Opinion is intended to facilitate communication between reader and author and reader and reader. Comments, viewpoints or suggestions arising from published papers are welcome. Discussion and debate about important issues in ecology, e.g. theory or terminology, may also be included. Contributions should be as precise as possible and references should be kept to a minimum. A summary is not required.

Nutritional ecology of insect–plant interactions: persistent handicaps and the need for innovative approaches

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Quantifying the flow of matter and energy in food webs is indispensable when assessing the effects of increases in atmospheric carbon dioxide, ozone level and temperature as a result of global climate change. In insect nutritional ecology, quantification of digestive and metabolic efficiency is performed using gravimetric methods in all published cases. A few cases combined these methods with calorimetric and respirometric techniques. Since 1986, methodological pitfalls and sources of error inherent to applying gravimetry as the only method to construct nutrient budgets have been addressed in a number of papers without noticeable impact on subsequent research. Especially for insects feeding on living plant tissues, the gravimetric method has inherent handicaps as it can only be used with excised plant tissues and does not allow for the dynamics of plant metabolism. We discuss the major constraints of the gravimetric method as it pertains to the physiological processes of both the insect and plant. We apply a relationship between relative metabolic rate and relative growth rate of the insect for an analysis of the gravimetric literature. The analysis reveals that gravimetry has given rise to physiologically unlikely results for poikilothermic insects. This points to serious constraints on progress in this field. We identify plant respiration as the major source of error in gravimetric studies. We establish that no single study has, thus far, determined the metabolic efficiency of a herbivore feeding on a photosynthetically active plant with its phyllosphere microclimate. We argue that a quantitative understanding of the ecophysiology and nutritional ecology of insect–plant interactions must rely on the adoption of a combination of existing and complementary methods such as the double labelled water method and infrared gas analysis.

Goals and concepts in nutritional ecology

Central to ecology are the processes by which organisms acquire nutrients and energy and the resulting fluxes of these nutrients and energy through food webs. For a proper understanding of the mechanisms governing trophic relationships between organisms, quantitative measures of food consumption and utilization are indispensable (Southwood and Henderson 2000). The interactions between plants and herbivorous insects comprise an important flow of matter and energy from a major group of autotrophic to a major group of heterotrophic organisms (Schoonhoven et al. 1998). Although in a qualitative sense green plants contain all nutrients required by herbivorous insects, inter- and intraspecific differences in plant nutrient composition are well documented. The term 'nutritional ecology' emphasises the importance of nutritional factors to understanding the behavioural strategies of herbivorous insects, which are directed toward optimizing of nutrient acquisition, utilization and allocation. This field provides indispensable basic knowledge to monitor the effects of climate change, such as increased atmospheric concentrations of carbon dioxide, ozone and higher temperatures on food and energy consumption and conversion efficiencies by plant feeding insects (reviewed by Whittaker 1999 and Führer 2003; Percy et al. 2002). Publications in the field of insect nutritional ecology during 1992–2003 have increased 3-fold compared with the number from 1982–1991 (Fig. 1).

Quantification of food utilization efficiency in insects was formalised by Waldbauer (1968) who proposed a number of indices derived from gravimetric determinations of insect body mass, food intake and faecal egesta. The basic equation of the nutritional budget is:

$$C = G + R + FU \qquad (1)$$

ISSN 0030-1299

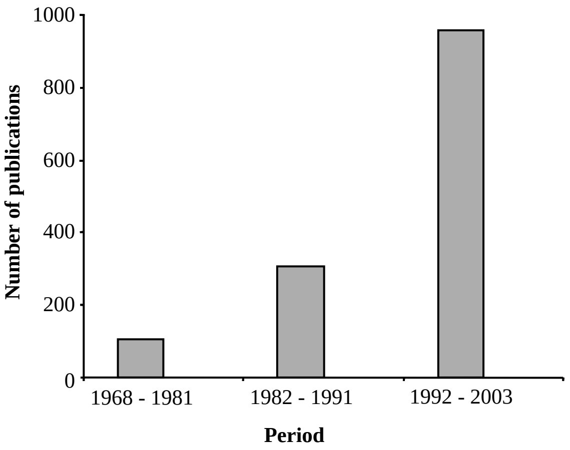

Fig. 1. Trend in the numbers of publications on insect herbivore nutritional ecology as returned by the CAB database in response to the search statement '((insect) and (plant) and (nutri*) and (ecolog*))'. Number for the period 1968–1981 was taken from Slansky and Scriber (1982).

where C is the amount of food consumed, G is insect biomass produced (including growth (somatic, reproductive and both secreted and excreted products), R is respiration (amount of carbon dioxide produced) and FU is faeces or egesta (mixed with urinary wastes in insects) and other metabolic waste products egested as faecal constituents. Nutritional budget items are routinely expressed in dry matter units, because water escapes as vapour from food, faeces and the insect body. Water losses via these respective routes are technically extremely difficult to quantify. Equation 1 is also valid for energy budgets as dry matter units can be converted to energy units through oxidative or combustive techniques (Southwood and Henderson 2000). Commonly employed measures of utilisation, often called nutritional indices, are efficiency of conversion of ingested food ($ECI = G/C$; also termed (gross) growth efficiency), approximate digestibility ($AD = (C - FU)/C$; also termed assimilation efficiency) and efficiency of conversion of digested food into body mass ($ECD = G/(C - FU)$; also termed metabolic efficiency or net growth efficiency). The gravimetric method has been, and still is, the prevalent method used to quantify food intake and utilisation. Gravimetry has been widely used by researchers, mainly because it is technically easy to perform and only basic equipment i.e. a stove and an accurate balance, are required. Extensive compilations on nutritional ecology of insects have been published twenty years after Waldbauers' 1968 review, summarising data from hundreds of primary articles (Slansky and Rodriguez 1987). The major outcome of such compilations is that particular feeding guilds, such as phloem feeders and folivores, can be characterised by their own set of values for consumption rates and food utilisation efficiencies. However, within guilds a large interspecific variation is evident, the cause of which is poorly understood. In this paper we propose that this large interspecific variation for insects feeding on living plants is physiologically improbable, and largely due to measurement errors. Nutritional ecology is logically linked to the level of the individual organism by physiological approaches of optimization of nutrient allocation, which is known to be achieved through a tight integration of behaviour and physiology (Simpson and Raubenheimer 1996, Lee et al. 2002).

Methodological handicaps inherent to gravimetry

A serious methodological constraint from the exclusive reliance on gravimetry to construct matter and energy budgets is the lack of an independent method to check the budget. Routinely, only C, G and FU are quantified, from which R is calculated as $R_c = C - G - FU$. Attempts at budget checks have been reported in relatively few cases and these uncovered considerable discrepancies in quantifying the R-term resulting from two independent methods: calculating it from the gravimetric budget equation (R_c) and measuring respiratory expenditure (R_m) by respirometric or calorimetric methods. Large discrepancies, with the R_c/R_m ratio ranging from 1.4–6 (Wightman 1981, van Loon 1991, Chaabane et al. 1999) have previously led to a discussion on methods in ecological energetics (Wightman 1981, McEvoy 1985) which has had no impact in the insect–plant literature. Here we took a physiological perspective and analysed a number of studies published over the last 12 years by focussing on the R-term of the budget that cannot be checked gravimetrically. A second serious constraint of gravimetry is of a physiological nature, due to the fact that plant material needs to be excised for accurate measurements to be taken. This issue will be addressed below.

Physiological analysis of published variation in metabolic efficiency

We analyzed gravimetry-based papers on herbivorous insects from a physiological perspective. The method we employed was to plot the relative metabolic rate as a function of the relative growth rate according to the equation:

$$RMR = RMR_M + (c \times RGR) \qquad (2)$$

where RMR is the relative metabolic rate, calculated using the R-term of budget Eq. 1 divided by the mean dry body weight and the total number of experiment days; RMR_M is the relative rate of maintenance metabolism; RGR is the relative growth rate, calculated based on the G-term of budget Eq. 1, divided by the mean dry body weight and the total number of experiment days; and c is a coefficient, termed the net cost of growth (Wieser 1994). This approach aims to partition the energy expenditure over maintenance and growth by using rate expressions for metabolism and growth, analogous to a budget equation commonly used for other groups of organisms (Wieser 1994, Peterson et al. 1999). We expect a linear relationship between RGR and RMR, with the intercept at the ordinate to represent the rate of maintenance metabolism. The slope of the line reflects conversion efficiency which may vary due to differences in food quality, but the intercept is expected to be similar at similar temperatures. Relevance of this equation is based on the following observations (van Loon 1991, Wieser 1994, Peterson et al. 1999): (1) the demonstration that anabolism resulting in growth is the major energy requiring process in fast growing, nonreproductive and relatively sedentary poikilothermic herbivorous larvae and (2) the values of maintenance metabolism are similar among herbivorous larvae subject to similar temperatures, as deduced from respirometric methods (below). The next step we took was to compare rate values obtained on artificial diets with those obtained for the same species feeding on plant material. This allowed us to rule out plant respiration as a major confounding factor in the estimation of food consumption, the largest budget parameter (below). Results are presented graphically in Fig. 2 for 3 species of lepidopterous larvae. In each scatterplot, the relationship between RMR and RGR has been plotted for larvae feeding on artificial diet and plant food. The experiments analyzed studied the effect of nutritional quality of different plant species, either by continuous feeding on a single plant species or after switching between plant species, of plant or leaf age, light intensity, atmospheric carbon dioxide concentration or soil nitrate levels, on metabolic efficiency.

For the artificial foods, trendlines have been plotted by least mean squares regression, with an intercept at the ordinate set to a RMR value of 0.1 mg mg^{-1} dry body weight day^{-1}. This value corresponds to the intensity of maintenance metabolism and was calculated from typical respiration rates at temperatures in the range of 20–25°C of nonfeeding lepidopterous larvae, assuming carbohydrates (average caloric content 16.5 J mg^{-1}) are used (van Loon 1991, below). The slope of the line represents the coefficient c in Eq. 2. It is apparent from Fig. 2 that for the artificial, non-respiring foods the

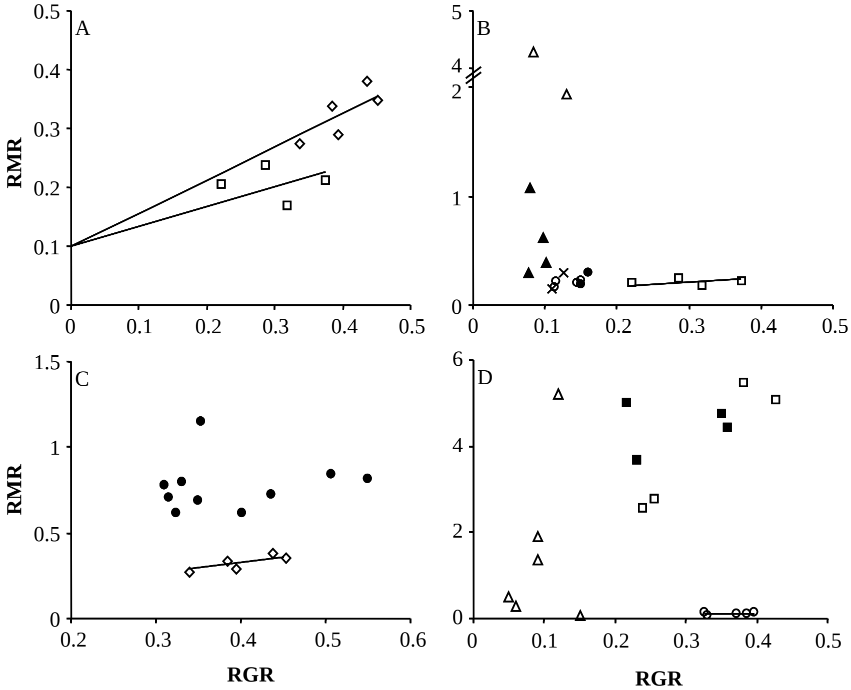

Fig. 2. Scatterplots of relative growth rate (RGR) vs relative metabolic rate (RMR), both expressed as mg dry weight g^{-1} dry body weight day^{-1}, based on published values. (A) For two caterpillar species, the gypsy moth *Lymantria dispar* L. females (□) and the southern armyworm *Spodoptera eridania* (◇) feeding on artificial diets during their final instar. Data from Karowe and Martin (1989) and Stockhoff (1992). Least square means regression lines were calculated with the ordinate intercept set at 0.1 (text). Each point represents the mean of an experimental treatment. (B) For the gypsy moth *Lymantria dispar* L. feeding on host plant: aspen from Kinney et al. 1997 (▲) and Lindroth et al. 1993 (△); maple from Kinney et al. 1997 (○) and Lindroth et al. 1993 (●); oak from Lindroth et al. 1993 (×). The least square means regression line for the artificial diet data (Stockhoff 1992) is also shown (□). Each point represents the mean of an experimental treatment. (C) For the southern armyworm *Spodoptera eridania* feeding on six different host plant species among which two chemotypes of *Lotus corniculatus* at two plant ages (calculated from data of Scriber 1978, 1981, 1982 (●)). Each data point represents the means for a host plant or a host plant chemotype and age. The least square means regression line for the artificial diet data (Karowe and Martin 1989) is also shown (◇). (D) For the forest tent caterpillar *Malacosoma disstria* feeding on an artificial diet for the first 70 h of the final instar (○) (Karowe 1989) and on three host plants (aspen, oak and maple) from Levesque et al. 2002, at 18 and 24°C in 1997 (□) and 1998 (■), and from Lindroth et al. 1993 (△) at ambient and elevated atmospheric carbon dioxide levels. The least square means regression line for the artificial diet data (Karowe 1989) is shown. Each point represents the means of an experimental treatment.

relationship between RGR and RMR matches the physiological expectation of a linear relationship whereas the data obtained for plant foods show considerable scatter for RMR and show no such relationship. As will be elaborated below, neglecting plant respiration is the most likely factor causing an overestimation of RMR, whereas random error in the determination of dry matter content of plant material is responsible for huge variability in RMR.

Variability in insect metabolic rates, poikilothermy and physiological realism

Calculation of the relative metabolic rate (best expressed in calories per unit of dry body weight per unit of time, but in the majority of studies expressed in dry matter units respired per unit of dry body weight per unit of time) is especially informative as this rate is constrained physiologically due to the poikilothermy of insects, implying that metabolic rates are strongly dependent on environmental temperature. Most studies in insect–plant nutritional ecology deal with larval insects that display sedentary habits on their food plant and spend little time and energy on locomotion. Based on discontinuous respirometric data, increase in respiration rate, by a factor of 1.5–5 for caterpillars, has been ascribed to the muscular activity of feeding itself or to an increase in digestive activity following feeding (Aidley 1976, McEvoy 1984, Kukal and Dawson 1989). However, during continuous flow-through indirect calorimetry, respiration rates declined prior to pupation when feeding had ceased and wandering behaviour had commenced, suggesting that digestive and anabolic activity require greater amounts of energy than locomotion in caterpillars (van Loon 1993). In locusts the calorigenic effect of feeding, observed as a factor of 3–4 increase in respiration, seems unassociated with energetic costs of digestion and absorption and has been ascribed to a state of neural arousal (Gouveia et al. 2000). Through continuous measurement of respiration integrated heat production can be obtained (indirect calorimetry), which allows the transient increases in metabolic rate that contribute to total metabolic expenditure during an entire developmental phase to be assessed. For caterpillars feeding on leaves, a significantly smaller variability in the calculation of total energy expenditure and metabolic efficiency was found using indirect calorimetry when compared with gravimetry, even though corrections for plant respiration were applied (van Loon 1993).

Calculated relative metabolic rates differ by a factor of 3–5 in many studies, approaching a factor of 10 in some studies where food quality was the main experimental factor. In view of the poikilothermic constraints to which insects are subjected, a 2–3-fold increase in relative metabolic rate, sustained over days, would require an increase in ambient temperature of 10°C, according to the Q_{10}–rule, derived from a substantial body of physiological literature on insect metabolism (Hoffmann 1985, Casey 1993). Comparisons at a single constant temperature in the range of 15–30°C are typically employed in nutritional studies on larval insects. The value for Q_{10} based on respirometric measurements on the tent caterpillar *Malacosoma americanum* is 2.5 (Casey and Knapp 1987; temperature range 15–25°C); in the same range of temperatures, highly variable Q_{10} values (0.9–2.3) arise from gravimetrically determined RMR for the tent caterpillar *M. disstria* and show no consistent temperature dependence (Levesque et al. 2002). In addition, it should be realised that physiological variation will be higher when expressed as relevant caloric equivalents, than expressed as dry matter equivalents, as a milligram of dry matter may represent a value between 16 J (carbohydrate oxidation) or 39 J (lipid oxidation). Physiological mechanisms regulate feeding behaviour, absorption and egestion such that, within certain limits, the body composition at the end of the growing phase consists of specific proportions of proteins, lipids and carbohydrates, which can be visualized as a target point in nutritional space (Raubenheimer and Simpson 1999, Lee et al. 2002). The energetic cost of producing insect dry matter body growth with a typical caloric content of 22–24 J mg^{-1} (Southwood and Henderson 2000) has been estimated at 8.5 J mg^{-1} for caterpillars in a size range of 45–110 mg, taking maintenance respiration into account (van Loon 1991). This value corresponds with the narrow range reported for ectotherms belonging to different animal classes (7–9 J mg^{-1} dry body weight; Peterson et al. 1999). This leads us to conclude that the amount of energy spent per unit of growth is remarkably constant and thus that variation in energetic efficiency of growth will depend mainly on the contribution of maintenance metabolism over the growth period.

Plant respiration: a major source of error in gravimetric studies

Based on the insect physiological analysis presented in the previous sections, it follows that data on energetic efficiency obtained along gravimetric methods are susceptible to systematic errors in absolute values. In this section we argue that plant respiration is the major source of these errors. The measurement of consumption, the largest budget item, in dry matter weight units seems a straightforward task, by establishing the amount of food that has disappeared after a pre-determined feeding period, but in reality this is not the case. Waldbauer (1968) already pointed to several sources of error inherent in the gravimetric determination of consumption. Sources of random and systematic errors in practising gravimetry have been outlined in detail by several authors (reviewed by van Loon 1991, Bowers et al. 1991). The extent of inaccuracies was explicitly noted for the first time in studies with artificial diets (Schmidt and Reese 1986). The excess of food left at the end of the experiment, combined with small errors in the estimation of dry matter content of the food offered (which can only be determined using aliquots) was shown to cause exaggerated errors in the estimation of consumption and consequently in the calculation of nutritional indices. Methodological problems are aggravated when insects feeding on living plant tissues are studied. Plant organs are inherently spatially and temporally variable, thereby invalidating assumptions on homogeneity and constancy of dry matter distribution. Moreover, plant tissues continuously respire, resulting in a decrease in dry matter content that cannot be ascribed to feeding. However, actively photosynthesising and growing tissue might partly compensate for dry matter loss (below). Waldbauer (1968) proposed a correction factor for respiratory dry matter loss, which has largely been neglected. In addition, Axelsson and Ågren (1979) noted that Waldbauer's advice on frequent changing of plant food to prevent detoriation, due to water loss and breakdown of nutrients over the course of the experiment, will increase the magnitude of errors. They proposed a correction factor for plant respiration that takes all relevant parameters into account: (1) the amount of food left over relative to the amount initially offered; (2) the respiration rate of the food; (3) the growth rate of the insect; (4) the total duration of the experiment and (5) the duration of the active feeding period. When feeding by the insect is deliberately interrupted to standardize the feeding period, the gut still contains food and this can lead to considerable error in estimating nutritional indices (Bowers et al. 1991). All these factors compromise accuracy when measuring the metabolic effciency of an insect herbivore feeding on respiring plant tissue. The magnitude of errors increases exponentially with increasing food excess (Axelsson and Ågren 1979, van Loon 1991). One could argue that such errors are only systematic in nature, and still allow the nutritional value of different plant foods to be compared reliably. This argument is incorrect because maintenance respiration differs between plant species, between leaves of varying age within an individual plant and is affected by temperature and light intensity (by a factor of 1.5–3.0; Penning de Vries 1975). Plant respiration rate can also be significantly increased in response to insect feeding damage (Lambers 1985, Zangerl et al. 1997). Failure to implement these necessary corrections may well lead to fortuitous conclusions on differences in nutritional quality which in fact result from differences in the aforementioned parameters (van Loon 1991). When an insect is feeding on old or young leaves of the same plant which differ by a factor of 1.5 in their respiration rate, the corrected values of ECD (above) differ by 10–20 percent when one third or two thirds, respectively, of the initial dry weight of leaf material is left at the end of the experiment. Examination of papers published over the last 12 years in an attempt to track the consequences of those papers that addressed methodological problems inherent to gravimetry, clearly show that they have not noticeably influenced this research field. Very few papers account for plant respiration or mention specific precautions to reduce errors by preventing excess food at the termination of the experiment, and none of the papers presents an error analysis. Many papers present only derived data or the results of statistical analyses making it impossible to calculate the basic budget items required to arrive at rate parameters. Advice that follows from these findings is that the excess of food offered should be minimized. However, it seems there is a limit to which this can be done without compromising consumption and growth (Stamp 1991). A straightforward way to account for these problems is to apply corrections for plant respiration (Axelsson and Ågren 1979, Candy and Baker 2002). Overestimation could be ruled out with an appropriate correction for plant respiration, as in the case of *Pieris brassicae* caterpillars feeding on excised leaves (van Loon 1993). Yet such corrections do not alleviate the constraints addressed in the next section.

Bringing plant ecophysiology into the picture: photosynthesis and phyllosphere microclimate

Gravimetry requires the excision of the leaf or other plant organ to enable accurate measurements of food intake, especially in the case of individual insects. Excision leads to rapid and significant changes in the physiological condition (e.g. turgescence) and biochemical composition of the plant organ offered as food (Bowers et al. 1991). Further, there are many other poorly studied phytochemical changes that are known to affect herbivore performance (McCaffery 1982). More importantly, however, physiologically realistic measurements of the nutritional quality of living plant tissue to an insect herbivore should be performed under natural light intensity, allowing for photosynthesis to occur. This condition is not met in any of the nutritional studies published to date. Insect herbivory has been shown to affect photosynthetic rate in the majority of cases, by either increasing or decreasing this rate, dependant upon the herbivores' mode of feeding (Welter 1989). These effects cannot in all cases be mimicked by mechanical damage and depend upon the insect species (Peterson et al. 1998). A recent paper by Zangerl et al. (2002) demonstrated that within a single leaf, the leaf area that displayed a reduced photosynthetic rate in response to herbivory was six times larger than the area removed by feeding. The exclusion of photosynthesis in gravimetric insect–plant nutritional studies seriously constrains the ecological relevance of published data. These observations have profound implications for our understanding of the dynamics of energy flow in insect–plant interactions and their associated food webs in nature. Generally, food consumption by insect herbivores has been overestimated with the exclusion of plant respiration. This means that their food conversion efficiency is higher than hitherto published, or, conversely the amount of plant biomass produced through photosynthesis that insect herbivores actually remove is lower than calculated based on extrapolations of experimental data from individual insects. We conclude that the exclusive use of gravimetry to quantify food utilisation and metabolic efficiency on plant foods should be considered obsolete.

There is another factor intimately associated with normal plant metabolism. Plants provide insects with a specific microclimate because they photosynthesise and transpire. Direct measurement and simulation of the leaf energy budget has shown that the temperature of the leaf surface can be higher or lower than the air temperature depending on climatic conditions and leaf evaporation rates (Campbell and Norman 1998). For example, on cool clear days, the leaf temperature of apple can reach 25°C while the air temperature is 15°C. Conversely, on hot days when the air temperature is 39°C, the leaf temperature may be 26°C (Ferro and Southwick 1984). Thus, an insect resting on the leaf surface can benefit from more favourable temperature conditions prevalent in the phyllosphere, and is expected to have a metabolic rate that differs from that predicted on the basis of air temperature only in the case of thermoconformers. Thermoregulators may greatly benefit from microclimatic gradients (Casey 1993). Moreover, the leaf surface is enveloped by a phyllosphere boundary layer of relatively still air where air currents have little effect and relative humidity levels can be markedly different from ambient (Willmer 1986, Campbell and Norman 1998). The boundary layer thickness can range from several micrometers to more than 10 mm. For example, relative humidity within this boundary layer can be 20% higher than ambient during the day in summer for leaves of cabbage (Willmer 1986). Such differences can clearly affect insect water balance and thus their metabolic rate.

However, little is known about how phyllosphere microclimate is affected by herbivore feeding. Herbivore activity either positively or negatively affects photosynthetic rates (Welter 1989, Zangerl et al. 2002, Raimondo et al. 2003) but also most of the parameters in the energetic budget of the leaf such as leaf size and stomatal conductance. These factors may cause changes in the thickness of the boundary layer. Stomata may be closed due to the physiological stress induced by feeding damage, leading to lower water vapour loss. These changes in the leaf energy budget may in turn have marked effects on herbivore metabolism through changes in surface temperature and relative humidity within the boundary layer. These plant physiological responses are absent or attenuated in excised leaves.

Towards improved methodology

We consider it important to resurrect the nutritional ecology of plant-feeding insects for reasons put forward in the introduction. To achieve this, only a combination of techniques, several of which have been optimised over the past 10 years, will ensure a methodologically sound approach. These include radiotracer, indicator and isotope methods (Southwood and Henderson 2000) and real-time gas exchange measurements. Biochemical radiotracer techniques are available that allow quantitative allocation of specific classes of nutrients, such as amino acids, carbohydrates and lipids, to reproduction and maintenance. This allows a dissection and physiologically relevant understanding of nutrient allocation strategies when compared with the commonly used overall dry matter conversion (Rivero and Casas 1999, Rivero et al. 2001). The use of the doubly labelled water method (Kam and Degen 1997) offers the possibility to study carbon dioxide production of organisms under natural foraging conditions, in which the condition of plants as a food source can be studied under realistic ecophysiological conditions. This technique, developed first for vertebrates, has been validated and applied successfully to freely foraging bumble bees (Wolf et al. 1996), although it has limitations in measuring inter-individual variability and it is unsuitable for real time recording (Butler et al. 2004). Technologies and methods commonly employed by plant ecophysiologists offer considerable potential for the development of combined, innovative approaches to quantify matter and energy budgets of plants and plant-feeding insects. Infrared gas analysis (IRGA) can be combined with gravimetric methods to minimize inherent random and systematic errors. Portable machines equipped with IRGA are available and allow simultaneous and real time recording under field–conditions of 1) respiration rate of the insect feeding on a leaf (i.e. production of CO_2), 2) net CO_2-assimilation rate of the leaf and 3) transpiration rates of the insect and the leaf. The insect feeding on the leaf which is attached to the plant is enclosed within a chamber. Gravimetry can be employed to monitor insect growth intermittently. Measurements of CO_2 concentration in the leaf chamber can be made when the insect is feeding on the leaf and can be resumed immediately after removal of the insect to determine the rates and dynamics of insect and plant metabolism. Climatic conditions within the leaf chamber (light, temperature, humidity and CO_2 concentration) can be tightly controlled. The IRGA method is most useful to quantify and dissect photosynthetic and metabolic rates of plant and insect intermittently. Care must be taken to avoid clip cage effects (Crafts and Chu 1999). IRGA measurements should be combined with the doubly labelled water method for longer term quantification of metabolic rates.

Conclusions

In this paper we have argued that interpretation of the substantial body of literature on nutritional and metabolic efficiencies published for herbivorous insects is problematic due to persistent methodological problems. Dry matter budgets which have not been checked by independent methods have led to hypotheses on energy allocation patterns which are physiologically improbable and cannot be rigorously tested. It is necessary to account for plant respiration, which differs between plant species and depends on environmental conditions. In our opinion, it is mandatory for workers in this field to adopt new techniques to achieve a correct quantification of metabolic expenditure and energy fluxes through plant based food webs. Sound nutritional ecology can only be based on sound methods. The first study on the metabolic efficiency of a plant feeding insect on an intact, photosynthetically active plant has yet to be performed.

Acknowledgements – We thank Suzanne Blatt, Steve Simpson and John Reese for their useful comments on drafts of this paper. We thank the Centre Nationale de la Recherche Scientifique for a Poste Rouge position granted to JJAvL.

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