The Role of Primary Nutrients in the Foraging Ecology of
the Forest Tent Caterpillar (*Malacosoma disstria* Hübner)

Meghan Noseworthy

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Abstract

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Levels of primary nutrients (protein and carbohydrate) and secondary metabolites

vary both within and between trembling aspen trees (*Populus tremuloides*), and affect the

feeding behaviour and performance of forest tent caterpillars (*Malacosoma disstria*). The

relationships between primary nutrient content, insect performance and dietary preference

were studied using both artificial and natural diets. In experiments with artificial diets of

controlled nutritional content, compensatory feeding abilities were tested. Caterpillars

were deprived of either protein or digestible carbohydrate to determine whether they

exhibit compensatory dietary self-selection when given a choice between foods

containing previously absent nutrients. In experiments using natural foods, caterpillar

performance and choice behaviour were tested using leaves treated with different

nutrient-boosting supplements: protein, carbohydrate, and a neutral control. Caterpillars

appeared to regulate for carbohydrate deficiencies only. When given a choice between

artificial carbohydrate- or protein-only diets, caterpillars took less time to contact the

carbohydrate diet, and consumed more upon first contact, suggesting an olfactory and

phagostimulatory response to carbohydrate. In the absence of choice, caterpillars ate

most and gained the most weight on a diet containing both protein and carbohydrate.

When given a choice between leaves, caterpillars showed no initial preference, but with

prolonged exposure ate more of the control leaf, followed by the carbohydrate leaf.

Under no-choice conditions, caterpillars on the control leaf experienced the shortest instar

duration and greatest growth rate, while on the carbohydrate leaf they exhibited increased survival. These findings suggest that carbohydrate concentration is the principal factor in forest tent caterpillar food choice.

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I would like to dedicate this thesis to my friend Sheldon Gill McDonell (1974-1996).

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Chapter 1
General Introduction

The first documented herbivorous insect outbreaks occurred thousands of years ago when locusts ravaged crops, often with devastating human consequences. Only in the last century have we begun to understand insects' impact on and interaction with natural vegetation (Hodkinson and Hughes 1982). Widespread outbreaks of herbivorous insects can have harmful and irreversible effects, especially in temperate forested areas of low diversity, and insect outbreaks threaten commercial forest industries, endanger public sanctuaries and can drive other organisms from their temperate forest habitats. However, forest pest outbreaks occur in cycles which allow most trees to recover from the defoliation effects. Defoliation can also benefit trees: the removal of excess foliage can stimulate foliar production, and foliage passing through the insect gut is broken down into a form that rapidly releases nutrients to the soil (Leonard 1981) where they are again available to trees. Central to this web of complex interactions is the basic relationship between an herbivorous insect and its food source: a fine-scale knowledge of an insect's food choice behaviour and performance on different diets can help in the development of models that predict outbreak potential, and aid in the design of pest management programs.

Each herbivore, in the course of its evolution, becomes associated with a particular plant or a group of plants while excluding others (Dethier 1982). When Lepidoptera (moths and butterflies) first appeared during the Cretaceous period, they fed on mosses, liverworts and pollen (Carpenter 1977; Strong et al. 1984). The

arrival of angiosperms (flowering plants) enabled Lepidoptera to prosper and radiate: today they make up 35% of all insect species (Smart and Hughs 1973; Southwood 1978). This thesis focuses on the dietary ecology of the forest tent caterpillar (*Malacosoma disstria* Hübner), a Lepidopteran of the Lasiocampidae family.

The forest tent caterpillar is a deciduous forest pest common to south-eastern Canada and most of the United States (Lindroth and Bloomer 1991). Reproductively mature moths eclose from their cocoons in late spring or early summer, females secrete a pheromone which attracts the males and mating takes place within hours of the female eclosion. Oviposition occurs soon after mating: the female disperses to find a suitable host plant twig on which to lay her single egg mass, containing 150-450 eggs. Within few weeks fully formed caterpillars are found within their eggs, there they stay until the following spring. The obligatory period of diapause is broken by the long exposure to cold. In the early spring (in North America the first week of May) the caterpillars emerge from their eggs as the buds on their natal host plant are opening. The new siblings cluster on the surface of the egg mass until most have eclosed and then set off in search of food and an aggregation site. Forest tent caterpillars are social species and the first three of the 5 or 6 instars are spent together. The duration of the larval stages, which varies according to climatic conditions, usually lasts about 7 to 8 weeks, the mature caterpillars seek pupation sites under overhangs or protected areas and spin a cocoon, they then metamorphose to the pupal stage and emerge as adult moths within two weeks. In the larval stage, forest tent caterpillars are active foragers and move in loose processions from aggregation site to feeding sites many times a day, traveling single- file or several side by side

(Fitzgerald 1995). These polyphagous herbivores prefer to eat trembling aspen (*Populus tremuloides* Michx.), but are also known to feed on sugar maple (*Acer saccarum* Marsh) during epidemics in south-eastern Canada (Panzuto et al. 2001). Outbreaks occur at 6-16 year intervals and may persist for two to six years (Batzer et al. 1995), during which time the caterpillars may defoliate thousands of acres of broadleaf trees (Lindroth and Bloomer 1991) resulting in smaller leaves, higher twig mortality, reduced tree growth and in severe cases, tree death (Duncan and Hodson 1958). The reduction in radial stem growth that can be attributed to severe outbreaks approaches 90%. A great deal of evidence suggests that trees weakened by heavy and repeated defoliation may be more vulnerable to disease, attack by secondary pest (including boring) insects, and fungal infestation. Interest in the forest tent caterpillar's effect on *Populus* species has increased due to aspen's importance to the wood pulp industry (Britt 1970). In 1991, during the peak of an outbreak in Ontario, Canada, this pest defoliated more than 18 million hectares of broadleaf trees (Anonymous 1994; Nicol et al. 1997).

To develop population models for herbivorous animals, food-plant nutritional and defensive characteristics must be considered (Rhoades 1983). Past forest tent caterpillar research has focused on the effects of the two dietary categories, primary nutrients and secondary metabolites. Primary nutrients are proteins and carbohydrates, essential compounds which play critical roles in metabolic processes. The main secondary metabolites of aspen are phenolic glycosides (e.g. salicortin, tremuloidin, tremulacin and salicin), compounds that are found in plant tissues but which do not appear to be critical to basic plant physiology (Palo 1984). Secondary

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metabolites often possess deterrent odours or tastes, and many are toxic to insects, causing injury or death in higher concentrations. We are interested in how the forest tent caterpillar responds to differences in the primary nutrient content of a food source. In the spring, when levels of primary nutrients are high, do the caterpillars make decisions based on the primary nutrient content of their leafy food source? This study focuses on the effects that primary nutrient content of both natural and artificial diets have on the foraging behaviour, growth, development and survival of forest tent caterpillars.

All calories, protein and other nutrients must be obtained from the host plant in order for an herbivore to successfully survive and reproduce (Bentley and Johnson 1991). Some plant tissues offer a better food source than others, in terms of energy and nitrogen content (Hodkinson and Hughes 1982), the primary nutrients under study here. The fresh weight of leaves contains approximately 90% water, 1 to 3 % protein, the balance being structural and digestible carbohydrate compounds (Hodkinson and Hughes 1982). Digestible or total non-structural (TNC) carbohydrates include sucrose, hexose and starch (Hemming and Lindroth 1999). Digestible carbohydrates are used for energy production and other metabolic functions, while protein serves mainly to build body tissues (Mattson 1980). Nitrogen is an essential building block of proteins and, although nitrogen is abundant in the earth's atmosphere, available plant nitrogen may be scarce during critical growth periods (Mattson 1980). The protein content is usually measured by nitrogen content and is much higher in insects than it is in their plant food sources. In addition, the available nitrogen is often bound up in plant proteins. Consequently, insects must find ways to access, utilize and

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maintain levels of nitrogen most efficiently. In response to this scarcity, some herbivores have evolved specific behavioural, morphological and physiological adaptations to make the best use of the ambient nitrogen levels of their host plants. Nitrogen has been the target of many herbivore nutritional studies due to its power to limit insect performance.

Carbohydrates are abundant in plants, but only the non-structural carbohydrates provide insects with energy-rich compounds that power metabolism and form the material basis of chitin in the cuticle. Glycogen, a long linear polymer of glucose, is the principal storage form of carbohydrates in insects. It is found in most tissues of the body but is most abundant in the fat body (Nijhout 1994). Many insects use carbohydrates, in the form of digestible sugars, to determine a good food source (Panzuto et al. 2001), while blood sugar levels may be used to regulate intake of food (Bernays and Chapman 2001; Bernays et al. 2004; Thompson et al. 2001).

The ability to detect the nutrient composition of a food source and select for the nutrients required is called dietary self-selection. This feeding behaviour involves a continuous regulation of food intake with frequent shifts between different foods. The two criteria for self-selection suggested by Waldbauer and Friedman (1991) are: (1) that the animal's choice of foods or nutrients is non-random and (2) that the animal benefits from self-selection (i.e., faring as well as or better than they would on a single diet having the same net nutrient content). Self-selection behaviour that compensates for missing nutrients can increase an insect's rate of survival through increased weight gain and accelerated development (Waldbauer and Friedman 1991). Nutritional feedback mechanisms have already been demonstrated, through dietary

compensation experiments, in a variety of outbreaking insects. When presented with a choice between foods, in diet selection experiments, individual insects regulated their intake of different food types to obtain the balance of nutrients they require.

Detection of the nutrients required to self-select a diet requires a mechanism for recognition. Electrophysiological studies of lepidopterous larvae have shown that gustatory organs consisting of groups of receptor cells exhibit specificity for different foliar compounds. The activity of each taste-sensitive neuron may be linked to feeding behaviour, e.g. stimulation of sugar-sensitive neurons often elicits feeding behaviour (Dethier 1982; Ishikawa 1966). Most Lepidopteran insects studied to date possess sugar-sensitive cells, the sensitivity of which can change according to previous meal sugar content (Bernays et al. 2004).

Dietary self-selection is helpful for insects in nutritionally heterogeneous habitats. The nutritional quality of trembling aspen, for example, varies both spatially and temporally. The life cycle of the forest tent caterpillar is synchronized so that the primary feeding stage occurs at the same time as the growth flush of their host (Fitzgerald 1995), when leaves are high in both primary nutrients, particularly proteins (Hemming and Lindroth 1999; Hunter 1991; Miller 1987). The quality of tree foliage also differs spatially, varying between species, among individuals of a species, between branches in a given tree, and between sun and shade leaves within the forest canopy (Denno and McClure 1983). In forests heavily affected by a forest tent caterpillar outbreak, not all host trees are equally defoliated. This phenomenon is believed to be the result of spatial variation in leaf nutrient content (Levesque et al. 2002), and suggests that forest tent caterpillars can differentiate and move between

high and low quality leaves on the same tree. The role of primary nutrients alone in the choice behaviour of these insects has not been closely studied.

In this thesis, Chapter 2 documents experiments that used artificial diets of known nutritional content to investigate whether fourth instar forest tent caterpillars are capable of self-selection. Food consumption, insect growth and foraging behaviour were measured to determine selection and compensation abilities. Additional studies on the compensation ability of second, third and fourth instar insects are outlined in Chapter 3. Natural foods were used in the experiments described in Chapter 4: fourth instar performance and food choice were tested using trembling aspen leaves that were treated with additional primary nutrients.

Chapter 2
Dietary Self-Selection: Do Forest Tent Caterpillars Compensate for Nutrient Deficient Diets?[*]

2.1   Abstract

Several generalist insects exhibit compensatory feeding, a powerful mechanism for achieving an optimal nutrient balance from diverse nutritionally unbalanced foods. We tested whether forest tent caterpillars (*Malacosoma disstria*) deprived of either protein or digestible carbohydrate exhibit compensatory dietary self-selection when given a choice between two artificial foods each containing one of these two nutrients. Insects were conditioned on one of four pre-treatment foods: a protein-only food (P), a balanced protein and carbohydrate food (PC), a carbohydrate-only food (C) and a no-nutrient food (O). Insects were then given a choice between the P and C foods. Insects on the balanced PC food exhibited the highest consumption and growth rate during the conditioning period. At the beginning of the choice period, most caterpillars contacted the C food first and fed for a longer period on the C food, irrespective of conditioning diet. Over the entire duration of the choice period, forest tent caterpillars compensated for carbohydrate deficiency but did not appear to compensate for protein deficiency. In Lepidopterans, changes in haemolymph trehalose concentration due to post-ingestive effects of both

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carbohydrate and protein consumption modulate the taste responses that control feeding behaviour. In the forest tent caterpillar, regulation of trehalose concentration in the haemolymph leads to higher food intake on balanced (PC) than on unbalanced foods (P or C) and to compensation for carbohydrate deficiency. Regulation of protein intake appears to be weaker than in previously studied ground-dwelling generalist caterpillars. For an oligophagous forest folivore that is confined to a single host plant and does not experience great diversity in food nutrient ratios, regulating protein and carbohydrate independently might not be necessary to meet nutritional requirements.

2.2 Introduction

Food quality is central to forest herbivore performance. High protein and digestible carbohydrate content in tree leaves strongly increase the growth of folivorous larvae (Fortin 1994; Scriber and Slansky 1981). Although protein is often believed to be the most important nutrient for growth (Mattson 1980; Slansky and Feeny 1977), a balance of protein and carbohydrate is required due to the different physiological roles of these two essential nutrients: protein for growth and carbohydrate for energy. In nature, leaves vary in nutrient content both within and between trees (Dudt and Shure 1994; Fitzgerald 1995; Lindroth 1991; Lindroth et al. 1987a). It follows that successful herbivore species should be able to select the best quality foliage according to their nutritional needs.

Choice behaviour on natural foods, such as foliage, can be regulated according to several nutritional components of the foods, including protein, carbohydrate as well as micronutrients and allelochemicals. Many animals regulate consumption of primary nutrients independently; a powerful method for achieving a balanced intake from multiple food sources with different nutrient concentrations (Simpson et al. 1995). If the intake of a given nutrient is independently regulated, an animal conditioned on artificial food deficient in that nutrient will later compensate by selecting a complementary unbalanced food that is rich in that previously deficient nutrient (Simpson et al. 1988a; Waldbauer et al. 1984). Independent dietary compensation for both protein and carbohydrate has been demonstrated in several ground-dwelling generalist insects including *Manduca sexta* L. (Lepidoptera) (Thompson and Redak 2000), *Spodoptera littoralis* Boisduval, (Lepidoptera) (Simpson et al. 1988a), *Heliothis zea* Boddie (Lepidoptera) (Friedman et al. 1991; Schiff et al. 1989) and *Locusta*

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migratoria L. (Orthoptera) (Simpson et al. 1988b). After being fed a food source deficient in either protein or carbohydrate, these insects, when presented with a choice between protein-only and carbohydrate-only foods, regulated their intake to compensate for prior imbalances.

We are testing the ability of the forest tent caterpillar (*Malacosoma disstria*) to compensate for deficiencies in protein and carbohydrate. The forest tent caterpillar is a forest folivore that feeds on a narrower range of foods than do the ground dwelling insects for which dietary compensation has been previously shown. This caterpillar is a gregarious outbreaking forest pest in Canada, its main hosts being trembling aspen (*Populus tremuloides* Michx.) and sugar maple (*Acer saccharum* Marsh). It is an early-season feeder whose life cycle is synchronized with host phenology such that the larvae emerge at the same time as the growth flush of their host, when leaves are high in nitrogen and sugar (Hunter 1991; Hunter and Elkinton 2000). During outbreaks, defoliation by the forest tent caterpillar is not random: certain trees and certain parts of trees are significantly more attacked than others, which may be due to the aforementioned variation in nutritional value of foliage (Denno and McClure 1983; Levesque et al. 2002). For instance, forest tent caterpillars have been shown both to prefer sugar maple sun leaves over shade leaves (Levesque et al. 2002), and to grow and develop better on sun leaves (Fortin 1994). Sun leaves have higher concentrations of both nitrogen and digestible sugars (Levesque et al. 2002). It seems likely that these differences in nutrient content contribute to the preference for sun leaves; however, the way in which forest tent caterpillars use nutrient composition to regulate food choices is not clear.

Regulating the intake of a given nutrient requires mechanisms to detect the presence of the nutrient in food sources, to monitor physiological requirements for the nutrient and to couple food selection behaviour to internal state (Simpson and

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Raubenheimer 2001). Forest tent caterpillars, like most Lepidopteran larvae, have sugar taste receptors (Panzuto et al. 2001) and the closely related eastern tent caterpillar (*Malacosoma americanum*) has been shown to detect both sugars and free amino acids (Schoonhoven and van Loon 2002). Changes in the sensitivity of these taste receptors underlie compensatory feeding (Simpson and Raubenheimer 1993). In *Locusta migratoria*, responsiveness of sugar and amino acid taste receptors is regulated by concentrations of sugar and amino acids in the haemolymph respectively, and haemolymph composition varies with food intake (Simpson et al. 1991; Simpson and Simpson 1992).

In Lepidopterans, nutrient intake is regulated by the concentration of trehalose in the haemolymph; trehalose is a disaccharide of glucose and the main storage sugar in insects (Mullens 1985; Thompson and Redak 2000; Thompson et al. 2001). *H. zea* fed low carbohydrate diets have low levels of trehalose, and consequently choose a diet rich in carbohydrate rather than a diet rich in protein, whereas caterpillars on high carbohydrate diets have high levels of trehalose in the haemolymph and selectively feed on protein rich diets (Friedman et al. 1991). Changes in the sensitivity of taste sensilla mediate these effects of haemolymph composition on caterpillar feeding: in *Spodoptera littoralis*, caterpillars fed protein-free food exhibited increased gustatory sensitivity to amino acid stimuli while insects fed a carbohydrate-free diet were more responsive to sucrose stimuli (Simmonds et al. 1992). However, independent regulation of protein and carbohydrate consumption via changes in the corresponding taste receptors appears not to be universal among Lepidopteran larvae: the generalist arctiid, *Grammia geneura* (Strecker), when fed diets of different composition, exhibits changes in the taste

sensitivity to sugars but not to amino acids and shows short-term compensatory feeding following carbohydrate, but not protein, deficiency (Bernays et al. 2004). Independent regulation of protein and carbohydrate is expected to be less important in oligophagous folivores such as the forest tent caterpillar than in the generalist species studied to date (Simpson and Raubenheimer 2001).

We examined responses of forest tent caterpillars to synthetic foods of varying protein and digestible carbohydrate content and tested whether the caterpillars compensate for deficiencies in either of these two nutrients by selecting the missing nutrient when given a choice between protein-only and carbohydrate-only foods. While it has been argued that the use of fresh leaves elicits a more natural response than is observed when artificial foods are used (Levesque et al. 2002), the use of artificial food enables us to chemically manipulate and standardize the nutrient content. Furthermore, the artificial food deliberately excludes allelochemicals in order to focus on the dynamics of the essential nutrients.

2.3 Methods and Materials

2.3.1 Experimental Insects

Forest tent caterpillar egg masses from the Canadian Forest Service Great Lakes Forestry Centre (Sault-Ste-Marie, Ontario) were hatched and reared on a nutritionally balanced, standard meridic artificial diet (Addy 1969) under controlled light and temperature regimes: 18 h photoperiod and 22°C. Experimental insects were removed from the culture 24 hours after moulting to the fourth instar.

2.3.2 Artificial diets

The chemically defined foods used in the experiments were based on the Simpson and Abisgold diet (Simpson and Abisgold 1985), used in previous work on *Spodoptera littoralis* (Simpson et al. 1988a; Simpson and Simpson 1992). The experimental food, unlike the rearing diet, was chemically defined. Four diets differing in content of protein and digestible carbohydrate were prepared: PC = protein 21% : carbohydrate 21%, P = protein 21% : carbohydrate 0%, C = protein 0% : carbohydrate 21%, and O = protein 0% : carbohydrate 0%. The protein content of the food consisted of a 3:1:1 ratio of casein, peptone and albumen, while the carbohydrate portion was made up of a 1:1 ratio of sucrose and dextrin. Other components of the food were Wesson's salt (2.4%), cholesterol (0.5%), linoleic acid (0.5%), ascorbic acid (0.3%) and 0.2% of a vitamin mixture. Cellulose, a non-nutritive bulk agent, was added to fill the remaining part of the food. The foods were presented to the insects in a 1% agar solution in a 6:1 agar solution : dry ingredients ratio. All experimental diets were novel to the insects.

2.3.3 Conditioning Period

100 caterpillars (25 per conditioning treatment) were deprived of food for 2 h, to ensure that all undigested material was expelled, then weighed to within 0.1 mg. A two-hour period of food deprivation is well within the normal inter-meal duration. The inter-meal duration of isolated fifth instar *Malacosoma americanum* is over 4 hours (Fitzgerald and Visscher 1996). Insects were placed individually in 10 cm diameter Petri dishes with a 0.3 x 1 x 1 cm block of one of four conditioning foods and a 2 x 2 cm square of paper towel moistened with 2 ml of distilled water to prevent dehydration. Petri dishes were sealed with Parafilm.

Individual insects were fed one of the four above-described experimental diets for a conditioning period of 16 or 48 h. Long conditioning periods were used due to the long inter-meal interval in this species; preliminary trials with 4 – 12 h conditioning periods showed no significant effects (see Chapter 3). Additional pieces of each conditioning food were set up in Petri dishes without insects and dried at the end of each experiment to establish a wet-to-dry weight regression.

At the end of the conditioning period the remaining food was removed, labeled and placed in a drying oven for 48 hours at 30°C. The initial dry weights of food pieces were determined from the dry: wet weight regression and compared to the final dry weights to determine the amount eaten by each insect. Insects were deprived of food for 2 h and weighed to find the weight change during the conditioning period.

2.3.4 Protein/Carbohydrate Choice Period

Individuals were then placed in the center of fresh Petri dishes, with moistened paper towel and a piece of each of the P and C foods. The two cubes of food were located on opposite sides of the Petri dish on the outside edge where the insects travel. Again food pieces were placed in Petri dishes without insects for the duration of the choice treatment and then dried to establish a wet-to-dry regression. The P/C choice treatment lasted for a total of 24 h. At the end of the choice treatment period insects were removed, food deprived for 2 h and weighed to determine the amount of weight gained during the choice treatment. The remaining P and C pieces of food were collected and placed in the drying oven at 30°C for 48 h, then weighed, and the dry amount of each piece of food consumed was calculated.

2.3.5 Observations

Short-term behavioural observations were conducted during the choice period in order to assess short-term taste responses to the two food types. A sub-sample of 48 insects (12 from each conditioning treatment) was observed during the first two hours of the choice treatment. Insect behaviour was recorded every 12 seconds using a laptop computer. The types of behaviour recorded were: walking, searching, resting, contacting protein, contacting carbohydrate, eating protein or eating carbohydrate:

- *walking*: the individual exhibits directed locomotion.

- *searching*: the caterpillar sways its anterior body in all directions.

- *resting*: the individual is not moving.

- *contact with either protein or carbohydrate*: the individual contacts one of the food

  blocks but does not eat.

- *eating protein or carbohydrate food*: the individual consumes one of the food blocks,

  as the caterpillar chews its head capsule moves (Fitzgerald 1995).

The following variables were calculated: latency to first contact with each food

type and duration of first contact. A good measure of a food's phagostimulatory power is

the duration of first contact, as this variable is not confounded by post-ingestive effects

(Simpson and Raubenheimer 2000). Latency to first contact (time elapsed before

contacting a food) is an index of short and long-distance attraction, or primarily olfaction,

to that food (Schoonhoven et al. 1998; Stadler and Hanson 1975).

2.3.6 Analysis

Analyses of covariance (ANCOVA) were used to examine how the protein and

carbohydrate content of the different conditioning diets influenced consumption and

growth during the conditioning period (Raubenheimer and Simpson 1992). The insects'

fresh mass was used as a covariate to adjust for size differences. Tukey's Post Hoc tests

were used to determine differences between treatments.

Paired sample t-tests were conducted on the consumption of protein and

carbohydrate food during the choice treatment for each conditioning treatment separately

to test for preferences for either of the two diets. Two-way analyses of variance on the

observational data tested the effects of conditioning and of food type on the time elapsed

to first contact with a food and on the duration of first contact on that food. SPSS version

11 software (SPSS Inc.) was used for statistical analyses.

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2.4 Results

2.4.1 Conditioning Period

There was a highly significant effect of conditioning treatment food type ($F_{3,96}$ = 10.21, P = 0.000) and duration of conditioning treatment ($F_{1,96}$ = 13, P = 0.000) on the amount of food eaten during the conditioning period. The quantities of the PC, P, C or O food eaten by fourth-instar forest tent caterpillars conditioned for 16 and 48 hours are shown in Fig. 2.1. Insects conditioned on the PC food ate significantly more overall, and those conditioned for 48 hours ate more than insects conditioned for 16 hours. Weight gain also differed ($F_{3,96}$ = 8, P = 0.000): caterpillars fed the PC food gained significantly more than those fed the unbalanced C, P or O food (see Fig. 2.2). There was no effect of insect original weight on consumption or weight gain.

2.4.2 Protein/Carbohydrate Choice Period.

When given a choice between protein and carbohydrate foods, the insects conditioned on the protein (P) food showed a significant preference for the carbohydrate food (t_{23} = -2.37, P = 0.027, see Table 2.1). For all other conditioning treatments, the amount of protein and carbohydrate eaten did not differ significantly from the null expectation of equal consumption of both food types (see Figs. 2.3 and 2.5).

The total amount of food eaten was not affected by conditioning treatment ($F_{3,96}$ = 0.133, P = 0.94). Weight gain during the choice treatment period was not significantly affected by the conditioning treatment ($F_{3,96}$ = 1, P = 0.4, see Fig. 2.4).

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2.4.3 Observations.

Conditioning treatment did not have a significant effect on the latency to contact the protein or carbohydrate foods during the choice treatment (see Table 2.2), or on the duration of the first contact (see Table 2.3). However, there was an effect of choice food type (P or C) on both latency to first contact and duration of first contact (see Tables 2.2 and 2.3): caterpillars generally contacted the C food first and spent more time on the C food upon first contact (see Figs. 2.6 and 2.7). The interaction term was not significant, suggesting that conditioning treatment did not influence the initial preference for the C food.

2.5 Discussion

Fourth-instar forest tent caterpillars exhibit an initial preference for carbohydrates regardless of nutrient deprivation when given a choice between P and C foods, and over the longer term, they compensate for a deficiency in carbohydrate but don't appear to regulate for protein deficiency.

Previous research has shown that other caterpillar species, who tend to show an initial preference for C food irrespective of nutritional deficiencies, over a longer period of time compensate for both protein and carbohydrate deficiencies (Bernays and Singer 2002; Thompson 2003). Caterpillars in general appear to be less sensitive to protein deficiency than they are to carbohydrate deficiency (Friedman et al. 1991; Simpson et al. 1990) and seem to regulate protein intake less tightly than do locusts (Thompson 2003). The present findings suggest that forest tent caterpillars regulate protein less strongly than they do carbohydrate and less strongly than do previously studied caterpillar species.

An initial preference for the C food irrespective of previous nutritional deficiency has also been observed in *Spodoptera littoralis* (Simmonds et al 1992) and *Grammia geneura* (Bernays et al. 2004). As in the present experiment, these caterpillar species contacted the C food first. Caterpillars thus appear to be capable of selecting between P and C foods before physically contacting the foods, believed to be due to responsiveness of an olfactory system (Simmonds et al. 1992). Whether this is a learned association or an internal negative feedback related to the gustatory system is not certain. A longer first feeding bout on the C food irrespective of conditioning treatment was also shown in *S. littoralis* (Simpson et al. 1988a) and *G. geneura* (Bernays et al. 2004) caterpillars. The

prolonged duration of the first contact on the carbohydrate diet reflects the phagostimulatory power of soluble sugars. Indeed, soluble sugars are used as a cue for the selection of a favourable food plant by many phytophagous caterpillars, and sugar-sensitive chemoreceptors are found in all lepidopteran larvae studied thus far (Albert and Parisella 1985; Schoonhoven 1967; Schoonhoven 1987), including forest tent caterpillars (Panzuto et al. 2001).

The maintenance of high trehalose levels in the haemolymph of Lepidopterans could explain the initial preference for C food as a rapid response to lowered haemolymph trehalose (Thompson 2003). When blood sugar drops in caterpillars, unlike with vertebrates, glycogen stores are not broken down to replace the sugar before 24 h starvation (Thompson 1998; Thompson 2003). The primary source of haemolymph trehalose is dietary carbohydrate, and caterpillars with low blood sugar show increased feeding on sugar sources long before they begin to break down glycogen or proteins (Thompson and Redak 2000). Caterpillars thus respond to sugars after a short time without them (Thompson 1998), as was observed in the present experiment. All insects in the present experiment were deprived of food for two hours prior to the choice treatment; however, previous research suggests that this food deprivation period is too brief to significantly lower the haemolymph trehalose of insects fed on high carbohydrate diets (Friedman et al. 1991; Thompson and Redak 2000). The initial preference for C food exhibited by the C pre-treated caterpillars is likely due to the fact that these insects ate very little during the conditioning period (Simpson et al. 1988a).

Over long-term exposure to the P/C choice, carbohydrate-deprived individuals compensated for that deficiency, whereas insects from the other conditioning diets ate

equal amounts of both protein and carbohydrates. Haemolymph trehalose concentrations increase and decrease when caterpillars are fed on C and P foods, respectively, leading the insects to switch between the two food types (Friedman et al. 1991). In the present experiment, low haemolymph trehalose concentrations at the beginning of the choice period would explain the initial feeding bout on the C food. Blood sugar would rise following consumption of the C food and the insects would switch to feeding on the P food, leading to a drop in blood sugar and resumption of feeding on the C food. Insects pre-treated on the P food would have lower haemolymph trehalose than those in the other treatments (Thompson and Redak 2000), and would therefore exhibit compensatory feeding on the C food. In *H. zea*, this alternation between C and P foods generated by changes in haemolymph trehalose produces stronger compensation for C than for P deprivation (Friedman et al. 1991). In the forest tent caterpillar, compensation for P deprivation appears to be even weaker.

Although forest tent caterpillars do not appear to compensate directly for protein deficiencies they do respond to protein in food sources. During the conditioning period, larvae fed the balanced PC diet consumed substantially more than those fed either of the deficient foods: *S. littoralis* take frequent small meals on deficient diets (P or C) and longer ones on balanced (PC) diets, resulting in higher consumption on the balanced diet (Simpson et al. 1988a). Indeed, nutrients interact to influence food intake via post-ingestive effects on haemolymph trehalose (Thompson 2003): *M. sexta* caterpillars fed balanced protein: carbohydrate diets have lower haemolymph trehalose levels than those fed carbohydrate rich diets and therefore consume larger amounts of food (Thompson and Redak 2000). The present experiment suggests that although forest tent caterpillars may

not regulate protein and carbohydrate consumption independently, post-ingestive effects of carbohydrates and protein on haemolymph trehalose can nonetheless promote consumption of balanced foods.

Independent regulation for protein and carbohydrate may not be necessary for forest tent caterpillars to achieve a balanced nutritional intake. To what extent do fourth instar forest tent caterpillars encounter foods with high protein-to-carbohydrate ratios in nature? High-protein plant tissues such as young leaves and reproductive tissues are one example (Lee et al. 2002). However, fourth-instar forest tent larvae normally feed on at least two-week old foliage, known to be lower in digestible protein than the foliage fed on by earlier instars. These insects are synchronized with their host phenology and have little variation to select from: their main choices will be between leaves on the same tree. These may vary in chemical composition according to their position on the tree. However, both protein and digestible carbohydrates are higher in the leaves preferred by forest tent caterpillars (Levesque et al. 2002). Regulating these two nutrients separately may not be necessary for the caterpillars if 'good' leaves are high in both essential nutrients, particularly if responses also need to be modulated according to secondary metabolites in the foliage.

Nutritional compensation is important in mobile, polyphagous species encountering a wide variety of food sources varying in nutrient content (Scriber and Slansky 1981; Slansky and Scriber 1985). Fourth-instar forest tent caterpillars are generally confined to one host plant and thus differ from the ground-dwelling generalist species where regulation has previously been studied. Generalist herbivores experience greater nutritional heterogeneity in their diets; they sometimes feed on unbalanced foods

and need to respond to deficient diets through compensatory behaviour (Simpson and Raubenheimer 2001). Studies comparing the specialist *Spodoptera exempta* to the generalist *S. littoralis* showed that responses of *S. exempta* were less flexible than those observed in *S. littoralis* (Lee et al. 2002). Similar results were found when comparing generalist versus more specialized locusts (Simpson and Raubenheimer 2000). Insects with a narrower niche breadth are less reliant on compensatory feeding than generalist insects with a wide variety of nutritionally different food sources.

For fourth-instar forest tent caterpillars with a relatively narrow diet breadth, where protein and carbohydrate content often co-vary between food sources, independent regulation of protein and carbohydrate intake might not be advantageous (Simpson and Raubenheimer 2000). Low trehalose concentrations in the haemolymph would stimulate forest tent caterpillars to feed on a food source high in digestible carbohydrates, which, in the context of food sources naturally available to them, would be high in protein content as well. These findings are consistent with food preference studies on forest tent caterpillar by Panzuto *et al* (2001) and Levesque *et al* (2002) and support the notion that nutritional compensation mechanisms vary among insects according to feeding ecology.

Table 2.1: Paired sample t test of protein (P) eaten versus carbohydrate (C) eaten during the choice period separated by conditioning treatment.

Conditioning Treatment	Mean	Standard error	t	df	P-value
C	-12.65	10.77	-1.17	23	0.252
P	-21.97	9.28	-2.37	23	0.027
PC	2.0575	11.12	0.19	23	0.855
O	11.16	8.47	1.28	23	0.214

Table 2.2: 2-way ANOVA showing the effect of food type and conditioning treatment on latency to first contact on the two food types during the choice period.

Source	df	MS	F	P-value
Conditioning Treatment	3	6110	1.45	0.235
Choice (P or C)	1	19145	4.56	0.037
Conditioning x Choice	3	5730	1.28	0.289
Error	65	4202		

Table 2.3: 2-way ANOVA showing the effect of food type and conditioning treatment on duration of the first contact on the two food types during the choice period.

Source	df	MS	F	P-value
Conditioning Treatment	3	533	1.18	0.444
Choice (P or C)	1	4933	11.05	0.045
Conditioning x Choice	3	445	0.29	0.835
Error	3.3	446		
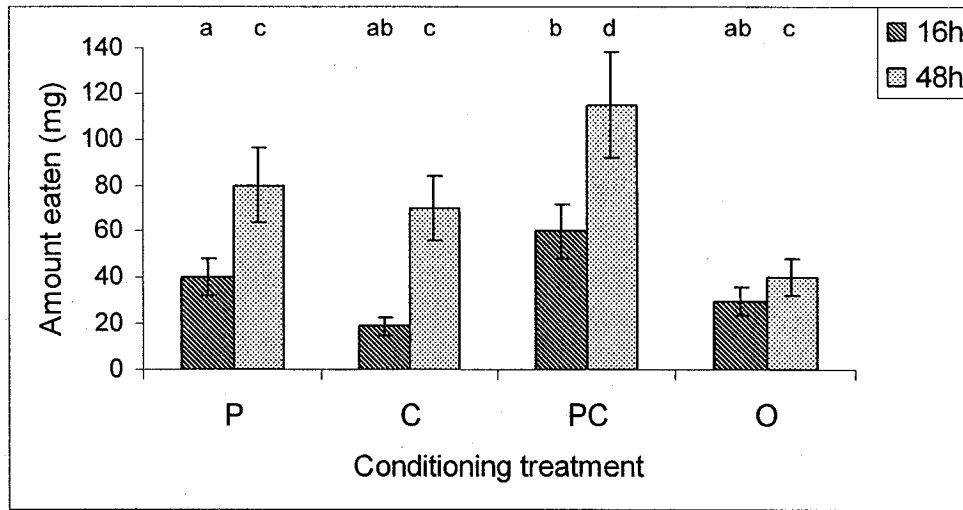
Figure 2.1: Dry weight of food eaten during the conditioning period. Food types: P- protein, C- carbohydrate, PC- protein and carbohydrate & O- no nutrients. Error bars = standard error. Letters represent differences between conditioning treatments based on Tukey's Post Hoc tests ($P < 0.05$).
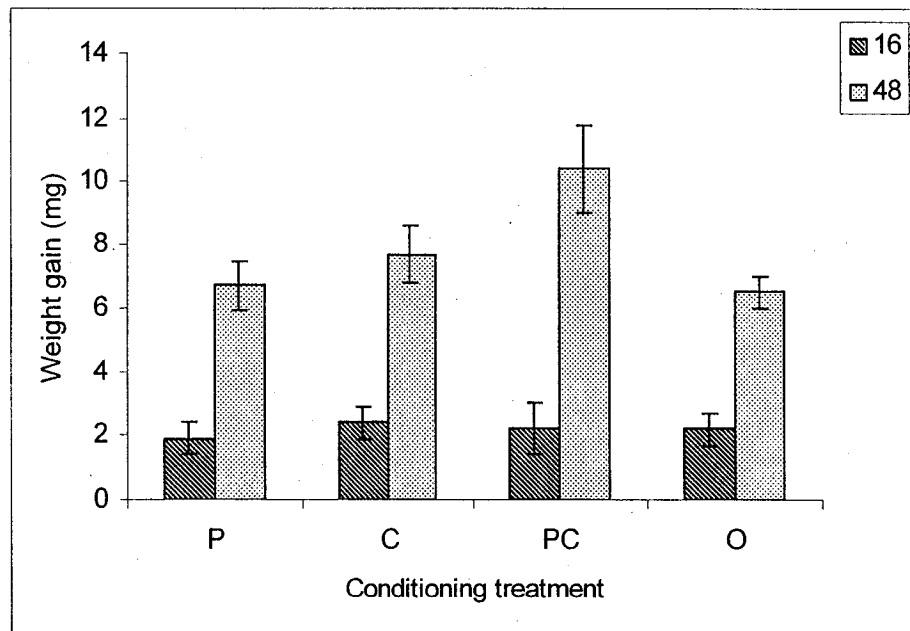
Figure 2.2: Weight gain on conditioning food.

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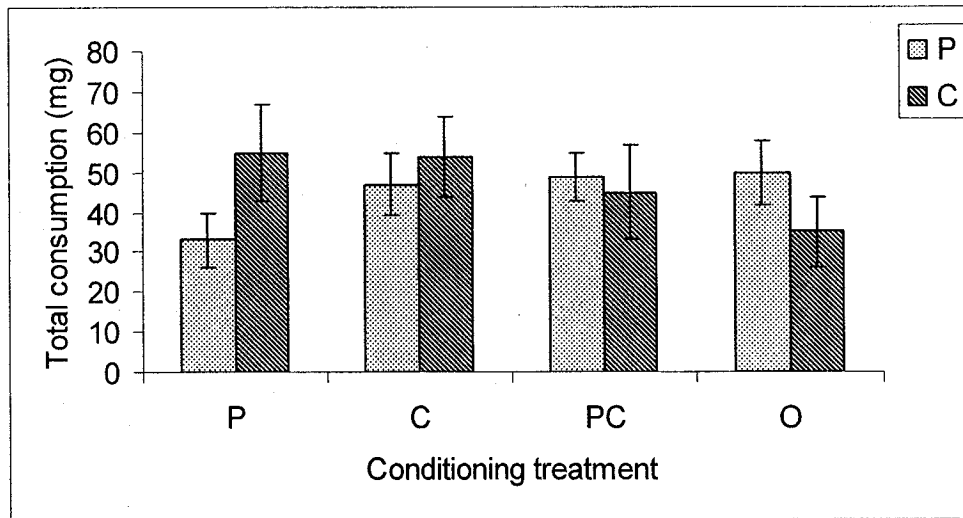
Figure. 2.3 Total food consumed during the choice treatment. No significant differences were detected between the two conditioning period durations, and these were pooled for the figure.
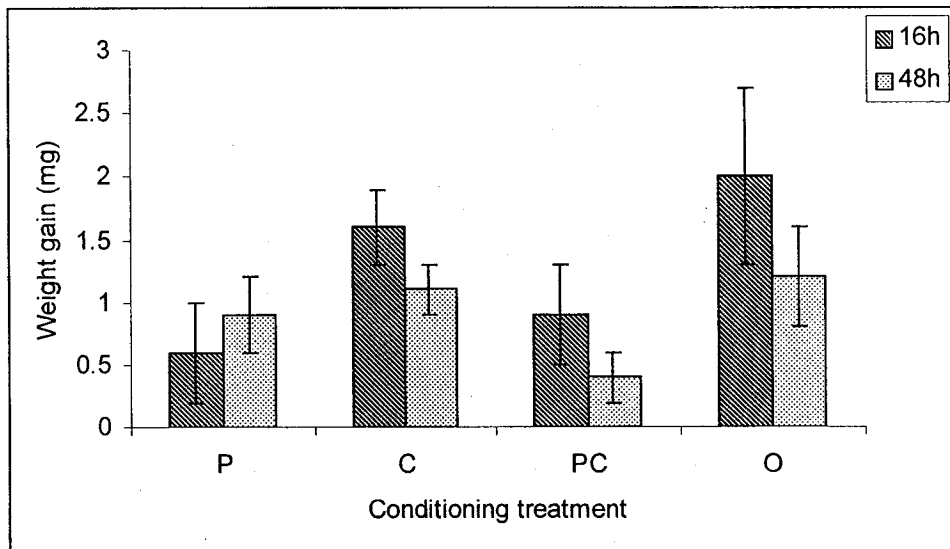
Figure 2.4: Weight gain on P/C choice treatment.
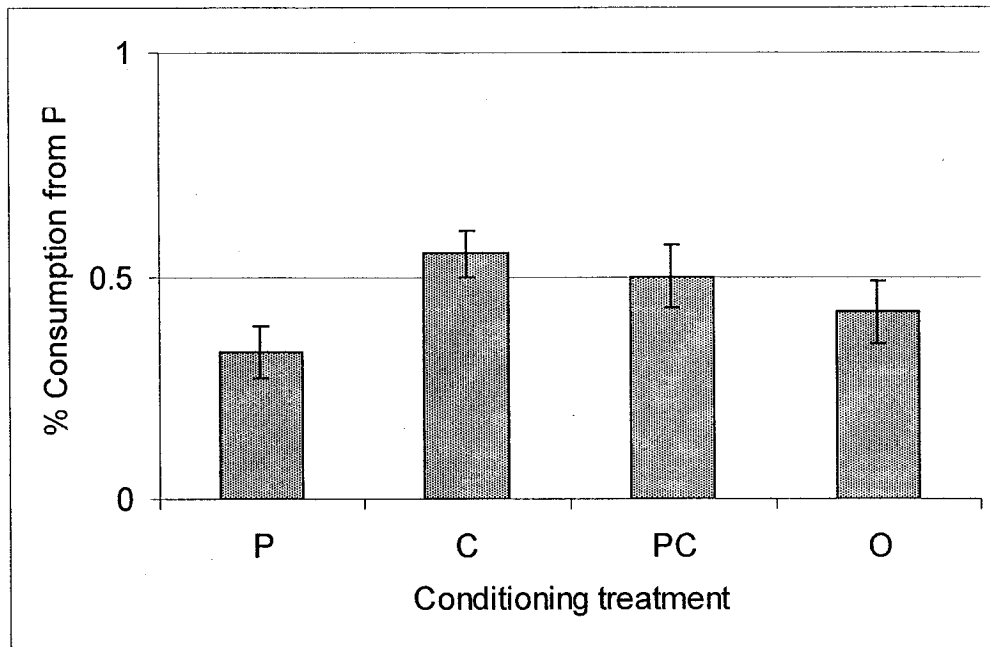
Figure 2.5: Proportion of protein food eaten during the choice treatments. Proportion of protein food eaten = amount of protein food eaten/total amount of both foods eaten. No significant differences were detected between the two conditioning period durations, and these were pooled for the figure.
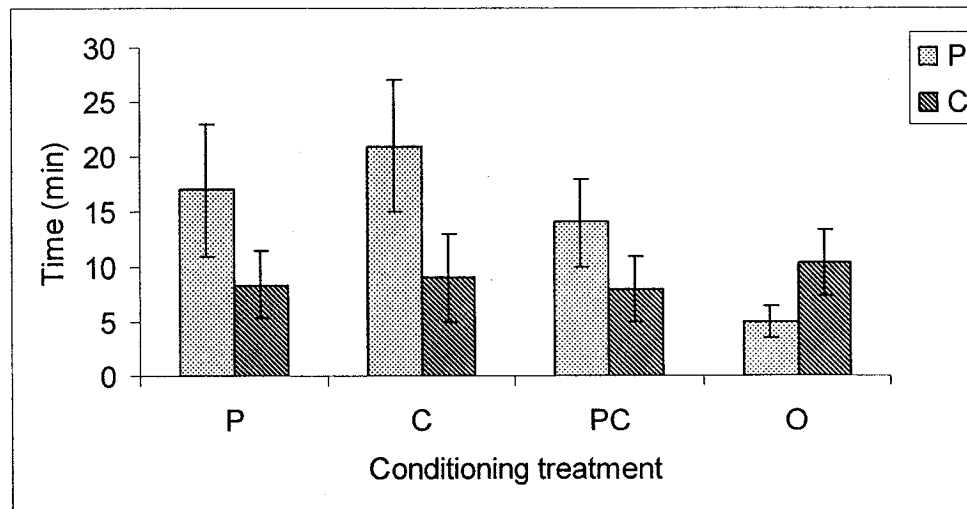
Figure 2.6: Latency to contact P- protein and C- carbohydrate foods during the choice treatment. No significant differences were detected between conditioning treatments. .
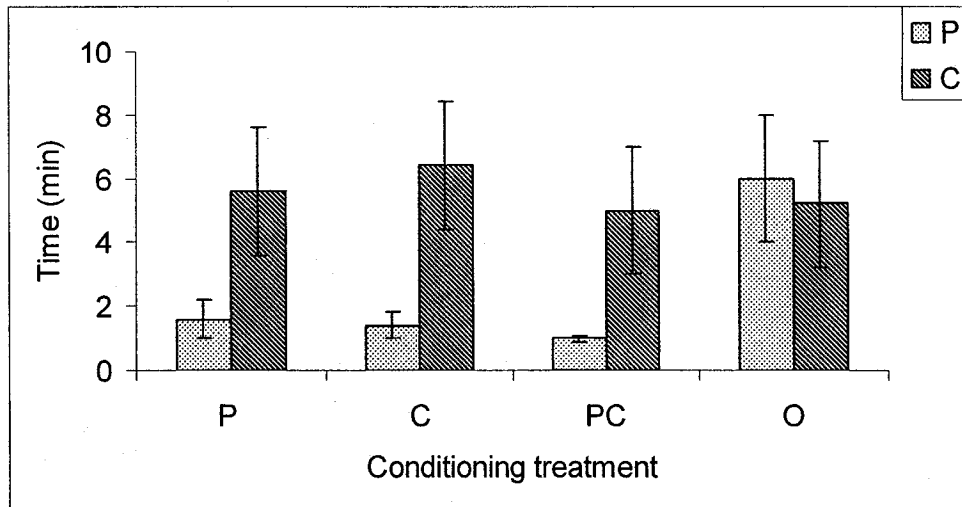
Figure 2.7: Duration of first contact on P or C foods during the choice treatment. No significant differences were detected between conditioning treatments.

Chapter 3
Additional Compensation Studies

3.1 Introduction

In an attempt to discover whether the forest tent caterpillar is able to compensate for diets deficient in primary essential nutrients, protein or carbohydrate, a series of preliminary studies were undertaken. Second, third and fourth instar caterpillars were conditioned on four artificial foods containing differing concentrations of protein and carbohydrates, for varying lengths of time, after which they were given a choice of protein only and carbohydrate only foods to determine whether the caterpillars would select the primary nutrients which were missing in their previous (conditioning) food. The food consumption and insect weight gain were measured for conditioning and choice treatments and observational studies were conducted to determine preference and performance. Many of the following results were not found to be statistically significant, however, the trends in the data support the results found in Chapter 2.

3.2 Methods

The methods for the additional studies were the same as those for Chapter 2 with a few exceptions, insects were food deprived for 4 hours, rather than 2 hours, before and after each treatment. Different instars (including 2nds and thirds) and conditioning times, depending on the study, were applied for each of the following additional studies.

3.3 Second instar Caterpillars: Group Compensation

Second instar forest tent caterpillars were conditioned for 16 hours on one of the four conditioning foods, and then given a choice between the protein- or carbohydrate-only diets for 24 hours. Caterpillars were tested in groups of five.

Univariate analyses of variance revealed no significant effect of the conditioning diet treatment on the amount of diet eaten (see Fig. 3.1a) or insect weight gain. While there does appear to be a difference in the amount of P and C diet eaten in the choice treatment (see Fig. 3.1b), these results are not reliable, due to overall low diet consumption and small number of test insects. The problems we experienced resolving low weights led us not to conduct further studies on second instar insects. The weight resolution problems make group studies a better option with these small caterpillars, although group results would not be directly comparable to those obtained using individuals.

3.4 Third Instar Caterpillars: Individual Compensation

Third instar forest tent caterpillars were conditioned on one of the four conditioning foods for 16 or 48 hours (n_{tot}=108). Univariate analyses of variance showed that the amount of conditioning food consumed for either conditioning time (16 or 48 h) was significantly affected by the conditioning treatment (see Table 3.1a). The trends are similar to those found for fourth instar caterpillars shown in Chapter 2. Those

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conditioned on the PC food ate more than insects on the P, C or O foods (see Fig. 3.2a).

Weight gain was significantly affected by the conditioning treatment and conditioning

duration (see Table 3.1b): those conditioned on the PC and C foods gained more than

those insects on the P or O foods (see Fig. 3.2b). There was also an effect of conditioning

time on the weight gained. These results also agree with those for fourth instar

caterpillars, shown in Chapter 2.

When given a choice between the P only and C only foods, the proportion of P

and C food eaten did not differ significantly from a 50/50 ratio (see Fig. 3.2d). However,

weight gain again did differ according to the previous conditioning treatment. Insects

conditioned on the non-carbohydrate foods P and O gained significantly more during the

choice period than insects on the C and PC foods (see Table 3.2c and Fig. 3.2d).

Third instar forest tent caterpillars are believed to continue foraging in groups due

to their small size. For this reason we proceeded to test fourth instar caterpillars, which

are believed to be more solitarious in their foraging ecology and perhaps more likely to

exhibit compensatory feeding.

3.5   Fourth Instar Caterpillars: Individual Compensation

Fourth instar forest tent caterpillars were conditioned for 6 hours on each of the

four conditioning foods after which they were given a choice between protein-only and

carbohydrate-only foods. Univariate analyses of variance showed that there was no

significant effect of the conditioning food treatment on the amount of conditioning food

eaten (see Fig 3.3a). Weight gain on the conditioning treatment, proportion of protein-

only and carbohydrate-only foods eaten in the choice period and weight gain during the choice treatment were not significantly affected by conditioning treatment (see Figs. 3.3b and c). Trends in the data suggest that there is a phagostimulatory effect of the carbohydrate food. Weight gain on the choice foods are similar to those from studies on third and fourth instars conditioned for 16 and 48 hours: insects conditioned on non-nutritive (O) foods gained more when given a choice between the protein- and carbohydrate-only foods (see Fig. 3.3d). It appears that the short conditioning treatment time may not be long enough to allow all the insects to have a meal on their conditioning food. The inter-meal duration of isolated fifth instar *Malacosoma americanum* is over 4 hours (Fitzgerald and Visscher 1996). Similar results were found for forest tent caterpillars conditioned for 4 and 12 hour periods: when given a choice they ate a balance of protein and carbohydrate.

3.6   Fourth Instar Caterpillars: Observation

87 fourth instar caterpillars conditioned for 16 hours on one of the four conditioning foods were then given a choice between protein only food and carbohydrate only food. Caterpillars were observed and their behaviour recorded on five-minute interval scans for six hours. In Chapter 2, observational studies were conducted on a 15-second interval for 2 hours. Although the five-minute scale is less fine, it shows the behaviour over a longer interval. The results are similar to those found for the 15-second interval data. Latency to contact the carbohydrate diet was shorter than latency to contact the protein diet (see Fig 3.4a) and duration of the first contact longer on the carbohydrate

diet than on the protein diet (see Fig. 3.4b). Univariate ANOVAs show that there was no effect of conditioning diet treatment on either behaviour variable.

3.7   Conclusion

From these additional studies it was concluded that food consumption by second and third instar insects was too small to be accurately measured with our equipment. When amounts eaten are small there is an increase in the size of the error which consequently obscures results (Schiff et al 1989). More significant results might be found if the insects were tested in large groups, however, group effects would have to be taken into consideration. In order to compare these results to those found for other herbivorous insects shown to exhibit compensatory feeding, we must test forest tent caterpillars individually. Fourth instar forest tent caterpillars are believed to be more solitarious than preceding instars; due to this and their greater size, we conducted subsequent compensation experiments on fourth instar insects. Food consumption and insect weight gain were more clearly defined, and differences in preference and performance were detectable.

The trends in both the consumption and growth data of these studies agrees with those found for fourth instar caterpillars conditioned over longer intervals. Preference was most significantly affected by the carbohydrate content of the food, whereas performance (weight gain) was greatest on a balanced protein/carbohydrate diet. In the additional studies, we found results similar to Chapter 2: the forest tent caterpillar does not seem to be able to compensate for protein deficiencies. However, those insects fed a

diet lacking in carbohydrate, when given a choice between carbohydrate and protein foods, did select slightly more carbohydrate food and those conditioned on the no-nutrient (O) food gained more weight during the choice treatment. Both of these results suggest that carbohydrates are phagostimulatory and increase weight gain, which is in keeping with the results found in other nutritional experiments on phytophagous insects. More careful measurement and observation are needed to establish if there are more sophisticated mechanisms at work in the choice behaviour and performance of these insects.

Table 3.1a: Univariate ANCOVA of conditioning food eaten by third instars with original weight as a cofactor.

Source	df	MS	F	P-value
Insect original weight	1	200	2.9	0.090
Conditioning treatment	3	407	6.0	<0.005
Hourly treatment (16, 48 hour)	1	1411	20.6	<0.005
Conditioning treatment * hour	3	199	2.9	0.038
Error	90	63		

Table 3.1b: Univariate ANCOVA of weight gained by third instars during the conditioning treatment.

Source	df	MS	F	P-value
Insect original weight	1	4.2	0.81	0.369
Conditioning treatment	3	44.9	8.61	< 0.005
Hourly treatment (16, 48 hour)	1	28.9	5.55	0.020
Cond. Treatment * Hour	3	11.3	2.16	0.009
Error	96	5.2		

Table 3.1c: Univariate ANCOVA of weight gained by third instars during the P/C choice treatment.

Source	df	MS	F	P-value
Insect original weight	1	0.46	0.13	0.71
Conditioning treatment	3	12.41	3.7	0.013
Hourly treatment (16, 48 hour)	1	1.3	0.39	0.53
Conditioning treatment * hour	3	1.5	0.45	0.72
Error	96	3.3		

Second Instar Caterpillars: Group Compensation
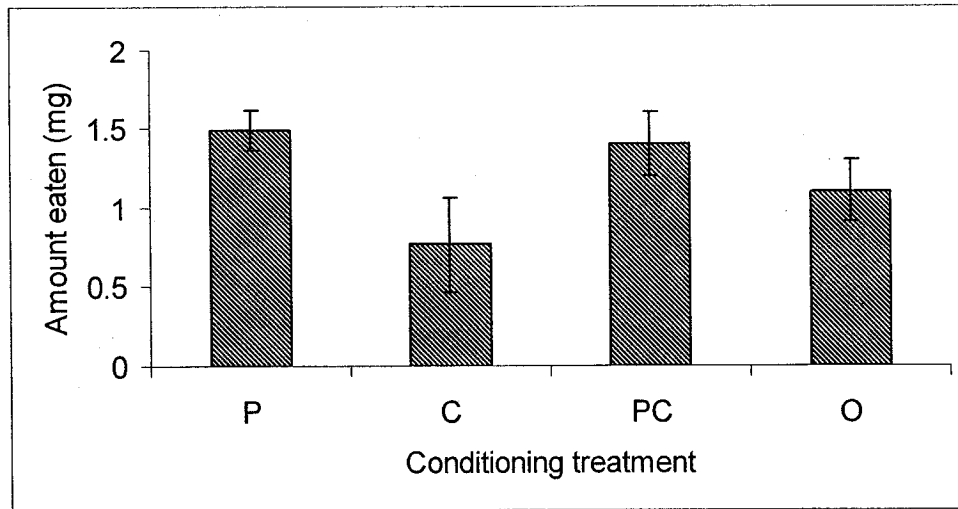
Figure 3.1a: Conditioning food consumed by second instar forest tent caterpillars in 16 hours.   P: protein food, C: carbohydrate food, PC: protein/carbohydrate food, O: no nutrient food.   Error bars represent standard error.
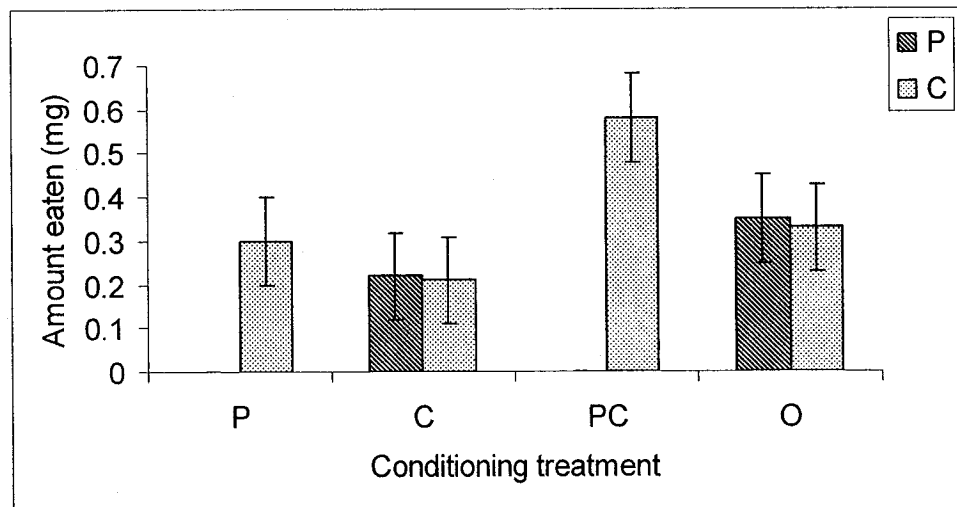
Figure 3.1b: Amount of protein (P) and carbohydrate (C) food consumed during the 24 hour choice treatment.

Third Instar Caterpillars: Individual Compensation
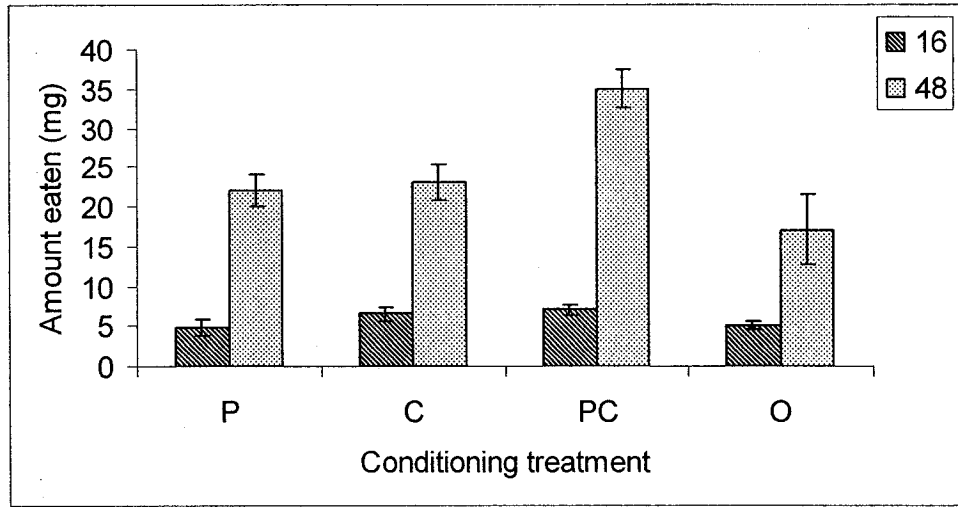
Figure 3.2a: Conditioning diet consumed by third instars in 16 and 48 hour conditioning treatments.
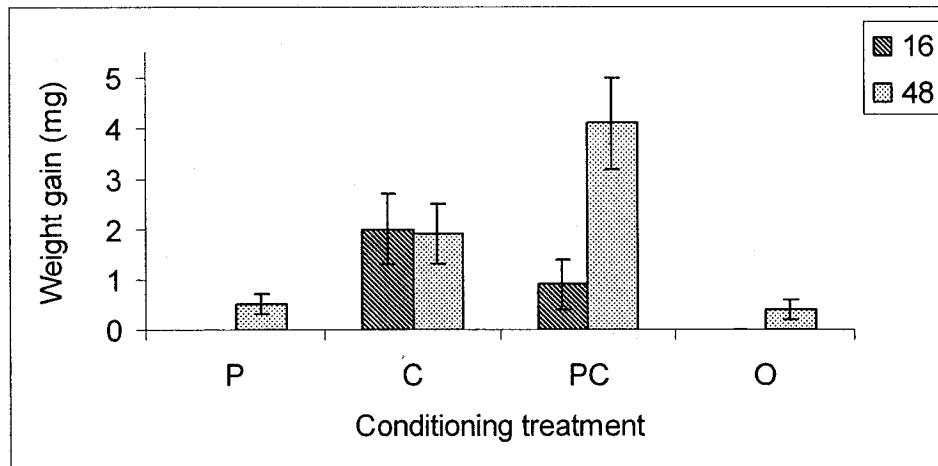
Figure 3.2b: Weight change on conditioning foods for the 16 and 48 hour conditioning periods.
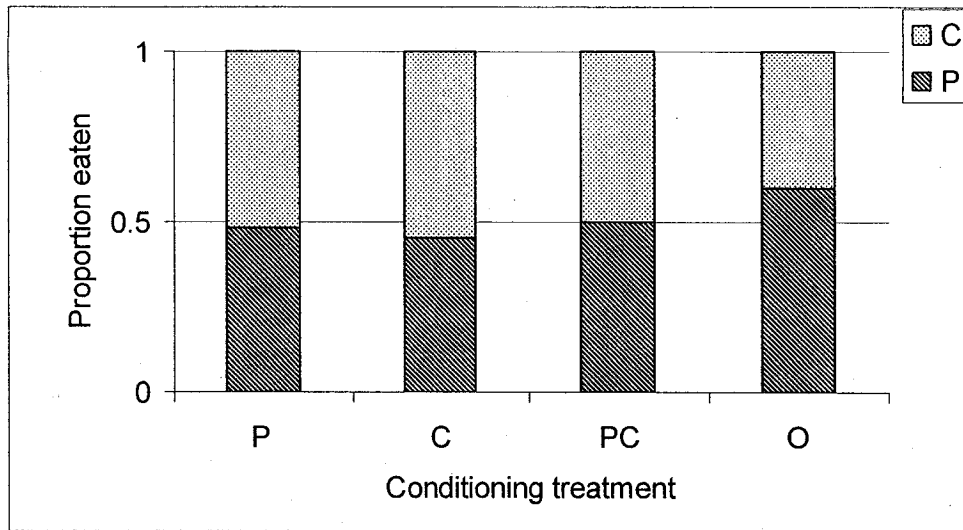
Figure 3.2c: The proportion of protein (P) and carbohydrate (C) diet consumed during the 24 hour choice period.
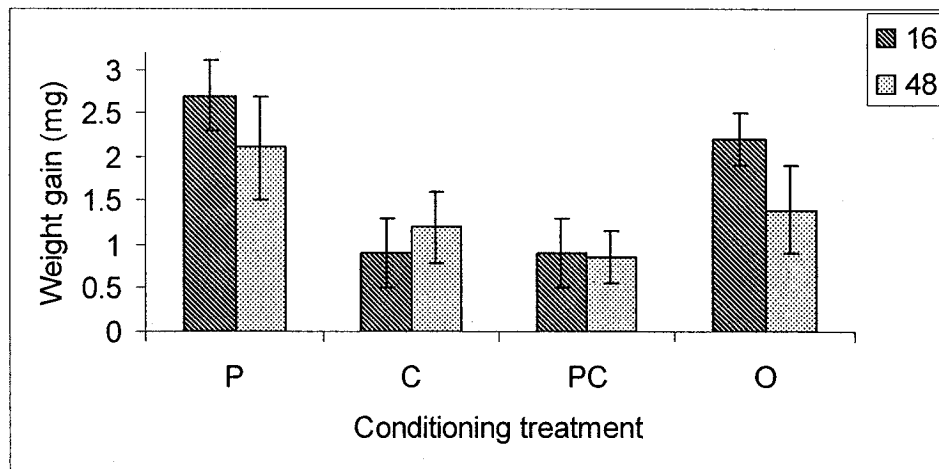
Figure 3.2d: The weight change on the choice diet.

Fourth Instar Caterpillars: Individual Compensation
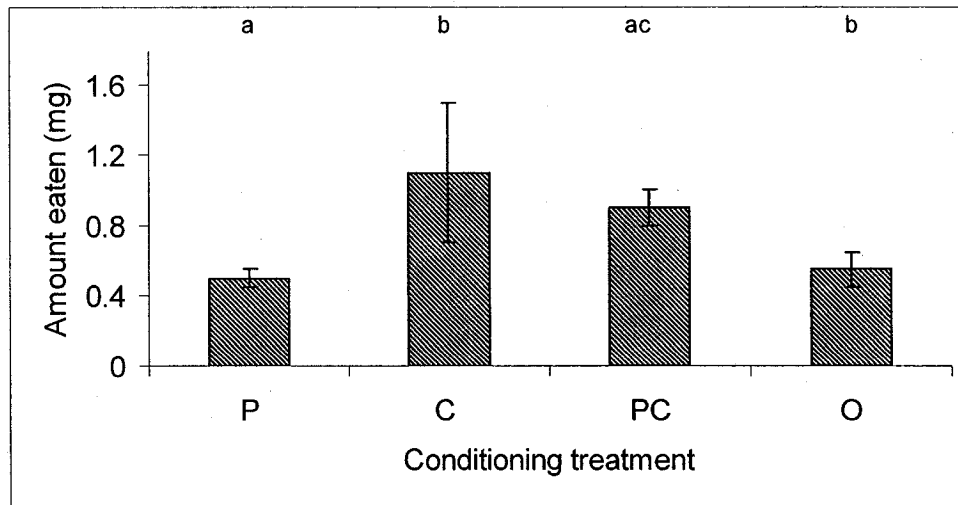
Figure 3.3a: Conditioning diet consumed by fourth instar caterpillars over 6 hours. Letters represent significant differences in consumption from Tukey post hoc test.
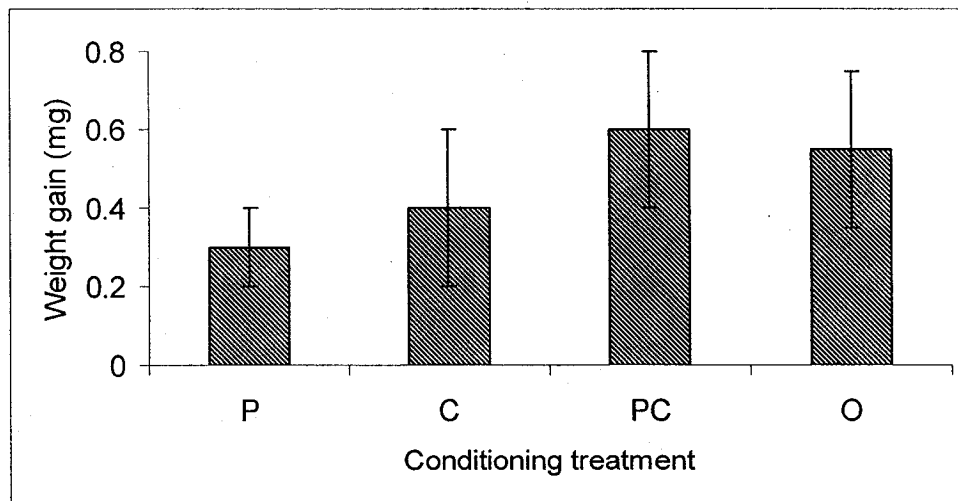
Figure 3.3b: Weight change experienced by insects treated on the conditioning diets for 6 hours.
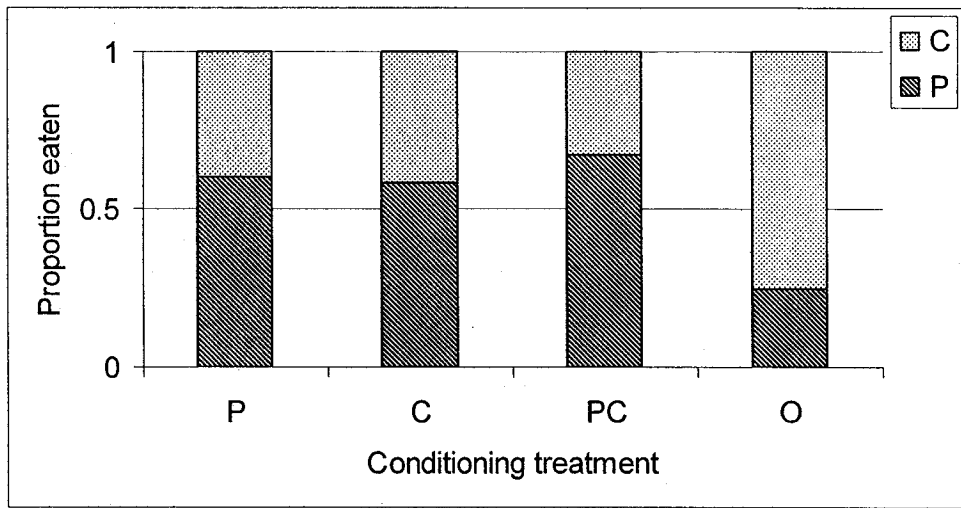
Figure 3.3c: Proportion of protein and carbohydrate food eaten by insects in 24 hours after being conditioned for 6 hours.
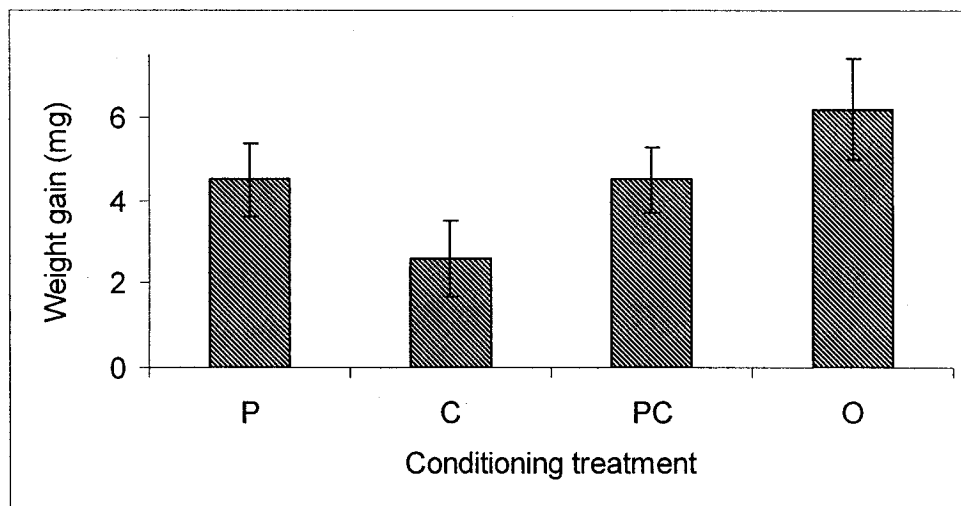
Figure 3.3d: Weight change on the P/C choice treatment.

Fourth Instar Caterpillars: Observation
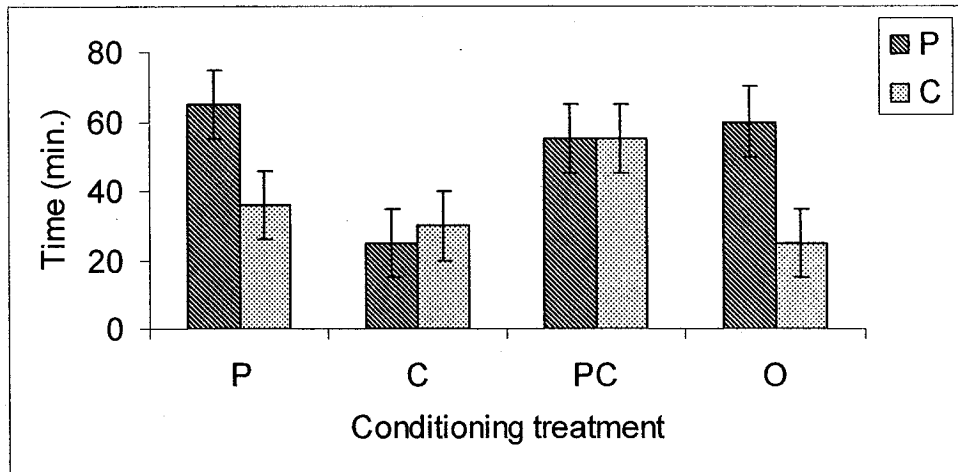
Figure 3.4a: Latency to first contact of the choice diets (P: protein and C: carbohydrate) during the first 6 hours of exposure. Insects conditioned for 16 hours.
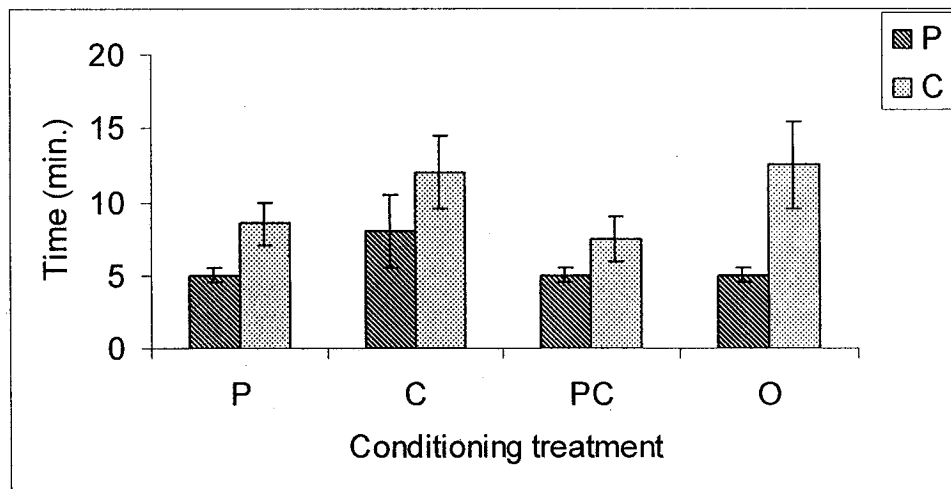
Figure 3.4b: The duration of the first contact on either choice diet (P or C) during the first 6 hours of exposure, insects conditioned for 16 hours.

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Chapter 4
Feeding Behaviour on Nutrient Supplemented Leaves

4.1 Abstract

Levels of primary nutrients and secondary metabolites vary both within and between trembling aspen trees (*Populus tremuloides*). Herbivores, including the forest tent caterpillar (*Malacosoma disstria*), exhibit differences in feeding behaviour and in performance on aspen foliage from different sources. Our study was designed to assess the effects of the ratio of the primary nutrients, protein and sugar, in foliage on the performance and choice behaviour of the forest tent caterpillar, an oligophagous forest folivore whose primary host is aspen. Fourth-stadium larvae were fed aspen leaves supplemented with either casein or sucrose. Sugar supplementation increased survivorship relative to control leaves, but decreased development and growth rates; protein supplementation had no significant effects. Behavioural observations showed that the insects did not differentiate between supplemented leaves over the short-term, but, over the longer term, they fed slightly less on the protein supplemented leaves than on control or sugar-supplemented leaves. Decreasing the protein:carbohydrate ratio via sugar supplementation does not appear to influence feeding behaviour and has ambiguous consequences on fitness. Increasing the protein:carbohydrate ratio via protein supplementation slightly decreases preference for that food but has no effect on performance.

4.2 Introduction

Host plant quality is essential to herbivore performance and fitness (Singer et al. 2002) and can also influence foraging patterns. Food quality of individual plants or plant parts varies due to differences in the content of the primary nutrients, protein and digestible carbohydrate (Awmack and Leather 2002; Slansky and Scriber 1985), and of secondary metabolic compounds (Bernays and Chapman 1994). Due to the complex chemical composition of plants, it is difficult to disentangle the roles of nutrients and secondary metabolites in governing herbivore performance (growth and survival) or preference (choice) on different foliage types.

Available nitrogen in the form of protein is often argued to be the most important nutrient in a herbivore's diet, due to its central role in all metabolic processes (Karowe and Martin 1989; Mattson 1980; Scriber 1984). Although nitrogen is important for herbivore performance, a balance of protein and sugar are in fact required because of their different physiological roles: proteins for growth and carbohydrates for energy. The ratio of protein to carbohydrate in a herbivore's diet has significant effects on growth, development and survival (Awmack and Leather 2002; Simpson and Raubenheimer 2000; Simpson and Simpson 1990).

Preference for a certain type of foliage may be dictated by a herbivore's ability to detect different components of the plant. Many herbivorous caterpillars use sugars as a cue for good quality foliage (Panzuto et al. 2001) and possess chemoreceptors for sugars (Schoonhoven 1967; Schoonhoven 1987; Schoonhoven and van Loon 2002). Chemoreceptor sensitivity to dietary sugars, in Lepidoptera and locusts, is modulated by

concentration changes in blood sugar (haemolymph trehalose- a disaccharide of glucose and the main storage sugar in insects) due to post-ingestive effects of both carbohydrate and protein consumption (Mullens 1985; Thompson et al. 2003; Thompson and Redak 2000). Insects with low trehalose in the blood show greater taste receptor sensitivity to dietary sugar and feed more readily on sugar-containing foods (Bernays et al. 2004). Some Lepidoptera also have taste receptors which respond to a number of free amino acids (Schoonhoven and van Loon 2002; Panzutto and Albert 1997); in some species, these exhibit changes in sensitivity in response to dietary protein that lead to regulation of protein consumption (Simpson et al. 1988a). However, in other species, only sugar chemoreceptors respond to changes in nutritional status; in these cases food selection in the absence of secondary metabolites is determined mainly by chemosensory responses to sugars (Bernays and Chapman 2001; Bernays et al. 2000).

We will investigate differences in the performance of lepidopteran larvae, *Malacosoma disstria* (the forest tent caterpillar; Hübner) when fed trembling aspen (*Populus tremuloides*) leaves supplemented with varying levels of proteins and carbohydrates, and will test for preferences for foliage with different protein to carbohydrate ratios.

Forest tent caterpillars (Lepidoptera: Lasiocampidae) are early season polyphagous defoliators of deciduous trees throughout eastern North America (Fitzgerald 1995; Hodson 1941), their preferred host being trembling aspen (Fitzgerald 1995). Compared to other host trees such as sugar maple (*Acer saccharum* Marsh), trembling aspen has higher concentrations of sugars and total non-structural carbohydrates (Lindroth et al. 1993; Lorenzetti 1993), as well as lower levels of condensed tannins and

no hydrolyzable tannins (Lindroth et al. 1987a; Lindroth et al. 1993). Forest tent caterpillars prefer the leaves on certain trees compared to other trees; on differing poplar clones they disperse from unsuitable trees in search of better quality trees (Robison and Raffa 1996). Host plant quality can vary within an individual aspen tree. Much of the within-tree variation may be related to the fact that *Populus* trees are indeterminate growers. As a result, after the spring flush of leaves, herbivores have a choice of leaves of various ages, the youngest having the highest phenolic glycoside concentrations (Lindroth 1991), while also having high nitrogen and sugar content. Leaf chemistry also varies according to the vertical position of the leaf (Dudt and Shure 1994; Futuyma and Saks 1981). Forest tent caterpillars perform better on sun leaves than they do on shade leaves (Fortin 1994): Fortin and Mauffette (2001) suggest that the increased leaf consumption and growth rates of forest tent caterpillars feeding on sugar maple sun leaves located at forest edges may be due to the 10% higher levels of nitrogen and soluble sugars in those leaves. Although sun leaves also have more total phenols than leaves from trees located within the forest interior, the rates of insect growth and leaf consumption do not seem to be affected by the increased levels of phenolic glycosides (Fortin and Mauffette 2001).

Recently studies of tree foliage quality effects on forest tent caterpillars have focused on plant physical stress caused by environmental changes such as increased ozone (O_3), carbon dioxide (CO_2) and high and low sunlight and levels of soil nutrients (Bryant et al. 1987; Bucker and Ballach 1992; Coleman et al. 1995; Fortin et al. 1997; Hemming and Lindroth 1999; Holton et al. 2003; Kopper and Lindroth 2003). While these studies have successfully shown that changing environmental factors affect the

nutrient content of foliage and subsequently affect herbivore consumption and performance, the effects of changes in protein and carbohydrate content of foliage have not been isolated from other environmentally induced changes in leaf composition. General trends of nutrient variation with environmental changes show that factors which increase non-structural carbohydrates tend to increase leaf consumption, and factors which increase nitrogen availability generally improve performance.

Specifically, increasing O_3 elevates levels of nitrogen in trembling aspen foliage, resulting in increased larval performance (Bucker and Ballach 1992). In general, increased levels of CO_2 result in a decrease in the P:S (protein:sugar) ratio, but also affect secondary metabolites such as tannins and phenolic glycosides (Awmack and Leather 2002). Therefore, poor herbivore performance at elevated CO_2 levels cannot be attributed to a decrease in the P:S ratio alone. Decreased P:S ratio may also increase palatability, thereby enhancing herbivore performance. On trembling aspen, an increased level of CO_2 results in decreased levels of nitrogen, and has no effect on forest tent caterpillar performance (Holton et al. 2003; Kopper and Lindroth 2003). Increased levels of sunlight and soil nutrients boost sugar and nitrogen content in the foliage of trees while lowering the levels of phenolic glycosides and starch, resulting in higher rates of leaf consumption and growth by forest tent caterpillars (Hemming and Lindroth 1999). In contrast, increased exposure to sunlight and lowered soil nutrient content yields leaves that are low in nitrogen but high in sugar, starch and phenolic glycosides, a composition that was found to hinder leaf consumption and growth rates by forest tent caterpillars. Secondary metabolites must also be considered as they usually vary with primary

nutrients (Hemming and Lindroth 1999; Lindroth et al. 1987b; Ricklefs and Matthew 1982).

In trembling aspen, secondary metabolites have been well studied and show a strong negative correlation with tent caterpillar performance (Haruta et al. 2001; Lindroth and Bloomer 1991): phenolic glycosides and tannins prolong development time and reduce growth rates (Lindroth and Bloomer 1991; Lindroth et al. 1987b). In a study by Hwang and Lindroth (1997) on variable trembling aspen clones, phenolic glycosides were the dominant factor responsible for variation in forest tent caterpillar performance while higher concentrations of carbohydrates were associated with increased consumption. However, it is difficult to distinguish between the effects of primary nutrients and secondary metabolites on herbivore performance. For example, in a study by Kopper and Lindroth (2003), increasing levels of ozone resulted in increased levels of foliar nitrogen and decreased levels of the phenolic glycoside, tremulacin. It was also found that increasing the atmospheric carbon dioxide levels lowered foliar nitrogen levels while boosting levels of tremulacin. Forest tent caterpillars performed better on leaves under elevated levels of ozone, but it is difficult to say whether this is due to increased levels of nitrogen or decreased levels of tremulacin.

Our study investigates the effect of varying the primary nutrient content of trembling aspen leaves on forest tent caterpillar growth and development, while keeping secondary metabolites relatively constant. We also examine the ability of the forest tent caterpillar to select foliage based on differing levels of primary nutrients. Experiments were conducted using trembling aspen leaves supplemented with protein (casein) or sugar (sucrose) to evaluate the roles of these two nutrients in forest tent caterpillar performance

and food choice. In the first experiment, 'the cafeteria experiment', fourth instar forest

tent caterpillars were given a choice between three leaves (protein supplemented, sugar

supplemented and a control leaf). In a second experiment, 'the no choice behaviour

experiment', caterpillars were given a single leaf and meal initiation was observed. In the

third experiment, 'the performance experiment', caterpillars were raised on one leaf type

for the duration of the fourth instar, and consumption, growth, instar duration and

survival were measured.

4.3 Methods and Materials

4.3.1 Experimental Insects

Forest tent caterpillar egg masses from the Canadian Forest Service Great Lakes

Forestry Centre (Sault-Ste-Marie, Ontario), were hatched and reared on a nutritionally

balanced, standard meridic artificial diet (Addy 1969) under controlled light and

temperature regimes: 18h photoperiod and 22°C. Experimental insects were removed

from the culture 24 hours after moulting to the fourth instar for the no-choice and

cafeteria experiments and immediately after moulting to the fourth instar for the

performance experiment.

4.3.2 Leaf Painting

Trembling aspen leaves, collected from a single tree on Ile Perrot, Quebec were

used in the study. Foliage was sterilized by soaking in a 1% bleach solution for 2 minutes

and then rinsed with water before painting (Hemming and Lindroth 2000). To maintain

leaf turgor, the petiole of each individual leaf was inserted through a hole in the top of a

pierced 2-ml microcentrifuge tube containing distilled water. To supplement the leaves,

0.125 g of casein or sucrose was dissolved per milliliter in KH_2PO_4 buffer (0.075 M, pH

8.0) with 3-mg/ml methylcellulose (Hemming and Lindroth 2000). Studies on the protein

and carbohydrate levels in aspen suggest that the addition of 9% casein and 10% sucrose

dry weight results in levels of protein and carbohydrate that fall within the variation

normally found in trembling aspen leaves (Hemming and Lindroth 1999; Miller 1987).

The leaves used for this experiment were sampled in mid-summer and therefore the levels

of protein and sugar, according to aspen literature, were approximately 19% and 15%

respectively (Miller 1987). The additional casein and sucrose would result in the

following theoretical values for each of the treatments. Buffer (B) painted leaves had no

nutrient supplement and were estimated to contain a protein/ sugar ratio of 19/15%

(Hemming and Lindroth 1999; Miller 1987), while sucrose (S) supplemented leaves

contained a ratio of 19/25% and protein (P) supplemented leaves contained a 28/15%

ratio. Each solution was applied to the leaves at 0.4-ml/g fresh leaf weight. It is

important that the solution concentration be such that it has sufficient volume to cover the

leaf area, but not so much that it floods the surface (Hemming and Lindroth 1999).

A micro-pipette was used to decant the appropriate volume of solution onto each

leaf surface. The solution was then spread evenly across the underside of each leaf. The

solution was left to air dry on the leaves for 1-2 hours while the petioles remained in

water-filled tubes.

4.3.3 Cafeteria Experiment

Single fourth instar caterpillars were deprived of food for 2 hours (del Campo C. and Renwick 1999) and placed in a 10 cm (diameter) Petri dish with three trembling aspen leaves. One leaf was supplemented with protein (P), one with sucrose (S), and the other with buffer (B). The insects were then observed for 2 hours, or until they had fed on any leaf continuously for more than 120 seconds. During the observation period, the observer performed scans every 60 seconds and recorded each individual's behavioural state using a laptop computer equipped with event recorder software. Thirty-three insects were observed in total. Nine behavioural states were recorded:

- *resting*: the individual is not moving.

- *walking*: the individual exhibits directed locomotion.

- *searching*: the caterpillar sways its anterior body in all directions.

- *contact with a leaf protein/buffer/carbohydrate*: the individual contacts one of the three leaves but does not eat.

- *eating a leaf protein/buffer/carbohydrate*: the individual cuts the leaf with its mandibles, as the caterpillar chews its head capsule moves (Fitzgerald 1995).

The difference between a nibble and a meal was determined by creating a scatter plot of the log-frequency distribution of number of meals against meal duration, the point of inflection of this graph provides a break between a nibble and a meal ((Sibly et al. 1990; Simpson and Raubenheimer 2000), see Fig. 4.1 below).
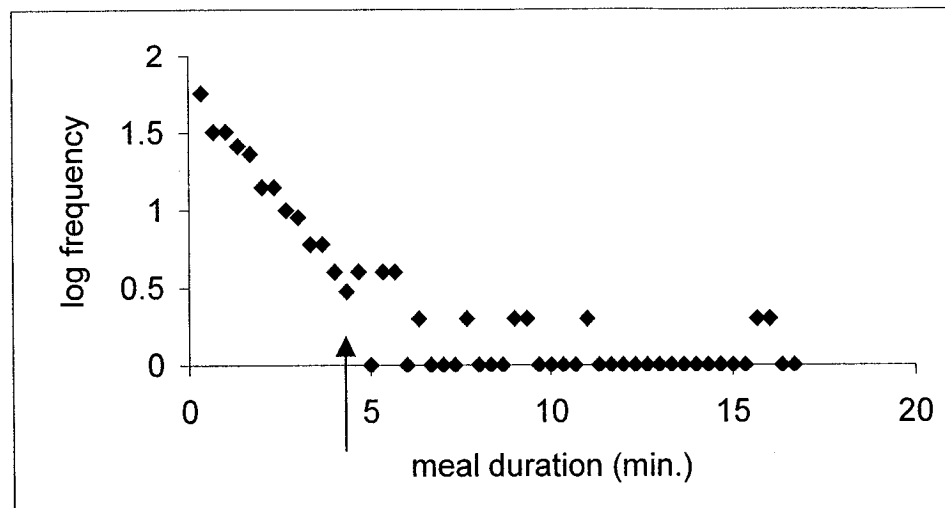
Figure 4.1: The differentiating point in time which separates nibbles from meals is 4 minutes, indicated by the arrow.

From the number and duration of bouts for each behavioural state, the following variables were recorded: a) latency to first contact (or time to commence feeding) on each of the three leaf types, b) probability of returning to first contacted leaf and c) total time spent eating each leaf in the first 2 hours of observation, d) first contact duration, e? first meal duration, e) number of meals and f) total time spent in contact with a leaf.

After the observation period, insects were left in the dishes for 24 hours, after which the leaves were removed, dried and weighed. A wet/dry regression was used to determine d) the amount of each leaf eaten.

4.3.4 No Choice Behaviour Experiment

Individual fourth instar (24 hours into the instar) forest tent caterpillars were deprived of food for 2 hours. Insects were then placed in 10-cm Petri dishes, each containing one of the three supplemented leaves, also for a period of 2 hours. Their

behaviour was recorded, and bouts of behaviour determined as in the previous experiment to examine responses to each leaf type. From the number and duration of bouts for each behavioural state, the following variables were recorded: a) the latency to first contact, b) duration of first meal, c) number of meals, d) total time spent eating in the first two hours, and e) first intermeal pause. The probability of initiating a meal upon contact is a good measure of the phagostimulatory power of a food. The time spent eating during the first two hours should include post-ingestive feedback from the food consumed. 66 insects were tested, 22 per treatment.

4.3.5 Performance Experiment

Freshly molted fourth instar forest tent caterpillars were fed leaves supplemented with either protein, carbohydrate or buffer for the duration of the instar to determine performance on the different leaf types. Individuals were weighed to within 0.1 mg and placed in a 10 cm Petri dish lined with a moistened paper towel under waxed paper (to prevent dehydration), with a leaf supplemented with one of the three solutions (casein, sucrose or buffer). Leaves were replaced every two days to ensure maintenance of the nutritional value of the foliage throughout the experiment (Miller 1987). Upon moulting to the fifth instar, a) instar duration, b) insect weight gain, c) RGR: relative growth rate (weight gain/(original weight * instar duration)) and d) survival were recorded. Of 142 insects tested, 54 were fed the protein-supplemented leaves, 44 were fed the sucrose leaves, and 44 were given buffer leaves.

4.3.6 Statisical Analysis

Non-parametric Friedman ANOVA tests were performed on the three-way choice cafeteria consumption data to determine deviation from the null hypothesis of equal feeding on the three supplemented leaves. Univariate ANOVAs were performed on the no-choice behavioural data to examine differences in consumption behaviour (time to contact, time to first meal, duration of first meal, number of meals, first inter-meal pause, total time spent eating an experimental leaf) between the three types of supplemented leaves. Tukey's Post Hoc tests were performed on all significant results. A univariate ANCOVAs were performed on the performance data: weight gain and instar duration and an ANOVA was performed on the relative growth rate. Survival was analyzed using a Pearson chi-square test. Analyses of the data were accomplished with SPSS version 11.0 software.

4.4 Results

4.4.1 Cafeteria Experiment

When given a choice between the three experimental supplemented leaves, caterpillars showed no significant difference in the latency to first contact (or time to commence feeding) each of the three leaf types (see Table 4.1 and Fig. 4.2b). The following variables also did not differ between the three leaf treatments: first contact duration, first meal duration, number of meals, total time spent eating (see Fig. 4.2c) or contacting a leaf (see Table 4.1). However, most insects returned to feed on the leaf that they contacted first: of the individuals that fed on a P, S, or B leaf, a significantly higher proportion took a second meal on that same leaf than fed on the other leaves (see Table 4.1 and Fig. 4.2a). Over 24 hours, insects ate significantly more of the B leaf than they did of the P leaf (Chi square = 9.7, df = 2, N = 20, asym. sig.= 0.008, see Fig. 4.2d).

4.4.2 No Choice Behaviour

Insects on all three experimental leaves exhibited very similar behaviour. There was no significant difference between the three treatment leaves in any of the variables examined: the latency to first contact, first contact duration, latency to commence eating, duration of first meal, number of meals, total time spent eating or contacting a leaf (see Figures 4.3 a-d). A multivariate ANOVA showed that the combination of variables were also not significant (for individual ANOVA results see Table 4.2). Trends in the data suggest that the total time spent eating and the number of meals taken were greater for individuals fed the sucrose-supplemented leaf, but these were not statistically significant.

4.4.3 Performance Experiment

Instar duration was significantly shorter on the control (B) and protein (P) leaves than on the sucrose (S) leaf ($F_{2,40} = 3.96$, $P = 0.028$, see Fig. 4.4a). Weight gain was significantly affected by leaf treatment: $F_{33,2} = 3.21$, $P = 0.05$ (see Figure 4.4b), insects on the B and S leaves gained significantly more than those fed the P leaf. Relative growth rate (RGR) was slightly lower for insects on the S leaf, but this was not significant ($P = 0.08$, see Fig. 4.4c). Survival was significantly affected by treatment: insects on the S leaf experienced a significantly higher survival rate than insects on either the B or P leaves (Pearson Chi-Square $_{58,2} = 5.764$, $P = 0.044$; see Figure 4.4d).

4.5 Discussion

Fourth instar forest tent caterpillars do not appear to differentiate initially between trembling aspen leaves having different nutrient compositions. Observation of caterpillar behaviour during the first two hours of exposure to leaves supplemented with sucrose (S), casein (P) or buffer (B) were similar in the cafeteria experiment to observations made in the no-choice experiment: insects exhibited no preference for any of the three supplemented leaves. This indifference suggests that the phagostimulatory power of the fresh leaves is equally attractive. Caterpillars in all studies contacted a leaf rapidly, and almost always initiated a meal within two hours but there is no difference due to post-ingestive effects within the first two hours. Insects in the three-way choice test tended to return to the first leaf eaten, throughout the first 100 minutes of choice. With increased

exposure time (24 hours), insects ate a greater proportion of the control (B) leaf, followed by the sucrose- supplemented (S) and protein- supplemented (P) leaf choices. Additionally, in a no-choice, long-term situation (the performance experiment), insects fed the buffer leaf experienced the greatest weight gain, relative growth rate and the shortest instar-duration, while insects fed the S leaf experienced the greatest survivorship.

It is not surprising that there is little, if any difference, in the initial response to the three supplemented leaves as specialist phytophagous insects are known to have a strong feeding preference for their natural food plants (del Campo C. and Renwick 1999). Surface concentrations of sugars and amino acids in plants directly elicit gustatory responses in insects (Bernays and Chapman 1994). In our experiment, supplements were added to the bottom side of the leaves, therefore surface taste does not represent the total concentration of digestible protein and carbohydrate, and random initial leaf choice exhibited by the caterpillars does not necessarily reflect an inability to make a choice. Food evaluation by forest tent caterpillars is based on sample feeding: insects will make many sample choices before commencing prolonged feeding (Robison and Raffa 1996). Two hours may not be sufficient time for lone fourth instar caterpillars to sufficiently sample the three choices presented to them. Repeated feeding on the first contact leaf may signify a response to pheromone- silk trails which lead the insect back to the first discovered leaf (Fitzgerald 1995; Fitzgerald and Costa 1986). With prolonged exposure, trails may be established to all leaves, thus the insect's choice and consumption patterns should be governed by the nutritional quality of the food. Our results suggest that this is the case: after 24 hours caterpillars in the three-way choice showed a significant preference for the buffer (B) and sucrose (S) leaves.

Insects in this study were tested individually. Decision-making may be stronger when insects are in groups and can share information about food quality (Fitzgerald and Costa 1986). Tent caterpillars produce trails that elicit following behaviour in colony-mates: sated individuals mark these trails with pheromone more strongly than do unfed individuals. Pheromone trails can thus serve to recruit other individuals to a food source, in proportion to the quality of that source (Bonabeau et al. 1998). Eastern tent caterpillars (*Malacosoma americanum*) lay two types of trails differentiating between food sources of differing quality (Fitzgerald 1976). Although trail strength does not vary as much with food quality in the forest tent caterpillar (Fitzgerald and Edgerly 1979), differential marking of trails can still serve to concentrate feeding on higher quality food sources (Fitzgerald and Costa 1986).

With prolonged exposure, forest tent caterpillars preferred the leaves with a lower protein to sugar ratio. Indeed, soluble sugars are used as a cue for the selection of a favorable food plant by many phytophagous caterpillars, and sugar-sensitive chemoreceptors are present in all lepidopteran larvae studied to date, including the forest tent caterpillar (Albert and Parisella 1985; Panzuto et al. 2001; Schoonhoven 1967; Schoonhoven 1987). Our previous studies with forest tent caterpillars fed artificial diets show that they respond most quickly to foods containing a high concentration of sugars (see Chapter 2). In Lepidopterans, nutrient intake is regulated by the concentration of trehalose in the haemolymph (Mullens 1985; Thompson and Redak 2000; Thompson et al. 2001). The maintenance of high haemolymph trehalose levels in Lepidopterans could explain their preference for a diet having a high concentration of sugar with respect to protein (Thompson 2003).

The effects of the protein/sugar ratio on caterpillar performance depend on the currency used to represent fitness. Indeed, instar duration was significantly shorter for the insects fed the buffer (B) or protein (P) leaves compared to those fed the sucrose (S) leaves. This increased development time also occurred in *Manduca sexta*. Thompson (1998) suggested that, on high sugar diets, these insects had high concentrations of trehalose in their haemolymph, which seemed to decrease the circulating Juvenile Hormone (JH). Low JH in turn results in low levels of ecdysone, which triggers moulting. Therefore insects with low ecdysone experience delayed moulting. Weight gain was greatest for insects on the B or S leaves, however relative growth rate (RGR) was highest for the P and B fed individuals: the difference is due to the longer instar duration of the S fed individuals. Survivorship was also significantly affected by the leaf supplement, with insects raised on the S leaf having the highest survivorship. Raubenheimer and Simpson (1999) also found that locusts (both *Locusta migratoria* and *Schistocerca gregaria*) developed more quickly when fed a diet having medium to high P:S ratio and that locusts, on sugar-biased diets, had higher survival rates and were more resistant to starvation than locusts on high P:S ratio diets (Raubenheimer and Simpson 1999).

As a stress response, plants often induce a shift from starch reserves to sugars and other compounds associated with tissue repair (Coleman et al. 1995), a shift which may result in more attractive leaves for forest tent caterpillars. Both protein and digestible carbohydrate have been shown to be higher in leaves preferred by forest tent caterpillars (Levesque et al. 2002), so choosing a leaf based on sugar content may be an effective way of differentiating between leaves if 'good' leaves are high in both essential nutrients. Our

findings suggest that leaf sugar content plays a key role in selecting a food source and that high sugar content can positively affect caterpillar performance. Sucrose is indeed a potent phagostimulant for most herbivores and low protein to sucrose ratios can overwhelm chemosensory responses to phagodeterrents (Singer et al. 2002). *Grammia geneura* (woolly bear, Strecker) caterpillars are deterred by secondary metabolites. They exhibit switching behaviour between foods containing different secondary metabolites to avoid toxin accumulation, with one exception: when sugar is added to their food choices, switching ceases (Singer et al. 2002). In addition, because *G. geneura* cannot taste protein directly, when fed two protein diets, the secondary metabolites dominated the flavor of the food and switching resumed. Similarly, studies of the taste chemoreceptors of the forest tent caterpillar, by Panzutto et al (2002), show that when the sugar, amino acid and salt cells of the maxillary sensillum, are tested, only the sugar cell exhibits a significant response to trembling aspen and sun and shade sugar maple extracts, which have a diverse array of chemicals, including amino acids. These results suggest that the forest tent caterpillar is primarily cued to the sugar content of its food source and responds strongly to sugar concentration.

In a study that aimed to differentiate between the effects of different components of natural foliage, Wright et al (2003) found that weevils (*Exophthalmus jekelianus;* Coleoptera: Curculionidae) also prefer high sugar leaves. Meal duration was found to be positively correlated with sucrose concentration whereas nitrogen content was negatively correlated with meal duration and secondary metabolites had no effect on meal duration (Wright et al. 2003). Hemming and Lindroth (2000) found that when high levels of protein were added to trembling aspen leaves, forest tent caterpillar performance was

hindered, an observation believed to be due in part to decreased consumption and growth rates. Although protein is generally thought to be important to herbivore performance and survival (Mattson 1980), insects don't seem to choose protein-biased leaves, nor do they exhibit enhanced performance when fed protein-biased, high P/S ratio, leaves.

The protein/sugar ratio is obviously important in determining food quality (Bernays and Chapman 1994; Simpson and Raubenheimer 1993; Simpson and Raubenheimer 2001). Some generalists have sophisticated mechanisms to regulate both nutrients and ensure consumption of an appropriate ratio of nutrients (Simpson et al. 2002; Simpson et al. 1995; Simpson and Simpson 1990). Forest tent caterpillars feeding on natural foliage however, seem to use high sugar as an index of foliage quality and to prefer low protein to sugar concentration foliage. Indeed, we show that the forest tent caterpillar does not benefit from a protein supplement (high P:S ratio) and might even benefit from sugar supplementation (low P:S ratio). Protein is essential to herbivores, but high protein, unbalanced with sugar, is not necessarily beneficial (Hemming and Lindroth 2000; Schroeder 1986). Increasing both protein and sugar is beneficial, even if there is a concomitant increase in secondary metabolites (Fortin and Mauffette 2001; Fortin et al. 1997; Wright et al. 2003). Natural foods consist of a complex mixture of nutrients and secondary metabolites: these can also interact to influence both choice and performance. Future studies addressing this interaction in the context of choice experiments are required to fully understand the role of primary and secondary metabolites in the feeding ecology of the forest tent caterpillar.

Table 4.1: Cafeteria Experiment- non-parametric Friedman ANOVA on the effect of supplemented leaf treatment (P: protein, S: sucrose or B: buffer) on behaviours exhibited during a three-way choice in the first two hours of exposure.

Analysis	df	Chi-square	n	P-value
Probability of returning to first leaf if P	2	38.0	33	< 0.005
Probability of returning to first leaf if S	2	24.5	33	< 0.005
Probability of returning to first leaf if B	2	21.1	33	< 0.005
Latency to contact each of the leaves	2	0.4	24	0.819
Total time spent eating each of the choice leaves	2	4.2	33	0.121
Proportion of leaves eaten in 24 hours	2	9.7	20	0.008

Table 4.2: No Choice Experiment- Univariate ANOVA on the effect of supplemented leaf treatment (P: protein, S: sucrose or B: buffer) on behaviours exhibited during the first two hours with a single experimental leaf.

Analysis	df	MS	F	P-value
Latency to Contact	2	6755	2.07	0.135
Duration of first meal	2	927	1.07	0.351
First intermeal pause	2	1746	1.85	0.169
Number of meals	2	1.34	0.88	0.422
Total time spent eating during first 2 hou	2	1341	0.87	0.426
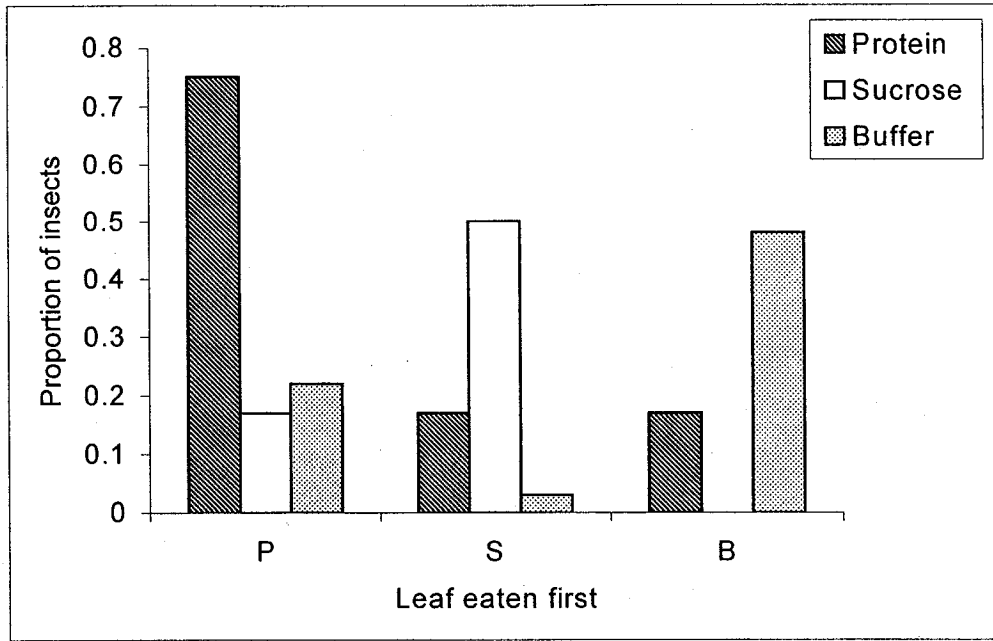
Figure 4.2a: Proportion of individuals that return to their first leaf choice.
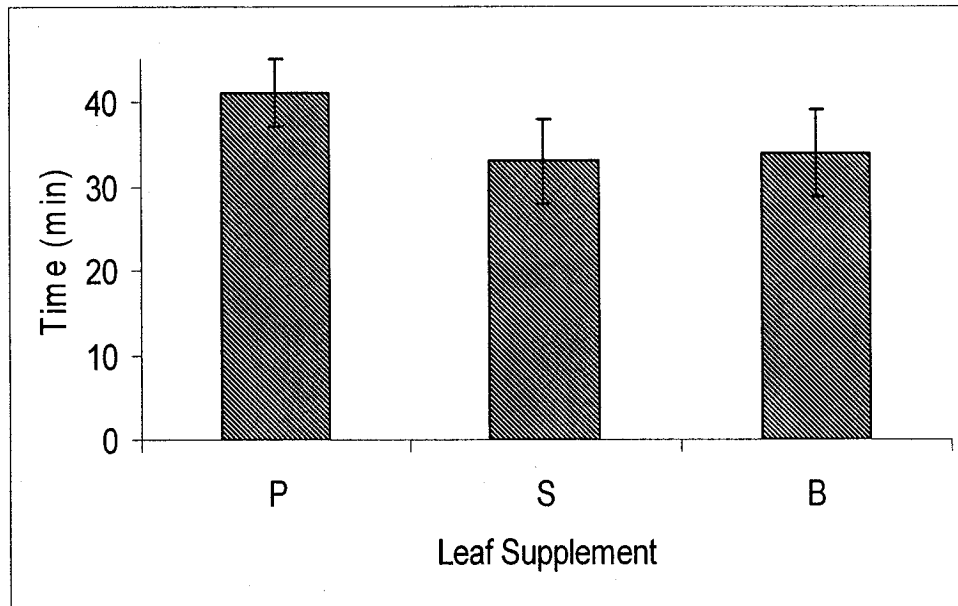
Figure 4.2b: Latency to contact first leaf. Error bars represent standard error.
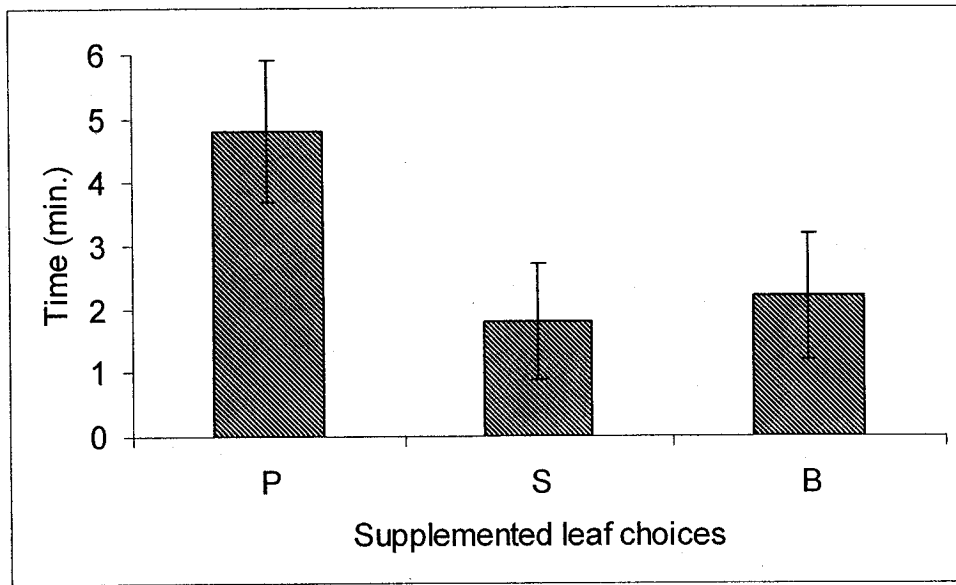
Figure 4.2c: Total time spent eating each of the three choice leaves in the first 100 minutes.
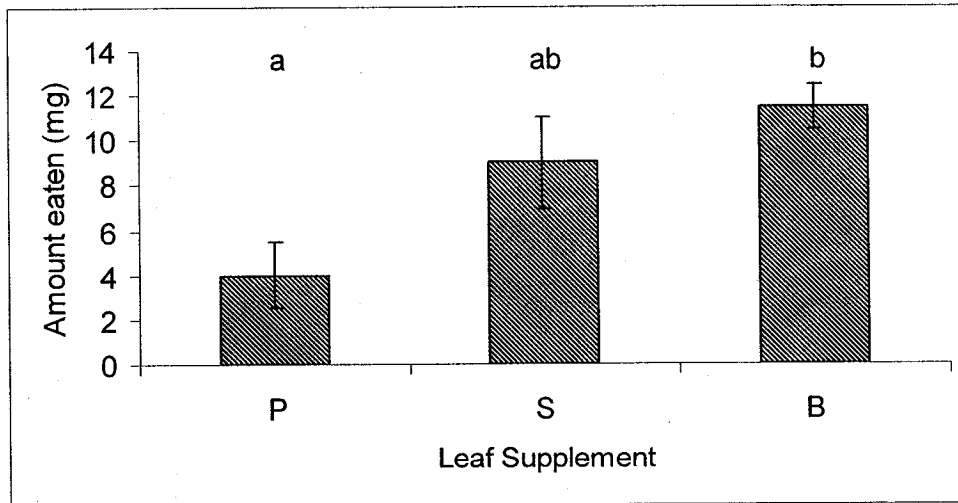
Figure 4.2d: Amount of each of the three choice supplemented leaves (P, S and B) eaten during the first 24 hours of choice exposure. Letters a, ab and b represent significantly different results. (from Tukey's post hoc test)
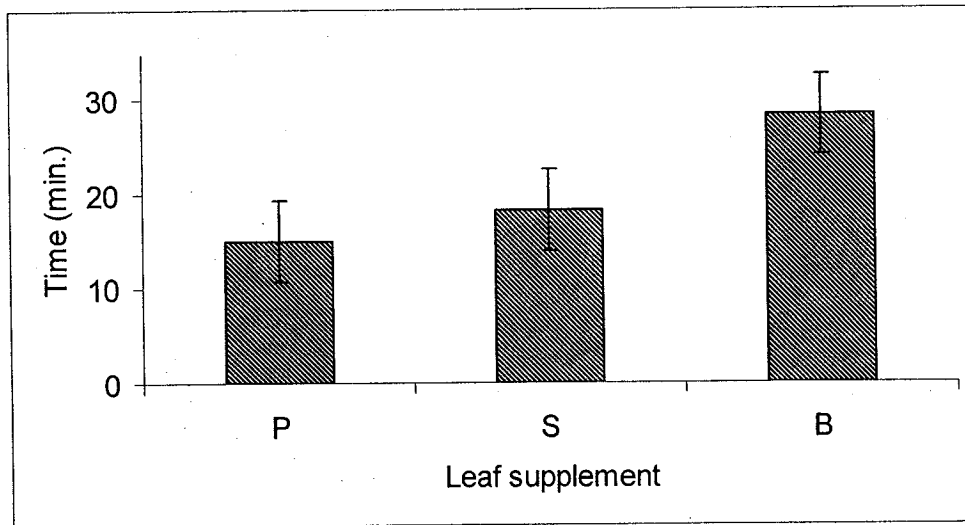
Figure 4.3a: Latency to contact experimental leaf.
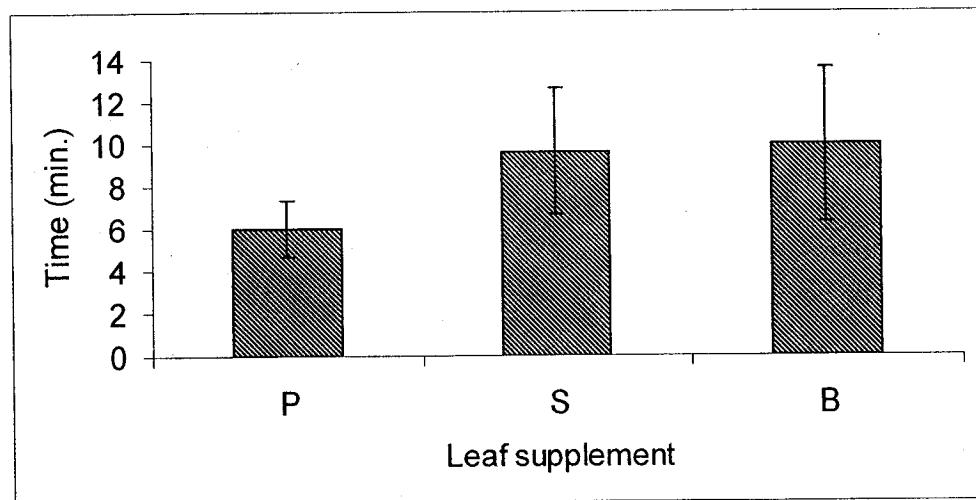
Figure 4.3b: Duration of first meal.
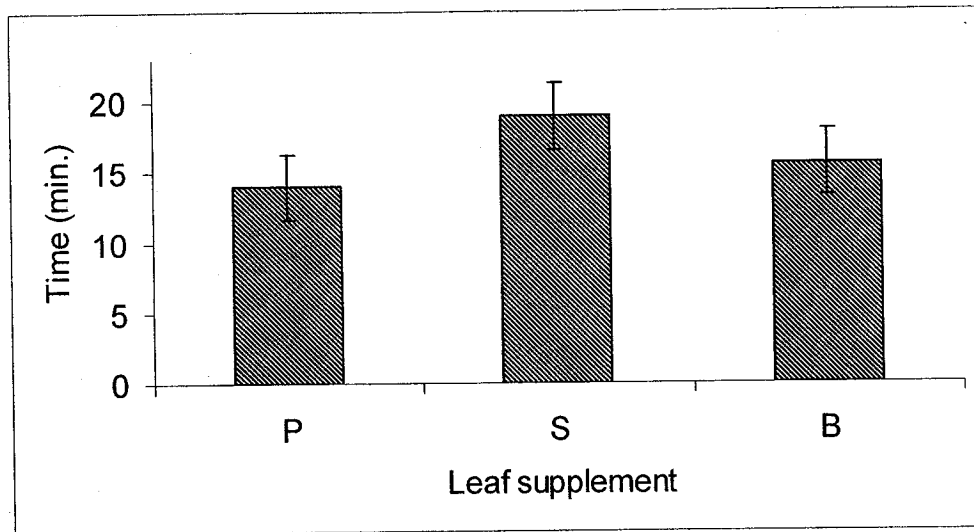
Figure 4.3c: Total time spent eating a leaf during the first 2 hours of exposure.
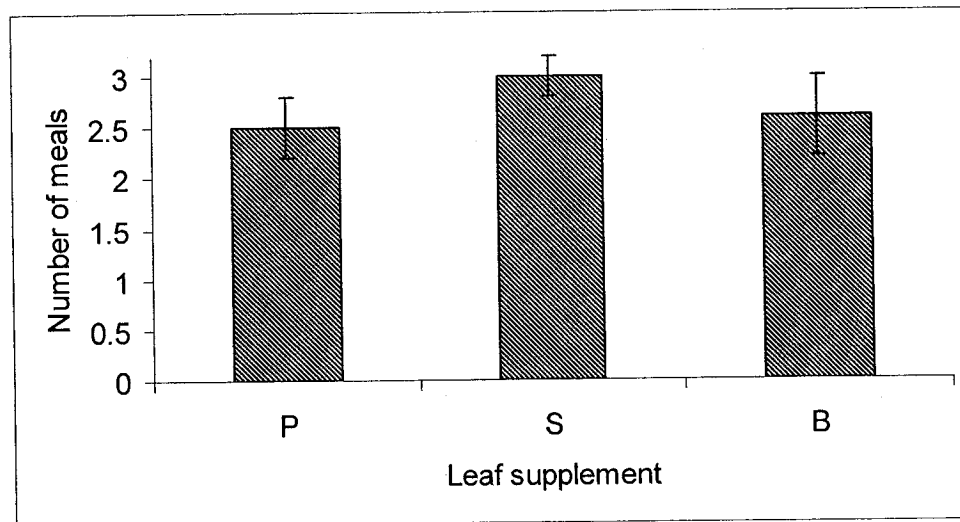
Figure 4.3d: Number of meals taken in the 2- hour observation period.
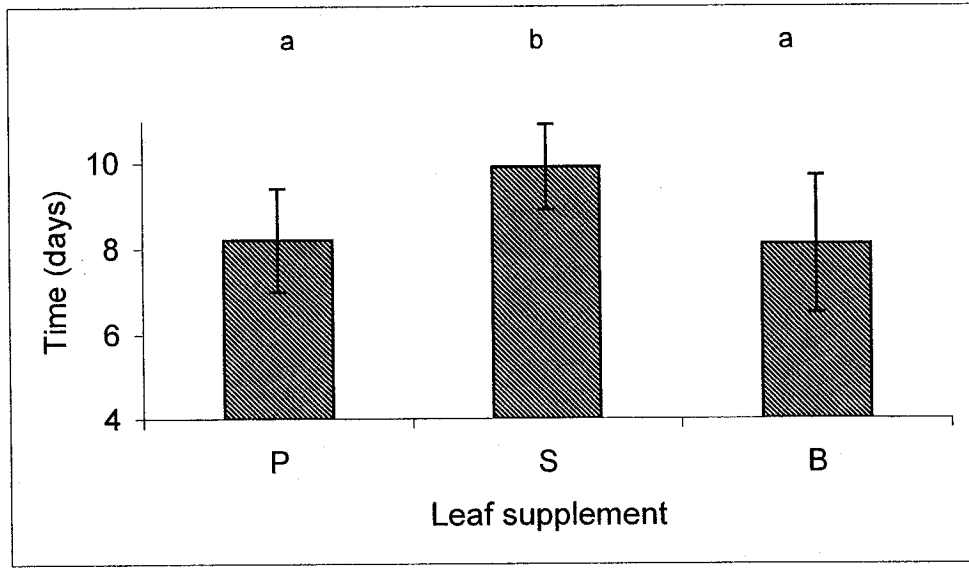
Figure 4.4a: Duration of the fourth instar in days. Letters represent significant differences from Tukey's Post Hoc test.
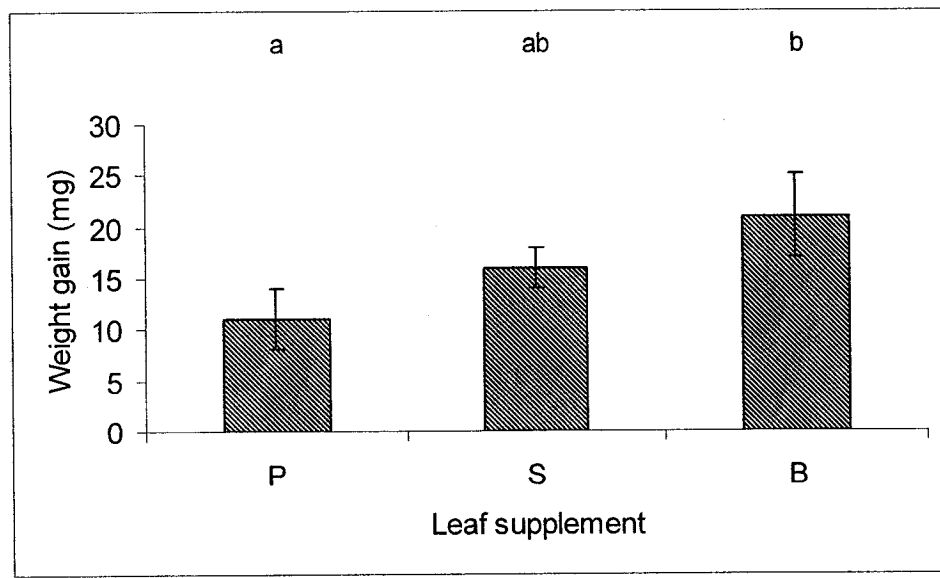
Figure 4.4 b: Weight gained over the fourth instar on one experimental leaf.
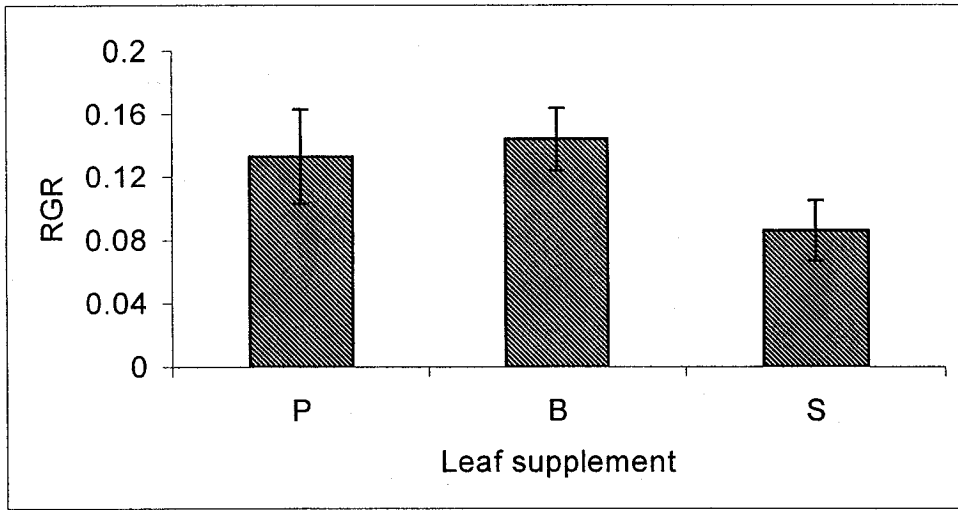
Figure 4.4c: Relative growth rate.
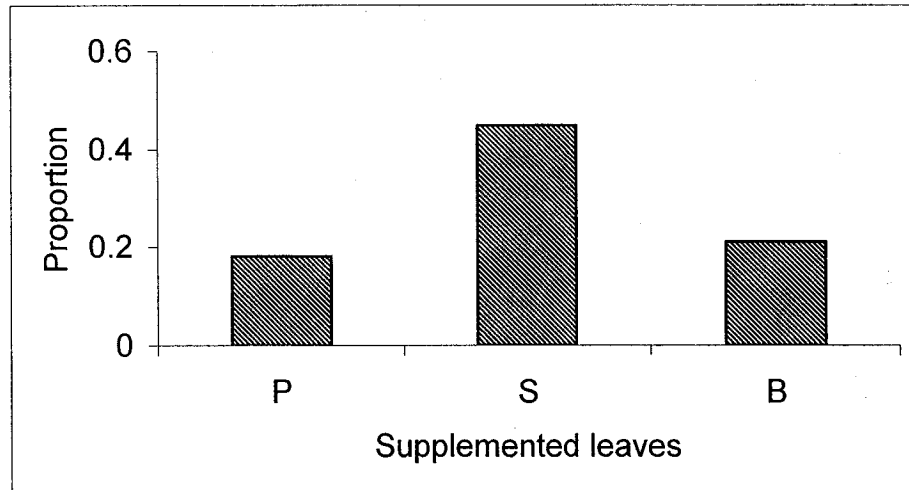
Figure 4.4d: Proportion of insects surviving until the moult to the fifth instar.

Chapter 5
General Discussion

The primary nutrient content of a food source, either natural or artificial, appears to have direct effects on forest tent caterpillar preference and performance. While it is argued that leaves elicit a more natural response, we have shown that by pairing natural and artificial diet studies, we can distinguish between the different primary nutrient constituents responsible for choice behaviour and performance. Artificial foods allowed us to manipulate nutrient concentration and ask specific questions regarding the caterpillars' food choices based on primary nutrient content alone. Experiments with natural foliage supplemented with nutrients confirmed the artificial diet findings in a more natural context.

In artificial food experiments, caterpillars exhibited an initial preference for carbohydrate food, consumed equal amounts of protein and carbohydrate over longer choice tests, and, in a no-choice situation, consumed larger amounts when given balanced than unbalanced food. These findings are consistent with food selection based on non-homeostatic regulation of haemolymph trehalose. Blood sugar levels depend on the consumption of both proteins and carbohydrates, and play a key role in directing feeding in other caterpillars (Bernays and Chapman 2001; Bernays et al. 2000; Bernays et al. 2004; Friedman et al. 1991; Thompson and Redak 2000). While we were not able to test this theory directly, it provides a reasonable explanation for our results.

Behavioural observations made during the first two hours of the artificial food choice treatment indicate that the caterpillars contacted the carbohydrate diet first, and

spent more time eating the carbohydrate diet upon first contact, irrespective of conditioning treatment. The initial contact on the carbohydrate diet suggests an olfactory sensitivity to sugars that has not been previously shown for this insect. The prolonged duration of the first contact on the carbohydrate diet reflects the phagostimulatory power of soluble sugars, and agrees with the results of many other studies on phytophagous caterpillars. All Lepidoptera that have been studied thus far have sugar-sensitive chemoreceptors (Albert and Parisella 1985; Schoonhoven 1967; Schoonhoven 1987), including forest tent caterpillars (Panzuto et al. 2001). In fact, studies by Panzuto et al (2001) showed that when the sugar, amino acid, and salt cells of the medial styloconic sensillum were tested with leaf extracts, only the sugar sensitive cell consistently showed differences in response to the variety of leaf chemicals. This study suggests that the sugar cell is the main chemosensory neuron used in the detection of nutrients by the forest tent caterpillar. Prior findings, combined with those of the present study, suggest that this caterpillar regulates its diet according to the sugar concentration of its food.

Using the caterpillar's natural food source, which contains both protein and sugar, and supplementing with additional primary nutrients (casein and sucrose), we attempted to establish which nutrient influences food choice and which is responsible for enhanced performance. In choice experiments, where the caterpillars had a choice between protein, sucrose and buffer supplemented leaves, we found that initially there was no difference in response, regardless of nutrient supplementation. However, with prolonged exposure, the caterpillars preferentially fed on the control (B) leaf, followed by the sucrose (S) supplemented and protein (P) supplemented leaves, again suggesting that post-ingestive processes lead to selection of a balance of protein and sugar and that the caterpillars need

more time to sample and differentiate between leaves of different quality. The buffer and sucrose leaves exhibited higher sugar to protein ratios, which likely stimulated the caterpillars to feed more than they did on the protein-supplemented leaf. On a longer time scale, in which caterpillars were given only one supplemented leaf type, we found that they experienced the shortest instar duration and greatest weight gain on the control (B) leaf. Performance tests showed that caterpillars did not benefit from protein supplementation. Similarly, Hemming and Lindroth (1999) found that caterpillars showed decreased performance on high protein diets. Schroeder (1986) suggested that the caterpillars were stressed by a high protein diet. On the sucrose-supplemented leaf, survival was greatest, but development was slowed. Similar results were found for locusts (Raubenheimer and Simpson 1999). Trembling aspen leaves contain high levels of sugars: two to three times the sugar concentrations of this insect's secondary host, sugar maple (Lindroth et al. 1993; Panzuto et al. 2001). Thus sugar may be the stimulant used by the caterpillars in host recognition. Perhaps sugar concentration is a good measure of foliage quality, in which case the lack of electrophysiological response to leaf protein content found by Panzuto et al (2001) and the lack of protein regulation shown in this and other studies are not surprising.

The role of sugars as nutrients to lepidopteran larvae has been emphasized in various nutritional studies (Clancy 1992; Harvey 1974). Dethier (1982) suggested that sugars increase growth by stimulating feeding. In addition, sucrose is a good source of energy and it spares the insect from metabolizing protein for energy (Simpson et al. 1988a), thereby directing protein towards growth (Shaw et al. 1978). There is often, in fact, a direct relationship between adult weights and rates of larval development with

increasing dietary levels of sugars (Harvey 1974). For forest tent caterpillars a balance of protein and carbohydrate is required for ideal growth and development. Although many outbreaking herbivorous insects can regulate protein and carbohydrate separately, it appears that separate regulation is not a necessity for all such insects. By eclosing in early spring, when their food is high in both protein and carbohydrates, these caterpillars thrive, initially. However, as the season progresses, the leaves begin to vary more in primary nutrient concentration. By responding to high sugar concentrations in the leaves, the caterpillars regulate intake so that they are able to get the balance of nutrients they need to survive. Many studies to date have acknowledged phenolic glycosides to be a key factor in forest tent caterpillar food selection. Carbohydrate content, however, is known to override deterrents (including phenolic glycosides) in other herbivorous insects, such as *G. geneura* (the woolly bear caterpillar; Bernays and Chapman 2001). Studies have shown similarly for the forest tent caterpillar that there is a preference for high nutrient and high allelochmicals to low nutrients and allelochemicals (Fortin and Mauffette 2001).

The purpose of this study was to determine the effects of primary nutrients separately from those of secondary metabolites. Most studies on the effects of food quality on herbivores are mainly correlational: the insects, instead of being given a dietary choice, are fed diets of differing phenolic glycoside and protein content, while their performance is measured (Hemming and Lindroth 1995; Hemming and Lindroth 2000; Lindroth 1991; Lindroth and Bloomer 1991): it is therefore not clear which food source the insect would choose. The present study investigates both the choices made by caterpillars and the effects of those choices on performance. The next step in solving the puzzle of differential aspen tree defoliation patterns by forest tent caterpillars should be to

combine differing levels of both primary nutrients and secondary metabolites while

observing both preference and performance of the forest tent caterpillar. Detailed work

on the neurophysiology of discrimination is also needed, particularly with respect to the

trehalose concentration in the haemolymph and its role in nutritional regulation. To fully

understand the mechanisms involved in the nutritional regulation of the forest tent

caterpillar, more research must be conducted to probe haemolymph composition and taste

receptor regulation based on nutritional deficiencies. The results of this study suggest

that the forest tent caterpillar regulates digestible carbohydrate only.

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