Journal of Insect Physiology 69 (2014) 35–40

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Regulation of water and macronutrients by the Australian plague locust, *Chortoicetes terminifera*

Fiona J. Clissold [a,b,*], Helena Kertesz [a], Amelia M. Saul [a], Julia L. Sheehan [a], Stephen J. Simpson [a,b]

[a] *School of Biological Sciences, A08, The University of Sydney, NSW 2006, Australia*
[b] *Charles Perkins Centre, The University of Sydney, NSW 2006, Australia*

ARTICLE INFO

Article history:
Available online 27 June 2014

Keywords:
Water
Protein
Carbohydrate
Nutrient balancing
Geometric framework
Insect–plant interactions

ABSTRACT

Nutritional outcomes for animals are best understood when the intake of multiple nutrients are considered together. The requirements for protein and carbohydrate and the consequences for development, growth and fitness when confined to sub-optimal amounts and ratios of these nutrients are well known for many herbivorous insects. Water is also essential for life, and it is known that herbivorous insects will actively ingest free water, have physiological mechanisms controlling thirst, and suffer fitness consequences if water is excessive or deficient in the diet. As herbivorous insects are thought to obtain the majority of their water from foliage, which can vary in protein, carbohydrate and water content, we investigated if the Australian plague locust, *Chortoicetes terminifera*, can select among complementary foods to attain a target intake across these three nutrient dimensions. Locusts demonstrated selection behaviour for protein, carbohydrate and water by eating non-randomly from different combinations of complementary foods. A ratio of $P:C:H_2O$ of 1:1.13:13.2 or 1(P + C): 6.2 H_2O was ingested. Given that locusts strongly regulate water intake, and its importance as an essential resource, we suggest future studies consider the single and interactive influences of water, protein and carbohydrate, when evaluating herbivorous insect host choice and foraging decisions.

1. Introduction

Nutritional outcomes are best understood when the intake of multiple nutrients are considered together (reviewed Behmer, 2009; Simpson and Raubenheimer, 2012). For example, in herbivorous insects rates of development, size at maturity, longevity and reproductive output are affected by the ratio and concentration of protein and carbohydrate ingested (e.g. Joern and Behmer, 1997; Lee et al., 2008; Simpson et al., 2004). Water is also essential for life, and herbivorous insects suffer reduced growth and slowed rates of development when water is over- or under-ingested when combined with other nutrients (e.g. Raubenheimer et al., 2005; Scriber, 1977, 1979; Simpson et al., 2004; Timmins et al., 1988; Van't Hof and Martin, 1989b).

Water is gained by terrestrial insects through the ingestion of food, by drinking free water, and from the catabolism of sources of energy ('metabolic water'), and is lost via transpiration, respiration and excretion (reviewed Bernays, 1990; Chown et al., 2011; Cohen, 2013; Harrison et al., 2012). In most if not all insects, metabolic water is insufficient for requirements, and water balance appears to be maintained primarily through food choice, drinking free water and by post-ingestive mechanisms (reviewed Bernays, 1990; Dow, 2013).

The ingestion of food and water are tightly linked (Bernays, 1990; Lewis and Bernays, 1985; Raubenheimer and Gäde, 1994; Roessingh et al., 1985). Meal size and intermeal duration are influenced by both free water availability and leaf moisture content (Baines et al., 1973; Ben Halima et al., 1983; Clissold et al., 2006; Raubenheimer and Gäde, 1994). Allowing the Australian plague locust, *Chortoicetes terminifera* Walker (Acridiae, Acridinae) to regulate water intake through the availability of free water increased the extraction efficiency of carbohydrates from the leaves of Mitchell grass (Clissold et al., 2006), and by increasing water intake *Drosophila* were able to minimize the deleterious effects of the consumption of excessive protein on lifespan (Ja et al., 2009).

The intake of nutrients (e.g. protein and carbohydrate) and water are primarily controlled by haemolymph factors (Bernays, 1990; Raubenheimer and Gäde, 1993, 1994; Simpson and Raubenheimer, 1996). It is thought feeding influences drinking behaviour through a direct effect on the osmotic pressure of the haemolymph and through volumetric changes mediated by blood volume, both factors which are influenced by fluxes of water and

---

* Corresponding author at: School of Biological Sciences, A08, The University of Sydney, NSW 2006, Australia. Tel.: +61 418 580 498.
E-mail address: fiona.clissold@sydney.edu.au (F.J. Clissold).

http://dx.doi.org/10.1016/j.jinsphys.2014.06.011
0022-1910/

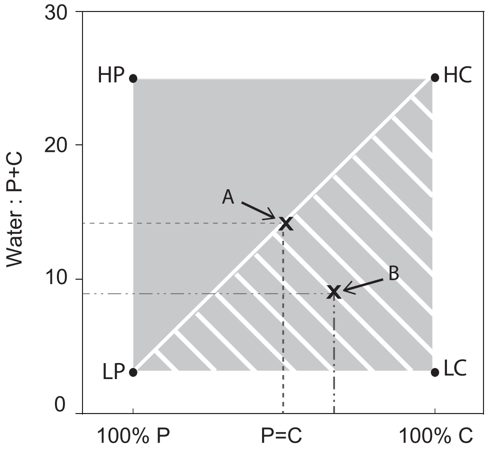

Fig. 1. Diagram illustrating the relative geometric nutrient and water space available to the locusts on treatments (1; the grey box) and (2; the white hatched triangle). The points labelled A and B are the ratios of protein (P):carbohydrate (C):water that the locusts would consume if they ate randomly from the foods available in each treatment. Locusts on treatment 1 have access to all four diets, (HP, LP, HC, and LC), while locusts on treatment 2 had access to diets LP, HC, and LC, where H and L refers to amount of water, high and low, respectively relative to the amount of nutrient (P or C).

solutes across the wall of the digestive tract and Malpighian tubules (Barton Browne and van Gerwen, 1976; Bernays, 1977, 1990; Raubenheimer and Gäde, 1993, 1994). How the physiological systems that regulate nutrients and water interact remains unclear. Increased intake of water and decreased intake of wet food by locusts has been recorded in response to increased haemolymph osmotic pressure (Bernays and Chapman, 1974; Barton Browne and van Gerwen, 1976; Bernays, 1977; Roessingh et al., 1985). Consequently, if free water is unavailable, feeding rates are retarded and locusts would not eat to gain water. Our aim was to discover whether *C. terminifera* is able to regulate intake of protein (P), carbohydrate (C) and water simultaneously when offered several complementary foods differing systematically in these three food dimensions. Like other locusts (e.g. Chambers et al., 1995), *C. terminifera* regulates both protein and carbohydrate to an intake target (*sensu* Raubenheimer and Simpson, 1993; Simpson and Raubenheimer, 1995) when offered a choice between dry foods varying in the ratio of P and C and with water provided separately (Clissold et al., 2009). The question is whether they can regulate P, C *and* water by food selection when these are combined within complementary foods.

To answer this question, we presented *C. terminifera* nymphs with two combinations of foods, such that if the locusts fed randomly among offered foods they would have consumed markedly different amounts and ratios of protein, carbohydrate and water (Fig. 1). Treatment 1 consisted of four foods, two protein (P) and two carbohydrate (C) sources, one high (H) and the other low (L) in water (i.e. HP, LP, HC and LC). Treatment 2 comprised three of these foods – LP, HC and LC.

2. Materials and methods

2.1. Locusts

Stock cultures of *C. terminifera* originally collected from outbreaks in southern New South Wales (February, 2005), north eastern Victoria (December, 2005), and southern Western Australia (October, 2006) were reared at The University of Sydney. Locusts were reared at a density of ca. 200 in fine mesh cages (30 × 30 × 30 cm) under a 14L:10D photoperiod in a room maintained at 32 °C. During the 'light-on' phase a 60 W heat lamp placed behind the cage allows the locusts to thermoregulate. Seedling wheatgrass and wheat germ were provided *ad lib.* daily.

2.2. Diets

Four chemically defined foods were prepared that differed in the ratio of protein (P) and carbohydrate (C) to water. The dry portion of the diets consisted of 64% protein or carbohydrate with 4% salts, sterols and vitamins and 32% indigestible cellulose. Protein consisted of a 3:1:1 mix of casein, bacteriological peptone and egg albumen, and carbohydrate was a 1:1 mix of sucrose and dextrin, as described in Simpson and Abisgold (1985). The dry portion of food was mixed with a 1% agar solution in the ratio of 1:3 [low water (L)] or 1:20 [high water (H)], giving a final ratio of protein or carbohydrate to water of 1:4.6 and 1:31.3, respectively. Diets were made fresh daily and while still liquid were poured into small (ca. 1 cm diameter) lids and allowed to set. Lids with a small diameter were used to minimize water loss from the surface of the food. The two concentrations of water used represented the extremes likely to be encountered in foliage. Water varies in leaves from ca. 50–90%, equivalent to a ratio of dry matter of between 1 and 9 parts water (Scriber and Slansky, 1981). However, for most foliage chewing insects not all of the leaf dry matter is digestible, with the macronutrients, protein and carbohydrate, accounting for approximately 30–60% of the dry matter (Van Soest, 1994). Therefore, the potential range of the ratio of nutrients [30% (60%)] to water is between 3.3 (1.7) and 10 (30). Typically, leaves low in water are also low in N, thus we made diets with the ratio of nutrient to water being ca. 3 and 30.

2.3. Experimental design

Twenty freshly moulted (within 3.5 h) 5th instar male and female nymphs were allocated to one of two treatments (i.e. each treatment consisted of 5 male and 5 female locusts). Each locust was confined alone within a clear plastic box (17 × 12 × 6 cm, $l \times w \times h$) with their treatment foods and a metal perch. The plastic boxes were housed within an incubator maintained at 32.3 °C (min–max: 31.1–33.0) with a 14L:10D photoperiod. Air was pumped through water containing a saturated solution of NaCl which maintained the humidity within the incubator and the plastic boxes at an average of 76.3 RH (min–max: 70.1–79.9).

Food was provided in two different combinations; these being Treatment 1, all four foods, and Treatment 2, LP, LC, HC (i.e. the HP food was omitted). Locusts were provided with fresh diets made daily. Four controls were set up for each diet daily as described above, except that the locust was omitted to determine the actual amount of water in each diet and water loss. Intake of each nutrient and water was determined daily by estimating the amount of dry food ingested (Eq. (1)) and then using this value to calculate protein and carbohydrate intake ($I \times 0.64$) and water as described below (Eq. (2)). Thus

$$\text{dry matter intake } (I_{\mathrm{DM}}) = F_i - F_r; \tag{1}$$

where F_i is the amount of dry food provided initially, estimated from a regression of dry wt. vs wet wt. based on the control samples taken daily, and F_r is the food remaining after drying the diets to a constant mass (60 °C for 48 h).

The amount of water ingested daily (I_W)

$$I_W = (1 - W_{\mathrm{L}}/2) \times I_{\mathrm{DM}} \times \text{Water/DM}; \tag{2}$$

where W_{L} = water loss [(water at $t = 0$ – water remaining at $t = 24$ h)/water at $t = 0$], Water = WM – DM, where WM = wet mass and DM = dry mass of food at time $t = 0$. See the Section 3 for the logic of this equation.