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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},