cross section (CS) of photosystem II is shown by the width of the corresponding arrow. The colouration of the foliage indicates the chlorophyll concentration per leaf cross section. Inactivation of the reaction centres\* per cross section is drawn in black. \*Specialized pair of chlorophyll *a* molecules, which promote electron transfer within photosystem II responsible for the water splitting and oxygen evolving reaction of photosynthesis.

**Table 2**  
The influence of HCLM activity on growth and storage of horse chestnut trees at the cessation of the 2008 growing season.

	Stem extension (cm)	Root carbohydrate concentration (g/100 g DW)	Stem starch concentration (g/kg)
HCLM	14.7	2.1	2.1
No HCLM	27.2*	2.8*	38.3*

All values mean of five trees, 15 stems per tree and four root cores per tree.

\* Significant at  $P < 0.05$  based on LSD.

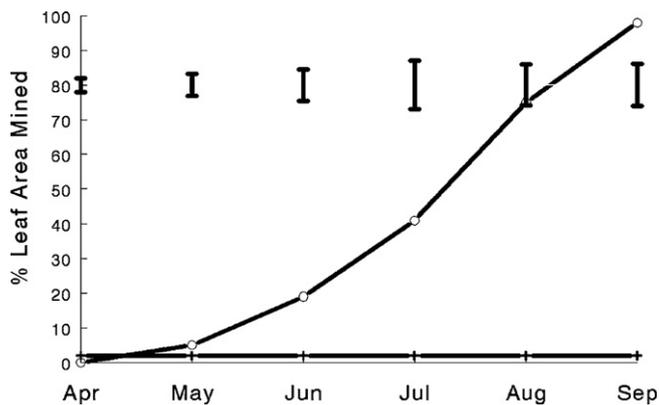


Fig. 4. Percent photosynthetic leaf area mined by HCLM activity of mature horse chestnut tree *Aesculus hippocastanum* L. over the 2008 growing season. All values mean of five trees, 45 leaves per tree. Error bars represent LSD significance at  $P < 0.05$ . + = insecticide treated tree, O = non-insecticide control tree.

(April–late June) energy loss ranged from 0% to 15%. However, this energy loss was not statistically different from HCLM infested trees. From late June/early July energy loss declined rapidly from 16% to 98%. Repeated studies of photosynthate allocation within deciduous trees over a growing season show that during the initial stages of growth, photosynthate allocation is primarily used for vegetative purposes such as leaf flush, expansion and growth, flower formation, etc. (Pallardy, 2008). This may account for the fact that horse chestnut trees consistently re-flushed annually following heavy HCLM infestation with no significant symptoms of physical deterioration such as branch or crown die-back (Salleo et al., 2003; Nardini et al., 2004; Straw and Bellet-Travers, 2004) i.e. the impact on energy loss from bud break to initial seed formation is insufficient to markedly affect these physiological processes. In addition it has been suggested that *A. hippocastanum* due its longevity would be expected to behave as typical K-strategists and therefore react to severe defoliation by ensuring its own survival and growth at the expense of reducing photosynthate allocation to reproduction (Pianka, 1970; Haukioja and Koricheva, 2000; Thalmann et al., 2003). Results of this study and that of others show that the impact of HCLM infestation on tree energy budget becomes significantly manifest later in the growing season when, at least in the UK, a switch in photosynthate allocation from vegetative growth to storage and reproduction is occurring (Fig. 1). In support of this, the greatest impacts on HCLM infestation and subsequent defoliation were recorded on seed size and

vigour (reproduction) and storage (root carbohydrate, stem starch content) in this study.

Studies of the impact of light, intermediate and heavy HCLM infestation on reproduction of *A. hippocastanum* i.e. seed and fruit weight have been undertaken by Thalmann et al. (2003). Results of their trials conducted on mature horse chestnut stands located in Munich and Bern concluded that dry weight of horse chestnut seed was on average 4.0 g on trees where HCLM infestation resulted in >75% lost leaf area. Similar to the results of Thalmann et al. (2003), average seed dry weight of trees heavily infested with HCLM in this study was 4.2 g where as control of HCLM resulted in an average seed dry weight of 8.0 g. Unlike the work of Thalmann et al. (2003) results of this study further demonstrated that this reduction in seed weight has a detrimental effect on germination and relative growth rates of germinated seedlings, indicating a significant role of HCLM infestation on reduced seed vitality and subsequent seedling growth performance. There is a general pattern in plants that large seeds perform better than small seeds in terms of height growth, survival and competition between other plants for light and nutrients. The ultimate consequence of reduced seed weight may be an alteration of plant succession that, in turn, may endanger the long term persistence of *A. hippocastanum* throughout South-East Europe (Thalmann et al., 2003). Results of this study provide further evidence to support this conclusion.

Carbohydrates are a major storage reserve of woody plants important in the growth and development of stress tolerance (Martínez-Trinidad et al., 2009). According to Tromp (1983), the importance of carbohydrate reserves for survival of the tree during winter and for resumption of growth activity in the spring cannot be overestimated. Woody plants accumulate carbohydrates during periods of excess production later in the growing season following leaf expansion and deplete these reserves when utilization exceeds production i.e. during bud break and early vegetative development (Tromp, 1983). The amount of reserve carbohydrates in young woody plants and their root system is crucial for successful establishment following out-planting (Ritchie and Dunlop, 1980; Struve, 1990). Likewise the process of recovery following root severance is dependent on the ability of a tree to manufacture abundant photosynthetic carbohydrates such as sucrose (Lonsdale, 2001; Lindqvist and Asp, 2002). Accumulation of carbohydrate reserves is very sensitive to late season stresses such as defoliation that in turn can profoundly affect a trees performance the growing year. Late season defoliation always results in smaller carbohydrate reserves and the roots are the most sensitive storage organs to such treatments (Loeschner et al., 1990; Pallardy, 2008; Martínez-Trinidad et al., 2009). In sugar maples late July defoliation resulted in a 90% reduction in root and shoot carbohydrate (sugar and starch) content. Similar responses have been shown in other tree species following late season defoliation to include pecan, sweet cherry and apple (Loeschner et al., 1990; Hoch et al., 2003). Effects on growth the following year included reduced root and shoot growth, smaller yellowed leaves and reduced fruit set (Loeschner et al., 1990; Lindqvist and Asp, 2002). In agreement with previous research, higher levels of HCLM infestation and subsequent loss of leaf area due to larvae mining activities resulted in significantly reduced shoot growth, root carbohydrate content and stem starch content in this study. While it could be argued that mature horse chestnut trees will be in a position to withstand repeated defoliation due to their larger storage reserves (Pallardy, 2008; Martínez-Trinidad et al., 2009) it is debatable whether smaller trees  $\leq 2.5$  m would be capable of such long term survival. Indeed within the UK there appears to be an increasing trend of removing young *A. hippocastanum* trees of such size and replacing with an alternative resistant species (*Castanea sativa* Mill) or *Aesculus* cultivar such as *A. carnea* Hayne (Percival, per-

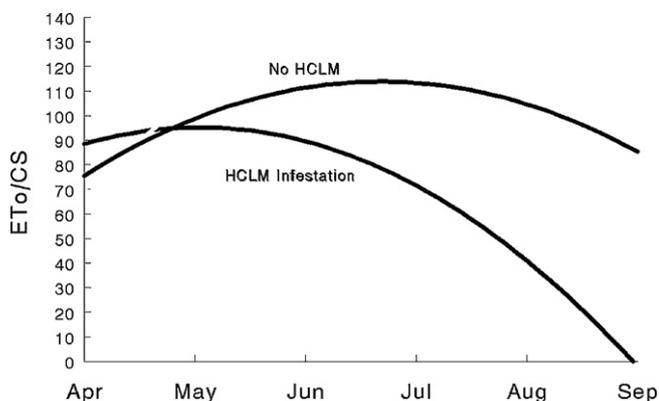


Fig. 5. The impact of HCLM activity on whole-tree energy loss based on electron transport (ETo) flux per cross section (CS) of photosystem II as measured by leaf chlorophyll fluorescence emissions of mature horse chestnut tree *Aesculus hippocastanum* L. over the 2008 growing season based on the area under a curve equation  $\text{Area} = \int_a^b f(x) dx$ . All values mean of five trees, 45 leaves per tree.

sonal observation). Similar observational losses and replacements have been recorded by Ferracini and Alma (2008). Consultation with leading tree suppliers in the UK indicates *A. hippocastanum* is no longer on their production schedule due to lack of demand. Such a management strategy whilst making good economic sense does raise concern regarding the long term persistence of *A. hippocastanum* within the UK landscape. If HCLM was more widely controlled, perhaps its value as a landscape tree would persist in the psychology of urban tree selection. Alternately greater research regarding provenance of seeds selected for nursery production with respect to HCLM resistance may offers an abundance of largely untapped genetic resource.

At present the consensus of opinion among researchers is that HCLM should be considered a cosmetic pest with the suggestion that control measures are not necessarily required (Salleo et al., 2003; Nardini et al., 2004; Straw and Bellet-Travers, 2004). Aesthetic quality (flowers, bark, berry, leaf colour) is an important consideration when planting trees within town and city streets, public recreation areas, parks, etc. (Ware, 1994; Percival and Hitchmough, 1995). With severe HCLM induced defoliation of horse chestnut trees observed by mid-July the aesthetic qualities of HCLM infested horse chestnut trees are now questionable. For these reasons development of HCLM control strategies may be warranted. Results of this study show that a single soil injection of the insecticide imidacloprid (Admire) had a highly significant influence on controlling leaf miner as no leaf mining activity was recorded through-out the 2008 growing season on treated trees. Ferracini and Alma (2008) demonstrated single systemic tree injection with imidacloprid applied immediately after flowering reduced leaf miner severity on average by 82% with, in many cases, this degree of control recorded over a two year period. Differences between the results recorded in this study (100% control) compared with 82% control (Ferracini and Alma, 2008) may relate to a higher concentration of imidacloprid applied via a soil injection system and the fact that imidacloprid was applied 6 months prior to the onset of bud break and subsequent leaf flush. Consequently a greater time period between application and the onset of HCLM miner activity existed in this study that in turn may have allowed for increased absorption and uptake of imidacloprid and a higher insecticide concentration within leaf tissue.

In conclusion, results of this study indicate that HCLM infestation and subsequent leaf mining activity resulted in an overall energy loss of 37.2% based on leaf electron transport flux (ETo) as a measure of photosynthetic metabolism as determined by chlorophyll fluorescence emissions. This loss of energy had a marked detrimental influence on growth (stem extension), storage (root carbohydrate, stem starch content) and reproduction (seed size and quality). While mature established trees due to their K-strategy survival mechanisms coupled with sufficient photosynthetic activity early in the growing season to provide energy for bud break, leaf flush and expansion may be able to cope with repeated HCLM infestations in the long term, the impact on reproduction is of particular concern. Reduced seed weight, germination rates and seedling vigour could detrimentally impact on the long term persistence of *A. hippocastanum* in the UK.

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