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Leaf miner-induced changes in leaf transmittance cause variations in insect respiration rates

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Abstract

Very little is known about alterations in microclimate when an herbivore feeds on host plant. Modifications of leaf transmittance properties induced by feeding activity of the leaf miner *Phyllonorycter blancardella* F. were measured using a spectrometer. Their effects on the herbivore's body temperature and respiration rate have been determined under controlled conditions and varying radiation level employing an infrared gas analyser. By feeding within leaf tissues, a miner induces the formation of feeding windows which transmit a large portion of incoming radiations within a mine. As a result, body temperature and respiration rate increase with radiation level when positioned below feeding windows. Therefore, the miner is not always protected from radiations despite living within plant tissues. The amount of CO_2 released by larvae below feeding windows at high radiation levels is about five-fold that recorded in the dark. By contrast, body temperature and respiration rate increase only slightly with radiation level when the insect is positioned below intact tissues through which radiation is only weakly transmitted. A mine offers its inhabitant a heterogeneous light environment that allows the insect larva to thermoregulate through behavioural modification. Our results highlight the importance of physical feedbacks induced by herbivory which alter significantly an insect's metabolism independently of its nutritional state.

Keywords: Body temperature; Herbivory; Leaf miner; Microclimate; Respiration

1. Introduction

Activity in all living organisms is highly constrained by temperature. Most biological functions of ectotherms are under the influence of body temperature (reviewed in Chown and Nicolson, 2004), such as metabolic rate (e.g. van Loon et al., 2005; Neven, 2000), feeding rate (e.g. Kingsolver, 2000) and development rate (e.g. Gilbert and Raworth, 1996). Two general adaptive mechanisms are used by organisms to cope with the wide and unpredictable range of ambient temperatures they experience: behavioural thermoregulation, e.g. the organism moves from a place to another which is more thermally favourable (May, 1979; Heinrich, 1999); Physiological thermoregulation, e.g. heat production by metabolic activity (e.g. Bartholomew and Heinrich, 1978; Ruf and Fiedler, 2000) and production of heat shock proteins allowing survival under extreme temperatures (e.g. Dahlhoff and Rank, 2000). Behavioural and physiological thermoregulation are often used together (e.g. Casey, 1992; Van Dyck et al., 1997). The metabolism of ectotherms is the result of complex interactions between environmental parameters, behavioural choices, and the physiological state of the organism. Accurate understanding of behavioural and physiological ecology of an organism demands us to determine: (i) the spatial scale allowing a complete evaluation of all environmental factors that impact the metabolism; (ii) the level of heterogeneity of the environment at this scale; and (iii) the existence of feedback loops between the animal and its environment. These aspects are detailed below.

The choice of study scale is clearly determinant in to find the environmental parameters altering significantly the insect metabolism. Body temperature is strongly altered by changes in the organism's physical environment, inducing a direct relationship between environmental parameters and

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the metabolism of the organism (Kingsolver, 2000). The use of biophysical heat budgets in ecophysiology has been particularly useful to predict the body temperature of an organism in the field (e.g. Kingsolver, 1979; Casey, 1992; Helmuth, 1998; Helmuth et al., 2005). This approach has revealed complex interactions between abiotic factors and physical properties of the organism in the determination of its body temperature at a microclimatic scale. The small size of insects allows them to exploit small-scale variations of microclimate that are not available to larger animals. Consequently, there is a great diversity in the microclimatic conditions experienced by insects. Accurate identification and characterisation of the experienced microclimate at the proper scale is therefore necessary to quantify and interpret the metabolism of an insect species.

Quantification of spatial and temporal heterogeneity in the insect microclimate is a crucial pathway when studying physiological processes. In insect–plant interactions, the microclimate of an insect is closely related to that of the plant. The plant provides small organisms with a specific microclimate which is temporally and spatially variable (Willmer, 1986). Leaf temperature can differ by several degrees from air temperature and a leaf surface is surrounded by a boundary layer of air which is nearly saturated for water vapour (Campbell and Norman, 1998; Nobel, 1999). For example, on clear days, the temperature of an apple leaf can reach 25 °C while air temperature is only 15 °C, but the same leaf can also be 10 °C colder than ambient air at air temperature 39 °C (Ferro and Southwick, 1984). Relative humidity within cabbage leaf boundary layers, which range in thickness from several micrometers to 10 mm depending mainly on leaf size and wind speed, is about 20% higher than ambient air during summer days (Willmer, 1986). Therefore, an insect resting on a leaf surface experiences a different microclimate than an insect living in ambient air. Although some studies have shown that insects clearly exploit the microclimatic variety of their food-plant by moving from a location to another within the plant during the day (Willmer, 1986), the effects of leaf microclimate on metabolic rate of an insect resting on a leaf have never been directly measured. This is unfortunate as many studies on nutritional ecology of an insect feeding on a plant have yielded wrong estimates of metabolic rates by neglecting the phyllosphere microclimate (van Loon et al., 2005).

Physiological feedbacks of feeding behaviour, related to nutrient acquisition, are well documented in insects (e.g. Edgecomb et al., 1994; Woods and Kingsolver, 1999; Casas et al., 2005; for review see also Chown and Nicolson, 2004), but very little is known about the existence of physical feedbacks which could be crucial for herbivores. The leaf microclimate is expected to be altered due to herbivore's attack as it modifies several leaf parameters that play a key role in a leaf heat budget. Characterisation of alterations in leaf microclimate and, subsequently, determination of their consequences for an insect's physiology might help to explain the evolution of feeding strategies. Externally chewing feeders alter plant physiology and reduce leaf size (Zangerl et al., 2002). Leaf tying and leaf rolling insects modify leaf shape (Berenbaum, 1978; Fitzgerald and Clark, 1994; Fitzgerald et al., 1991). Herbivory by several sap sucking insects (acarids and aphids) could alter the leaf optical properties (S. Pincebourde, personal observation). The feedback effects of such plant modifications on insect microclimate have however never been reported.

Leaf mining insects, as sessile organisms, are particularly suitable biological models to investigate the link between feeding pattern, microclimate and insect metabolism. Leaf mining insects develop inside leaf tissues by tunnelling within to produce a structure called a mine, and feeding on the various chlorophyll-containing tissues within the leaf. Their feeding behaviour results in alteration of leaf coloration in the fed areas. In some species the mine appears brown (e.g. Raimondo et al., 2003) while in others the mine is greenish with white spots corresponding to the areas eaten (e.g. Djemai et al., 2000). The endophagous way of life of leaf mining insects has been assumed to protect them from radiation, especially harmful ultraviolet radiation, as well as protecting them against natural enemies (Connor and Taverner, 1997).

We measured the changes in optical properties of mine tissues due to the feeding activity of the spotted tentiform leaf miner *Phyllonorycter blancardella* F. (Lepidoptera: Gracillariidae). We tested whether fed areas transmit more radiation than unfed areas within a mine. The effects of modifications in leaf optical properties on insect body temperature and respiration rate were investigated. We employed an infrared gas analyser to measure the CO_2 released by a larva under varying radiation level and when positioned below either uneaten tissues or fed areas. The method allowed us to study insect respiration under conditions as near as possible to the natural microclimate of the mine.

2. Materials and methods

2.1. Biology of the leaf miner

P. blancardella is a microlepidopteran herbivore with larval development divided into five instars (Pottinger and LeRoux, 1971). During the first three sap feeding instars, larvae define the outline of their mine by separating the two leaf integuments. During the fourth and fifth instars larvae are tissue feeders and this feeding behaviour results in the formation of feeding windows (Djemai et al., 2000). Feeding windows are translucent patches remaining after tissues containing chlorophyll have been consumed (Fig. 1A). Feeding events are mainly located at the periphery of the mine, leaving out a relatively large unfed area in the centre of the mine (Djemai et al., 2000). The leaf miner was reared on one-year old apple seedlings (*Malus communis*) within a greenhouse under temperate conditions (daily natural variations: mean air temperature 22 °C, mean relative humidity 59.5%, irradiance up to 718 $W m^{-2}$,

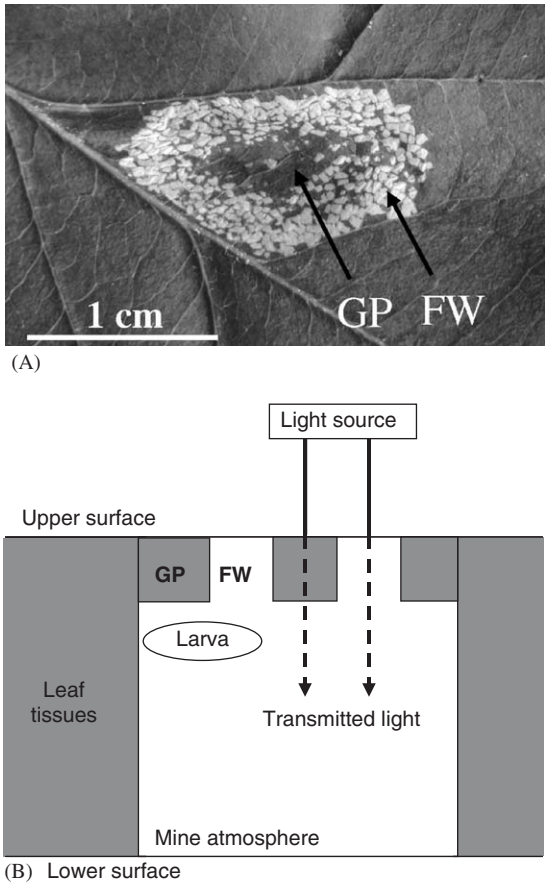

Fig. 1. (A) Upper surface of a mine containing a larva of *Phyllonorycter blancardella*. Insect feeding activity results in the formation of feeding windows (FW). Green patches (GP) correspond to the chlorophyll-containing tissues of the mine. The particular feeding pattern of a larva leads to the appearance of a large unfed area in the middle of the mine surface. A mine contains only one larva. (B) Schematic cross section of a mine. We measured the portion of incoming radiation which is transmitted within the mine space by feeding windows (FW) and green patches (GP). A larva moves within the mine on the upper side such that it could be positioned below feeding windows or green patches as well.

and natural light/dark cycle 11:13 during experiments). Seedlings were watered daily with a nutritive solution (N:P:K 6:6:6). All experiments were made on last-instar larvae.

2.2. Optical properties

Transmittance spectra of feeding windows and green patches (Fig. 1B) were measured using an Ocean Optics S2000 (Dunedin, FL, USA) directional spectroradiometer and a deuterium–halogen lamp DH-2000 relative to a 99% (300–700 nm) reflectance standard (Spectralon^{TM}). A directional spectroradiometer measures the amount of light transmitted perpendicularly to a surface. Transmittance was measured using a 1.5 mm diameter light emitter sensor equipped with a quartz window cut at 45° angle to avoid specular reflectance. The measured surface was illuminated from the top. A 1.5 mm diameter receptor sensor was positioned below the illuminated surface. Spectral measurements were made on freshly isolated upper integuments of last instar mines. The transmittance was determined at each wavelength from 300 to 850 nm on 43 feeding windows (from 20 different mines), 30 green patches (from the same 20 mines) and 50 intact leaf tissues (from 25 different intact leaves).

2.3. Insect respiration rate

The amount of CO_2 released by leaf miner larvae at different radiation levels was measured using the LI-6400 infrared gas analyzer (IRGA)-chamber system (Li-Cor Inc., Lincoln, NE, USA). This system precisely measures the amounts of water vapour and CO_2 in the incoming air flux and in the air leaving the sample chamber. Temperature, relative humidity and CO_2 concentration within the sample chamber are under control. The computer associated to the IRGA system calculates assimilation or production of CO_2 by the sample using the general gas exchange formula of von Caemmerer and Farquhar (1981). A slow flow rate of 52 μmol s^{-1} was used to improve the signal-to-noise ratio of the system (see Salvucci and Crafts-Brandner, 2000). Respiration of single larvae was too low to be measured individually and a group of ten larvae was used for each measurement.

Mines of last instar larvae were excised and living larvae were collected. Each larva was placed in a separate open plastic capsule (7 mm in depth and 5 mm in diameter). The capsules were translucent and reflected less than 2% incoming radiation. Four small holes (1 mm in diameter) were made through the capsule wall to allow CO_2 to diffuse without resistance into the measuring chamber. The ten capsules were transferred into the LI-6400 leaf chamber. Conditions within capsules were therefore similar to that within the whole chamber. To recreate mine conditions within the capsules, relative humidity was high (maintained between 80% and 90%), capsule temperature as measured with a copper–constantan thermocouple (0.2 mm in diameter) inserted within the capsules was 25 °C and CO_2 concentration was kept at the same level as that predicted within mines for different irradiance levels (Pincebourde et al., in revision). CO_2 concentration was 350 μmol CO_2 mol^{-1} in the dark, 270 μmol CO_2 mol^{-1} at radiation level 45 W m^{-2} and 240 μmol CO_2 mol^{-1} at radiation level 673 W m^{-2}, with a linear decrease between each point. The radiation level was altered by changing the distance between the sample and a 250 W metal iodide bulb positioned above it. Larvae were not directly irradiated and two groups were formed. In a first group of larvae, pieces of upper mine integuments, having at least 90% of their surface occupied by feeding windows, were cut out, dried to ensure that they stopped respiring and placed above each open capsule with the external surface facing the light source. In a second group, the capsules were covered with a dry piece of intact leaf tissues following the same method.

The amount of CO_2 released by larvae (μL $CO_2\,g^{-1}\,min^{-1}$, see below for method of conversion) was measured at several radiation levels ranging from 0 to $673\,W\,m^{-2}$ (this value corresponded to about full sun light in the field) for seven groups of ten larvae below feeding windows (total $N = 70$) and for five groups of ten larvae below intact leaf tissues (total $N = 50$). For each radiation level, samples were allowed to stabilise for 20 min before measurements were taken. The values given are the mean of ten measurements taken every ten seconds. Insects respire cyclically and discontinuously (Lighton, 1996), but we ensured to measure an accurate mean respiration rate by measuring ten larvae simultaneously.

The LI-6400 IRGA measures respiration rate per individual or per unit of sample surface. For example, Salvucci and Crafts-Brandner (2000) gave respiration rates in mol CO_2 $time^{-1}$ $insect^{-1}$ using the same apparatus. In order to express the amount of CO_2 release by unit of body mass, we first expressed respiration rates per unit of insect body surface. Eventually, it was translated using a relationship between body surface area and body mass. This transformation was applied on each group of ten larvae and allowed us to eliminate the between-group variation induced by a difference in the whole body mass. Dimensions of each larva (length and diameter) were measured to quantify the gas exchange surface of the insect. The body of the larva was considered as a cylinder for the purpose of these calculations. We established a relation between body mass and body surface on a further set of 14 freshly excised larvae. Larvae were weighed individually (Supermicro Sartorius, Richmond, UK) and their body length and diameter were immediately measured. Body mass was correlated positively and linearly to body surface (linear regression: body mass (mg) = 0.1179 body surface (mm^2)−0.1048, $R^2 = 0.93$, $P < 0.001$). This strong relationship indicates that even if body surface area is not a suitable unit to express respiration rate, it is helpful to convert values from the IRGA program into body mass unit. Finally, respiration rate was converted in μL $g^{-1}\,min^{-1}$ which is the usual unit.

2.4. Insect body temperature

In a further set of experiments, larval body temperature was measured as a function of radiation level. Three open plastic capsules, each containing four last instar larvae, were prepared as described above. The capsules were transferred to the LI-6400 sample chamber and each capsule was covered with a dry upper mine or leaf integument prepared as above. Conditions within the sample chamber were the same than before. Two fine copper–constantan thermocouples (0.2 mm in diameter) were inserted into each capsule, the first being in contact with the body of larvae while the second measured air temperature within the capsules. Body temperatures were recorded for three groups of three capsules covered with dry mine integuments containing feeding windows ($N = 9$), and for three groups of three capsules covered with dry intact leaf integuments ($N = 9$), at several radiation levels ranging from 0 to $673\,W\,m^{-2}$ using a Campbell data logger (CR10X, Campbell Scientific Ltd., Leicestershire, UK). All samples were allowed to stabilise for 20 min before measurements were taken. This stabilisation time permitted us to determine the body temperature excess compared to temperature within the capsule. We concentrated on the body temperature excess compared to mine (or capsule) temperature to establish a comparative analysis between the two positions, below feeding windows vs. green patches.

2.5. Statistical analysis

Linear regression models were used to assess the significance of the measured relationships between: (i) body temperature excess and radiation level; (ii) respiration rate and radiation level; and (iii) mean body temperature excess and mean respiration rate. We tested for the difference between the slopes of the regression lines using a one sided *t*-test (Zar, 1998). For all statistical tests, the probability threshold was 0.05.

3. Results

3.1. Transmittance of upper surfaces

The mean transmittance spectrum of green patches was similar to that of intact leaf tissues (Fig. 2A). Green patches transmitted very low amount of radiation within a mine in the visible range. By contrast, feeding windows transmitted a large portion of radiation within a mine. Higher transmittance levels were obtained at long wavelengths (Fig. 2B). Feeding windows transmitted a mean of 34.5% incident light whereas intact leaf tissues transmitted only a mean 1% incident light in the visible range.

3.2. Body temperature and respiration rate

Larvae ($N = 120$) had a mean length of 3.25 ± 0.71 mm and a mean diameter of 0.55 ± 0.10 mm, resulting in a mean body surface area of 6.10 mm^2 and mean body mass 0.61 mg.

At a constant capsule temperature of 25 °C, the body temperature of fully protected larvae (i.e. positioned below green patches) increased only slightly with radiation level (Fig. 3A; linear regression: $y = 0.0013x - 0.017$, $R^2 = 0.97$, $P = 0.01$). By contrast, body temperature of larvae below feeding windows increased strongly with radiation level (Fig. 3A; linear regression: $y = 0.0034x + 0.0293$, $R^2 = 0.99$, $P < 0.001$). The slopes of the two regression lines were significantly different (*t*-test: $t_{38} = 9.34$, $P < 0.0001$). At a radiation level of $673\,W\,m^{-2}$, the body of a larva below feeding windows was 2.4 °C warmer than the capsule temperature, and 1.5 °C warmer than the body of a protected larva.

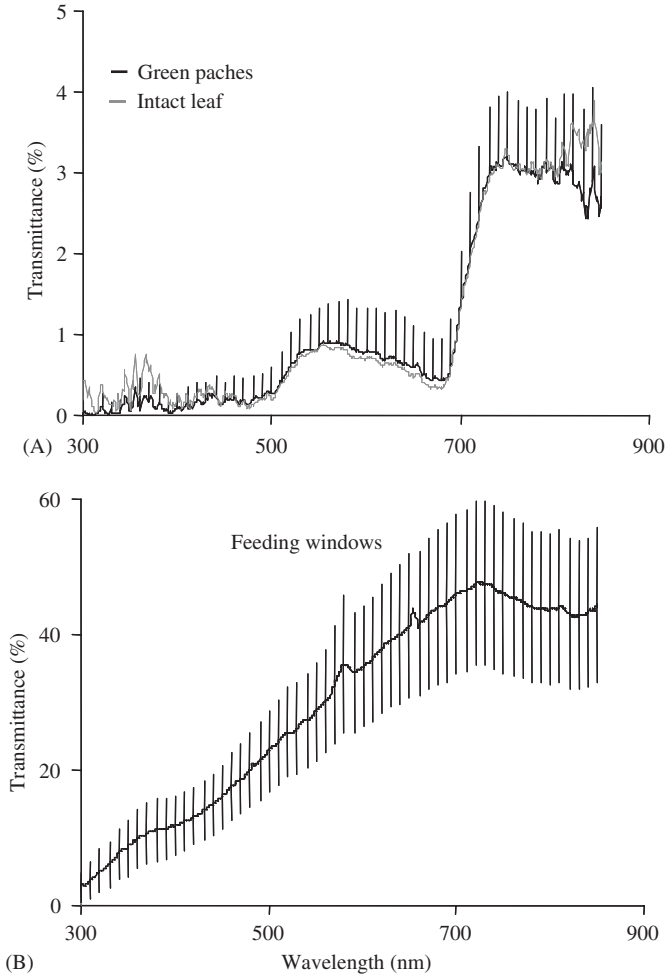

Fig. 2. Optical properties of upper integuments: (A) mean transmittance spectra (±SD) of green patches at the mine surface (black dots) and of intact leaf tissues (grey dots, SD not shown for clarity) in the ultraviolet, visible and near infrared ranges. (B) Mean transmittance spectrum (±SD) of feeding windows at the mine surface in the ultraviolet, visible and near infrared ranges. For all, standard deviations are shown every 10 nm.

The respiration rate of protected larvae increased with radiation level (linear regression: $y = 0.0468x + 33.916$, $R^2 = 0.99$, $P = 0.002$) and was less than two-fold higher at radiation level 673 W m^{-2} than in the dark (Fig. 3B). The CO_2 production of a fully exposed larva also increased linearly with radiation level (Fig. 3B; linear regression: $y = 0.114x + 30.329$, $R^2 = 0.97$, $P < 0.001$). At radiation level 673 W m^{-2}, the amount of CO_2 released reached 109.5 µL CO_2 g^{-1} min^{-1}. Respiration rate increased of about 480% from a dark situation to radiation level 673 W m^{-2}. The respiration rate of larvae below feeding windows was about twice that below uneaten leaf tissues at high radiation level and the slopes of the two regression lines were statistically different (*t*-test: $t_{86} = 8.39$, $P < 0.0001$). The respiration rate of larvae below feeding windows was strongly correlated with body temperature excess (Fig. 3C; linear regression: $y = 32.603x + 29.934$, $R^2 = 0.94$, $P < 0.001$). A similar correlation was obtained between respiration rate and body temperature excess for

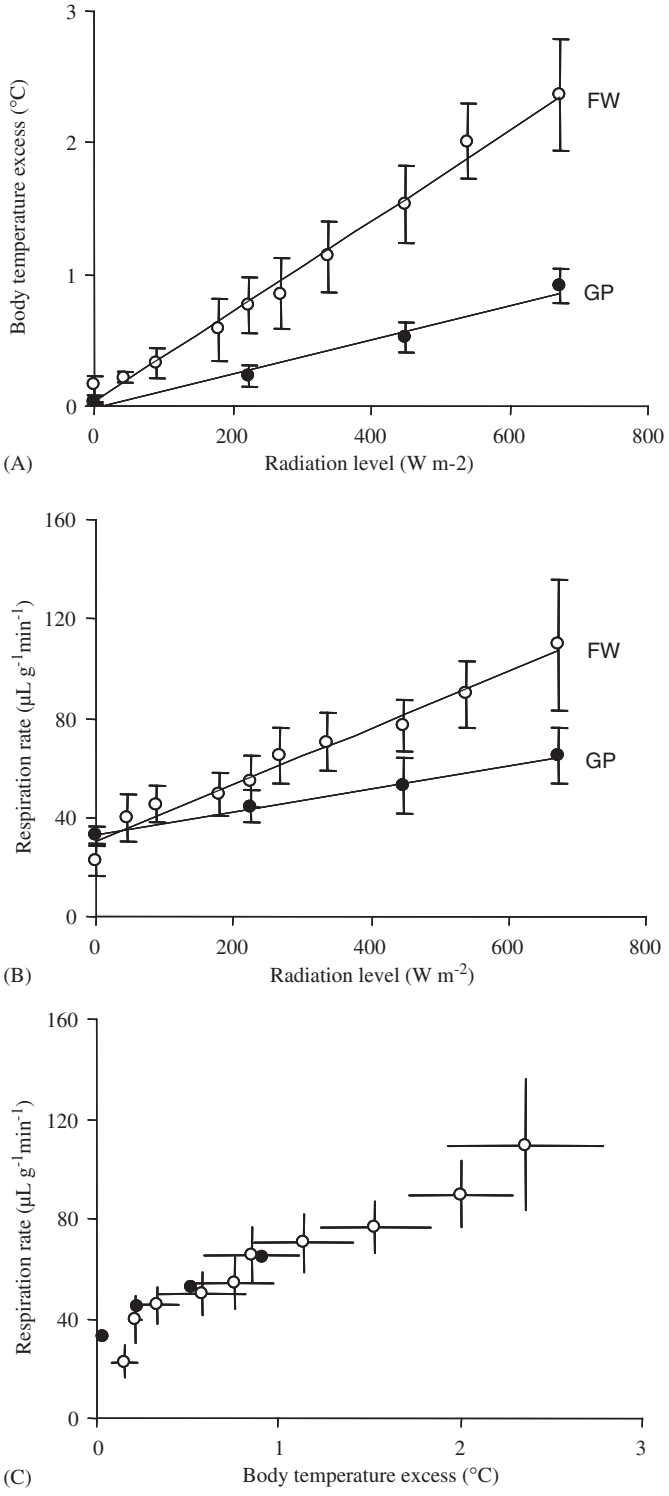

Fig. 3. Insect body temperature and respiration rate: (A) mean body temperature excess (±SD). (B) Mean respiration rate (±SD) of larvae as a function of radiation level at constant air temperature of 25 °C, as measured for fully exposed larvae (FW) and for protected larvae (GP). (C) Mean respiration rate as a function of mean body temperature excess for a protected larva (●) and for a fully exposed larva (○). Bars represent standard deviations on each axis and are shown only for exposed larvae, for clarity.

larvae positioned below intact leaf tissues (Fig. 3C; linear regression: $y = 35.12x + 33.825$, $R^2 = 0.97$, $P = 0.01$).

4. Discussion

Larvae of *P. blancardella* feed on leaf tissues in a meticulous way. Fourth and fifth instar larvae eat the palisade tissue such that chlorophyll-containing tissues are absent below the feeding windows. The transmittance spectrum we recorded for feeding windows is highly similar to that measured by Manetas (2004) for isolated periderm from twigs, two elements of similar structural properties. Optical properties of the green patches do not differ from that of intact and non-infested leaf tissues. Because the chlorophyll content of leaf tissues is well correlated with optical properties (Maccioni et al., 2001), we conclude that the chlorophyll content in the green patches is not affected nor is the capacity of the pigment to absorb radiation. This was confirmed by measurements of photosynthetic activity (Pincebourde et al., in revision). However, measurements made with a directional spectroradiometer, which records the light transmitted perpendicularly to the surface, might under-estimate the transmittance of the surfaces. Leaves usually transmit a scattered and isotropic light, i.e. in all directions (180° solid angle), below the surface (Breece and Holmes, 1971). It is not known whether feeding windows and green patches transmit scattered light; unfortunately these areas are too small areas to be measured with a hemispherical spectroradiometer. However, the transmittance spectra of intact leaf tissues, as measured with a hemispherical spectroradiometer (Pincebourde, unpublished data), was also lower than the potentially under-estimated spectrum of feeding windows presented here, indicating that the use of a directional spectrometer was sufficient to show the higher transmittance of feeding windows.

Changes in optical properties of the mine surface due to the formation of feeding windows have strong implications for the light environment of the leaf miner. Two aspects are considered below: (i) the quality of light and its heterogeneity within a mine, and (ii) the role of light heterogeneity at the mine scale as an interface between the physiology and the behaviour of larvae.

The strategy employed by leaf miners, galling organisms, leaf tiers and leaf rollers has been often seen as a mean to avoid radiation (Berenbaum, 1978; Connor and Taverner, 1997; Stone and Schönrogge, 2003; Hansell, 2005). Here we clearly show that it is not always the case. Feeding windows transmit a predominantly red light within the mine whereas green patches transmit a small quantity of green light. Therefore, larvae live within a small but highly heterogeneous light environment. The mine could be seen from the inside as a mosaic of small areas transmitting different qualities of radiation. Moreover, feeding windows transmit waves in the ultraviolet range. Up to 11% of incident light is transmitted in the ultraviolet range (300–400 nm, Fig. 2B). By contrast, ultraviolet radiation is not transmitted within the mine by the green patches. A protective role against harmful ultraviolet radiation has been evoked to explain the advantages of living within a mine (Connor and Taverner, 1997). For example, it was found in four leaf miner species that the mine surface transmitted less than 5% of ultraviolet radiations (Connor and Taverner, 1997). The leaf miner *P. blancardella* is obviously more protected even when positioned below feeding windows from ultraviolet radiations than a free-air living insect. However, it is not known whether the observed significant amount of ultraviolet radiation transmitted by feeding windows (11%) is sufficient to induce physiological damages in caterpillars having a very thin integument.

The heterogeneity of the mine's light environment translates into a larval position-dependent metabolism. The transmittance of radiation through feeding windows alters the physiological state of larvae whereas green patches do not transmit enough radiation to impact severely on larval metabolism. Body temperature of caterpillars is determined by the amount of radiation they receive and absorb through physical processes (Casey, 1992). The observed increase in body temperature leads to an increase in respiration rate. This is due to the ectothermy of the leaf miner which results in a strong link between body temperature and general metabolism, including respiration. The relationship between body temperature and respiration rate was linear rather than exponential as it is usually the case, certainly because the range of body temperature tested here is too narrow (25–27.5 °C). Because both body temperature and respiration rate were measured under constant air temperature but varying radiation regime within capsules, our results clearly reveal the direct effect of radiations transmitted by feeding windows on larval metabolism. Temperature within a mine is however also expected to increase with radiation level, as it is the case for leaf temperature (Nobel, 1999), leading to higher alterations in larval metabolism than that we measured at constant capsule temperature. The heterogeneous light environment within the mine offers larvae the possibility to regulate their metabolism behaviourally by moving within the mine between exposed locations and positions where they are protected from radiation. Behavioural thermoregulation could be significant when mine temperature approaches lethal temperature of the larvae since they are able to decrease their temperature by about 1.5 °C at high radiation levels (Fig. 3A). It is well known that solar radiation corresponds to an important heat source, and numerous insect species develop thermoregulatory behaviours in order to maximise or minimise the amount of radiative heat absorbed according to their thermal needs (May, 1979; Chapman, 1998; Heinrich, 1999; Chown and Nicolson, 2004). The pattern of the leaf miner's behavioural thermoregulation would be closely related to the mine design, i.e. the geometry of the mine surface or relative position of feeding windows and green patches. Indeed, Djemai et al. (2000)

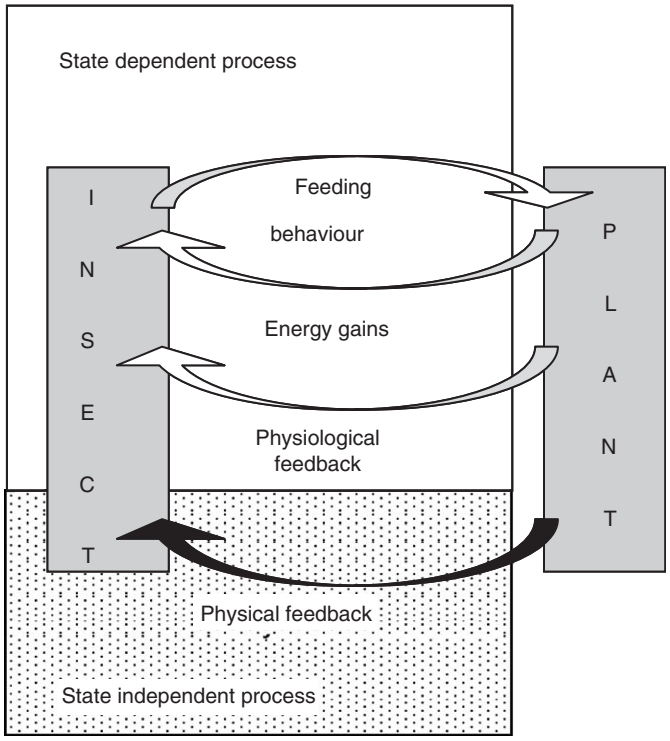

Fig. 4. Feedbacks of herbivory in insect–plant relationships from the insect' point of view. Feeding behaviour induces two physiological nutritional (or state) dependent feedbacks: the first is energy acquisition and the second comprises the effects related to the production of plant secondary compounds. Physical feedbacks are insect state independent and correspond to modifications in the insect microclimate.

demonstrated that the mine is built in such a way that a large area occupied exclusively by green patches remains in the middle of the mine surface, allowing a larva to be fully exposed or fully protected according to its position (Djemai et al., 2000).

Feeding activity has a large and direct impact on insect metabolism. For example, respiration rate increases during feeding and digestive activities (e.g. McEvoy, 1984; Kukal and Dawson, 1989). Food quality also directly alters metabolic efficiency of insects (e.g. Levesque et al., 2002) and production of secondary compounds by plant tissues had been shown to affect larval development (e.g. Beninger and Abou-Zaid, 1997). These nutritional state-dependent mechanisms usually affect respiration rates by a factor of 1.5–5 in caterpillars (van Loon et al., 2005). The indirect effects of feeding activity, acting independently of the organism's nutritional state, on the insect metabolism have been however so far largely ignored (Fig. 4). The feeding activity of the leaf miner *P. blancardella* results in the formation of feeding windows in the upper integument of its mine. Radiation transmitted by feeding windows induces larval body to warm through radiative energy absorption, a process independent from nutritional state, leading subsequently to a five-fold increase in respiration rate. Therefore, such physical feedbacks of herbivory have to be taken into consideration when attempting to predict the physiological state of an insect since they could be as important as nutritional state-dependent feedbacks. Physical feedbacks of herbivory determine key physiological parameters like growth rate and metabolic efficiency. In response to these feedbacks, a herbivore might develop a second layer of adaptive behavioural (e.g. thermoregulation) or physiological (e.g. heat shock protein synthesis) mechanisms. The understanding of intimate insect–plant relationships requires the consideration of physical feedbacks and ensuing behavioural adaptations.

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