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NUTRITIONAL ECOLOGY OF THE AUSTRALIAN PLAGUE LOCUST, *CHORTOICETES TERMINIFERA*

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ABSTRACT

The majority of locust nutritional physiology studies have been undertaken using artificial diets. This study has focussed on digestibility of natural diets by comparing dietary utilization and growth and development of the Australian plague locust on two grasses with contrasting life history strategies.

Australian plague locusts develop in Mitchell grasslands following sufficient rain, to allow growth of the two components of Mitchell grasslands, long-lived perennials, mostly Mitchell grass, and a suite of ephemerals of which Button grass dominates. Australian plague locusts appear to preferentially feed on Button grass, but as this grass mostly completes its lifecycle before the locust, the later instars and adults rely on Mitchell grass, which remains greener for longer, to complete development, migrate and to lay eggs in a suitable nutritious environment. Australian Plague Locust Commission field officers have noted that the longer Button grass remains green the more likely plagues are to develop.

Both grasses are chemically very similar, but structurally different with Mitchell grass requiring significantly more work to fracture than Button grass. The two grasses had a similar ratio of cell wall, protein and non-structural carbohydrates in the dry matter, while the specific leaf area and ratio of water to dry matter for Button grass was *c.* twice that of Mitchell grass. Button grass cells were on average larger than those in Mitchell grass.

Consequently, nymphs consuming Button grass gained more weight, developed faster with higher survival rates compared to those consuming Mitchell grass, as they were able to consume and assimilate significantly more dry matter. This appeared to be due to increased consumption (pre-ingestive mechanisms) rather than differences in diet digestibility or metabolic costs associated with processing (post-ingestive mechanisms). Early instars were able to digest and grow on either diet equally, but the later instars consuming Mitchell grass experienced reduced growth and increased instar durations. With increasing age, nymphs grew faster per unit body mass as they consumed dry matter more rapidly and although diet digestibility was lower, dry matter was assimilated faster. Older instar nymphs feeding on Mitchell grass

assimilated less nutrients and at a slower rate, as they had a longer intermeal duration than nymphs on Button grass. This could have been due to anatomical differences between Mitchell grass and Button grass causing the nutrients to be released and absorbed more slowly from Mitchell grass and/or the reduced water consumed with Mitchell grass resulted in increased nutrient concentration in the haemolymph.

Locusts were unable to digest the cell wall, which thus appeared to act as a mechanical barrier to the attainment of nutrients. The percentage of non-cell wall dry matter digested from either grass was the same. However, when the barrier effect of cell wall was removed by grinding, locusts digested less Button grass than Mitchell grass, although no difference in growth or development resulted. Grinding the diet increased diet digestibility resulting in increased dry matter assimilation and, with reduced metabolic costs of converting these nutrients to biomass growth was increased. Increased water consumption did not alter growth and development because while diet digestibility increased, so did the costs of converting assimilates to biomass. Processing (biting and chewing) the diet appears to impose a significant metabolic cost.

Decreased digestibility of the diets with increasing age could be due to proportionately fewer cells being fractured due to the increased size of the mandibles and decreased dietary processing (bites and chews) per unit intake and/or to a relative reduction in food retention time. The older nymphs consuming Mitchell grass took double the number of chews per bite of food than those consuming Button grass. A model describing how the locusts consume a grass blade is proposed.

Nutrient transfer from the environment to insect herbivores, and thus insect population dynamics, appears to be influenced by the anatomical and biomechanical properties of plants interacting with insect size. Although, plant cellular structure affects diet digestibility and post-digestive processing costs, the plant anatomy appears to influence the rate of nutrient digestion and assimilation, which consequently affects the amount of dry matter consumed. Small changes of nutrient concentration within the diets also appears to affect assimilation rates.

STATEMENT OF RESPONSIBILITY

To the best of my knowledge, this thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other institution, nor material previously published or written by any other person, except where due reference is made in the text of thesis.

[redacted]

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CHAPTER 1. GENERAL INTRODUCTION

Herbivores play a central role in most terrestrial ecosystems (Hartley and Jones 1997; Olff *et al.* 1999). Herbivory is usually considered to be detrimental to plant growth (Crawley 1993) and can influence plant population dynamics (e.g. Augustine and McNaughton 1998; Belsky *et al.* 1993; Crawley 1989; McNaughton 1983). Despite continuous 'predation' by herbivores, plant communities on the whole remain 'green'. Many herbivores, particularly insects, have high reproductive potential, as demonstrated by occasional outbreaks. Several hypotheses have been proposed to explain how plant communities remain 'green' in spite of the presence of herbivores (e.g. Feeny 1976; Hairston *et al.* 1960; Lawton and McNeill 1979; White 1985). In regard to insect herbivores, it is argued that plants have a larger impact on insect population dynamics than insects have on plant population dynamics (e.g. Crawley 1989; Schoonhoven *et al.* 1998).

Plants have evolved physiological, morphological and behavioural traits to avoid or tolerate the stresses that herbivory imposes. These traits influence the nutritional quality of food and affect insect herbivore survival, rates of development and growth, and reproductive output (e.g. Joern and Behmer 1997; Scriber and Slansky 1981; Simpson and Raubenheimer 1993b). Both the nutritional quality of plants and the nutritional requirements of insects vary in time, which has meant that insect herbivores have evolved an array of 'strategies' that allow them to maximize development and reproductive output. Characterizing the nature of compensation in response to changes in the nutritional environment is important in understanding insect population dynamics.

Many hypotheses have been proposed to explain the host choice of herbivores (e.g. Behmer and Joern 1993; Berenbaum 1995; Bernays and Graham 1988). Essentially, host choice can be limited by (1) abiotic and biotic factors other than plant dietary factors, and/or (2) quantity and quality of the host plant, i.e. not all plants or plant parts are edible and what is edible can be of sub-optimal quality. Plants can be a poor nutritional source for insect herbivores because available nutrients are diluted in a matrix of indigestible cell wall components and often contain toxic allelochemicals.

Food intake, utilization and allocation is a dynamic process for insects (e.g. Abisgold and Simpson 1987; Simpson *et al.* 1995; Slansky and Rodriguez 1987; Yang and Joern 1994c). It has been proposed that feeding behaviour be considered in terms of intake, nutrient and growth targets (Fig. 1.1). These targets can be considered 'global' optima in that they represent the best outcome for that genotype (over a specified period during its ontogeny) under the environmental conditions prevailing during its evolution (Raubenheimer and Simpson 1994). The growth target is the optimal amount and blend of nutrients required to build new tissue; the nutrient target is the optimal amount and blend of nutrients required for growth plus maintenance and all other activities; the intake target is the amount of nutrients that needs to be ingested in order to reach the nutrient target, given that not all ingested nutrients are accessible (Raubenheimer and Simpson 1993; Raubenheimer and Simpson 1994; Simpson and Raubenheimer 1993b; Simpson and Raubenheimer 1995).

In a nutritionally heterogeneous environment insects can employ pre- and/or post-ingestive mechanisms to reach the growth target. Pre-ingestive mechanisms include diet choice and regulation of the amount consumed (e.g. Abisgold *et al.* 1994; Chambers *et al.* 1995; McGinnis and Kasting 1967). Post-ingestive mechanisms involve regulation of extracellular digestion, assimilation and excretion (e.g. Simpson 1982a; Yang and Joern 1994c; Zanotto *et al.* 1993; Zudaire *et al.* 1998). Phenotypic variation can increase niche feeding breadth (Bradshaw 1965), especially in insects because they can alter resource allocation at each moult (e.g. Thompson 1992; Yang and Joern 1994b). If an insect cannot reach a target due to either the properties of the diet or other ecological factors (e.g. temperature), it will be forced to compromise and may moult or reproduce at a sub-optimal size and condition.

Plant constituents can be divided into two main categories, the easily digested cell contents and the relatively indigestible cell wall (Bacic *et al.* 1988). The majority of herbivorous insects cannot digest cellulose, the major component of cell wall (Martin 1991). It is suggested that insects have not evolved the capacity to liberate the energy in cellulose because in gaining sufficient nitrogen, carbohydrates from the cell contents are gained in excess of requirements. Evidence shows that growth and reproduction in herbivorous insects is limited by nitrogen and/or water not carbohydrate (Martin 1991; Slansky and Scriber 1985). While larval lepidopterans appear able to access cell contents without rupturing the cell wall (Barbehenn 1992),

orthopterans have different gut conditions (Evans and Payne 1964; Ferreira *et al.* 1990; Uvarov 1966) that suggest the cell wall must be ruptured to liberate nutrients. Evidence to date for the mechanism causing rupture is equivocal (Ferreira *et al.* 1992b; Hochuli *et al.* 1993), and while it is thought that the mandibles are primarily responsible, it has been suggested that enzymes that degrade cell wall may assist cell rupture (Ferreira *et al.* 1992b). Orthopterans, when consuming plant material, leave a considerable amount of diet unabsorbed and are also very inefficient at converting absorbed material to biomass (Slansky and Scriber 1985). Thus, plant cell wall may be a limiting factor in nutrient gain and therefore may be an important factor defining host choice (Abe and Higashi 1991; Martin 1991; Murdoch 1966).

Mandible morphology is important in defining feeding niche breadth (Bennack 1981; Boys 1981; Gangwere 1966) and dietary processing may be affected by mandible wear (Chapman 1964). Models of insect nutrition include nutrient limitation only as a result of insect mandible morphology. Distinctive oral morphology is associated with preferred dietary plant types (monocotyledons and dicotyledons) with both herbivorous mammals (Archer and Sanson 2002) and locusts (Chapman 1964; Isely 1944). For mammals, functional dental morphology is tightly linked to processing strategy and diet choice, and mastication processes limit nutrient gain (e.g. Sanson 1985). Very few studies of grasshoppers have investigated the effect of plant anatomical structure on its digestibility (e.g. Caswell and Reed 1976; Heidorn and Joern 1984), and the role it plays in grasshopper feeding strategies is unclear. A study that combined plant structure and locust mandible morphology showed that assimilation was significantly influenced by mandible morphology, but not by plant structure (Bennack 1981). Artificial diets are almost always produced from ground components. Consequently they require less processing to access the nutrients, as the nutrients are not 'sequestered' within the plant cells. Secondly, artificial diets commonly have lower concentrations of indigestible material and higher concentrations of nutrients than a natural diet. Hence less investment is required to gain nutrients when feeding on an artificial diet. As with mammalian nutritional models, the oesophageal boundary between the oral models and those limited by the boundaries of the gastro-intestinal tract needs to be surmounted (Sanson 1985).

Grasses have many characteristics that enable them to tolerate tissue loss better than the majority of dicotyledons (Rosenthal and Kotanen 1994; Tscharntke and Greiler

1995). In addition, the majority of grasses lack the variety of secondary compounds suggested to deter herbivores feeding on dicotyledons, and if present, they are often in very low quantities (Tscharntke and Greiler 1995). Grasses can provide all the nutrients necessary for insects (Bernays and Barbehenn 1987) but their nutritional value is affected by the amount of cell wall and the anatomical structure and biomechanical properties of the tissue (e.g. Boutton *et al.* 1978; Caswell and Reed 1976; Hedin *et al.* 1990; Hehn and Grafius 1949; Heidorn and Joern 1984; Van Soest 1994b; Wright and Vincent 1996) and the presence of silica (Peterson *et al.* 1988; Vicari and Bazely 1993).

Few studies on insects have included body size as a factor when considering insect nutritional requirements (e.g. Bernays and Simpson 1990; Reavey 1993; Simpson and Simpson 1990), which is surprising because of the 'gating' effect of insect moulting. However, it is difficult to quantify the diminutive amounts consumed by early instars. Insects, unlike mammals, are in a constant state of growth and for most insects success depends on their ability to complete their life cycle within a small 'window of opportunity'. Generally, as insects increase in age, both relative intake per gram body mass and diet digestibility are reduced, but the efficiency of converting assimilate to growth is increased (e.g. Slansky and Scriber 1985). The mechanisms behind this trend have not been elucidated and are likely to be complex (Simpson and Simpson 1990).

Understanding plant-herbivore interactions requires laboratory studies of the nutritional physiology of the insect as well as field studies of the plant, its herbivores and the community in which they both live. This thesis will concentrate on factors that limit nutrient transfer from the environment to an insect herbivore and the effects on insect performance and life history dynamics. The aim of this thesis is to investigate the nutritional relationship between the Australian plague locust, *Chortoicetes terminifera* (Walker), and the two dominant grasses of the Mitchell grass community they inhabit.

The Australian plague locust and Mitchell grasslands

The Australian plague locust is the most damaging of the four locust pest species in Australia, due to its area of infestation and frequency of plaguing (Australian Plague Locust Commission 1986). In eastern Australia, Australian plague locusts frequently

breed in the Mitchell (*Astrebla* spp.) grasslands of the arid and semi-arid interior (Australian Plague Locust Commission 1986; Wright 1987) (Fig. 1.2). Mitchell grasslands are endemic to Australia and occur on cracking clay soils extending in an arc from the Kimberley region in Western Australia through the Northern Territory, Queensland and into northern New South Wales (Orr 1986). The arid and semi-arid interior is characterized by having highly fluctuating rainfall in both time and space. To exploit this environment a plant must either avoid drought stress by only growing when conditions are favourable, e.g. an annual life cycle, or by tolerating long periods of drought. Mitchell grasslands comprise two floral components: (1) long-lived tussocks of predominantly *Astrebla* spp. (perennial grasses), and (2) a suite of ephemerals (mostly annual forbs and grasses) whose populations fluctuate in size over a relatively short time. The biomass and relative composition of these two components is determined primarily by the interaction between rainfall and season, and secondarily by sheep and cattle grazing (Orr 1975).

Curly Mitchell grass (*Astrebla lappacea* Lindl.) and Button grass (*Dactyloctenium radulans* (R. Br.) P. Beauv.) are two grasses with contrasting life history strategies that occur predominantly in Mitchell grasslands. Curly Mitchell grass (referred to hereafter as Mitchell grass) is the dominant species in the regions where locusts develop, while Button grass is typically the predominant annual that often forms dense swards between the Mitchell grass tussocks (*pers. obs.*; Orr 1986). Observations suggest that early instar nymphs select Button grass and other ephemerals in preference to the perennial species (Hunter *pers. comm.*).

Soil moisture is the major factor limiting plant growth in Mitchell grasslands, although, temperature and nitrogen also influence the rate of plant growth (Christie 1981; Orr 1986). While nitrogen concentration in these soils is low and phosphorus concentration is variable, plant growth is not thought to be nutrient limited until after 8-12 weeks of continuous summer growth (Christie 1979a).

Both grasses are from the same tribe (Eragrosteae) in the Poaceae (Wheeler *et al.* 1990) but differ markedly in the strategy they use to avoid or tolerate the highly stochastic environment they inhabit (Watson and Dallwitz 1985). Both utilize the C_4 photosynthetic pathway and have similar anatomy. Mitchell grass, a long-lived perennial, has extremely deep roots enabling the plant to survive long periods of

drought (Orr 1986). In contrast, Button grass is an annual that emerges following summer rain, matures quickly, flowers and dies unless follow-up rain occurs.

Annuals typically have more nutrients (protein and water) relative to indigestible cell wall material than perennials (Chapin III 1991; Garnier 1992), therefore Button grass may be expected to be a higher quality resource for locusts than Mitchell grass. While the nutrients are likely to be in similar anatomical locations within the leaf tissue, it is hypothesized that the ability to access them will differ due to a higher water content and lower cell wall content of Button grass, making accessibility easier compared to Mitchell grass.

Both Australian plague locusts and the two grass species have very similar requirements for growth. Mitchell grass and Button grass are both summer-growing species, with Mitchell grass reported to have maximum growth at 30°C (Christie 1975; Christie 1979b; Jozwik 1970). Australian plague locusts reared under constant temperature conditions only survive to fledging between 23°C and 41°C (Gregg 1981). The grasses respond rapidly to rainfall with both species producing green shoots two days after a minimum of 20-40 mm of rainfall. Unless further rain falls Button grass dries off in 4-6 weeks having completed its lifecycle, while Mitchell grass still has some green foliage 8-10 weeks later (Hunter 1989; Hunter and Melville 1994) (Fig. 1.3).

The Australian plague locust's life cycle passes through three main developmental stages, egg, nymph and adult (Fig. 1.4). Quiescent locust eggs require a minimum of 15 mm of rainfall to complete embryonic development and hatch, slightly less than that required for sustained growth of Mitchell grass (Wardhaugh 1980). Under optimal conditions nymphs take 20-25 days (with females taking longer than males) undergoing five nymphal instars to reach the adult stage. If conditions are dry or cold, an extra instar can occur (Australian Plague Locust Commission 1986). Mortality is generally highest during the first instar (Farrow 1982). If green pasture is available, the adults grow, accumulate fat, and unless further rain falls, they migrate and lay in areas where it has recently rained (Hunter *et al.* 1981; Hunter *et al.* 2001). If sufficient follow-up rain falls, the resident locust population will lay where they developed and their progeny will migrate.

Lipids are required to fuel long distance night flight (up to several hundred kilometres). If only one fall of rain occurs, Button grass dries off and dies before the locust has reached the fat accumulation stage in the adult phase. Since follow-up rain rarely falls, locusts usually have to switch diets, from the annual Button grass to the perennial Mitchell grass (Hunter 1989; Hunter *et al.* 2001). Field studies suggest that less than 10 % of nymphs survive to reproductive adult stage with rainfall explaining 79% of the variation in mortality (Farrow 1982). In the only laboratory study to date on locusts reared on Mitchell grass grown under four different water regimes, it was found that while tiller nitrogen correlated with tiller moisture, nymphal survival correlated better with tiller moisture than tiller nitrogen (Phelps and Gregg 1991). Thus, it has been hypothesized that access to 'good' quality food increases nymphal survival and therefore population survival.

During their lifespan, Australian plague locusts thus have to switch from a diet that is hypothesized to be soft and high in nitrogen, to a tougher diet with lower nutrient concentrations. This is possibly a more severe change than that which would occur with the increase in cell wall material and decrease in N that occurs within a plant as it ages. In addition, Mitchell grass tiller quality decreases as the soil dries out (Phelps and Gregg 1991). It is thought the size of the adults and potentially the number of eggs laid is affected by the duration of Button grass growth (Farrow 1982), while Mitchell grass allows locusts to migrate and lay (Hunter 1989). In a dietary environment that can deteriorate rapidly, if locusts are unable to maintain development by altered pre- or post-ingestive mechanisms, that population may become locally extinct.

The major aims of this thesis are to investigate

1. dietary utilization by the Australian plague locust feeding on the two dominant grasses of their natural habitat,
2. the effect of insect instar and body size on dietary utilization, and
3. the effect of plant structure on utilization by the locusts.

Thesis structure

This thesis explores the relationship between locust age and feeding requirements, the interaction of this with two diets of contrasting life history strategies, and how this might explain locust dynamics in the field. Diet digestibility and utilization were used to evaluate the performance of the locusts on the two grasses. Studies of diet digestibility and utilization have technical limitations that must be addressed (Bowers *et al.* 1991; van Loon 1991). In Chapter 2, I describe how methodologies were established that minimized changes in grass chemistry and obtained correction factors for any changes occurring during digestibility trials. Knowledge of how an insect overcomes the plant cell wall barrier is required to understand what may limit nutrient transfer from the environment to an insect. Therefore, in Chapter 3, I examine the locust's ability to digest plant cell wall. In Chapter 4, I report the chemistry, and in Chapter 5 the biomechanical, anatomical properties of the two grasses and the digestive capacity of the locusts. Diet digestibility and utilization was measured for Instar II to adult locusts feeding on two grasses (Chapter 4) and the feeding strategy employed (Chapter 5). Phenotypic plasticity can increase the niche breadth that an organism can occupy. In Chapter 6 I investigate phenotypic plasticity and the effect of body size, controlled for instar, on utilization and digestion of grass. The effect of plant structure on the intake, nutritional and growth targets of Instar V locusts is reported in Chapter 7. Finally, a general discussion synthesizes and integrates my findings with respect to the biology and ecology of Australian plague locust and locust digestive physiology and biology.

FIGURES

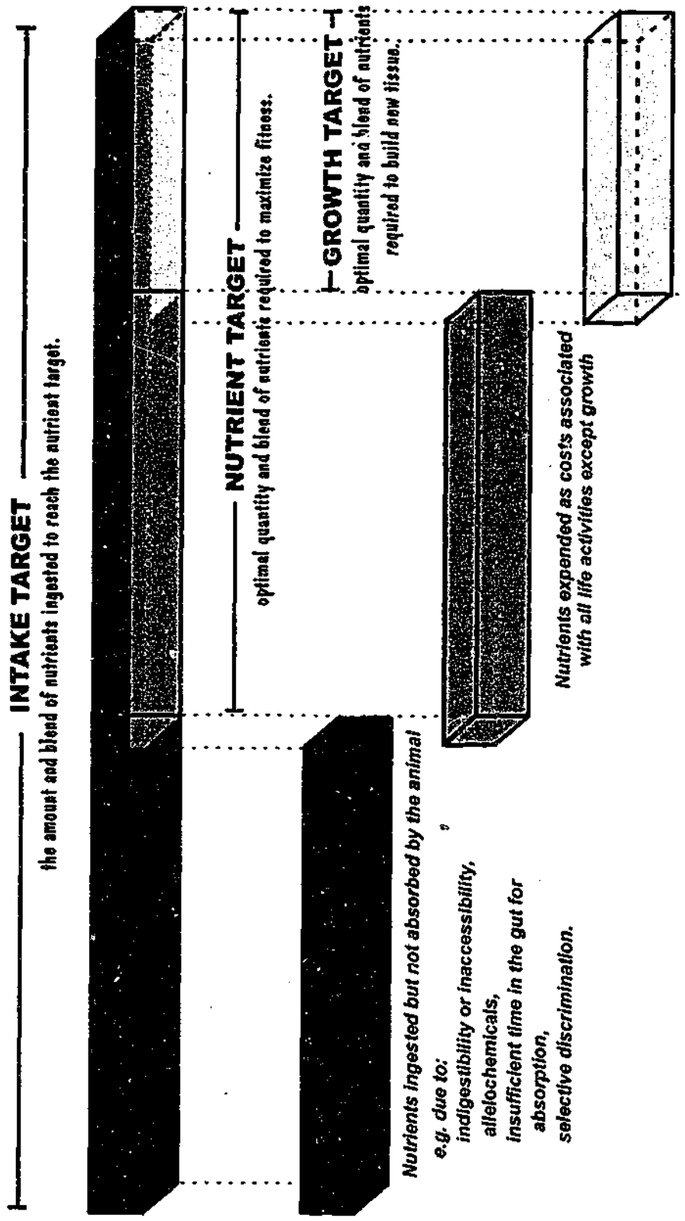


Figure 1.1 Growth of an organism utilizes consumed nutrients that are assimilated but not used for metabolic processes. Not all nutrients absorbed can be allocated to growth because of metabolic costs associated with life activities. Not all nutrients consumed are absorbed for many reasons. A diet can be categorized by the efficiency with which nutrients are absorbed and growth achieved given total nutrient consumption.

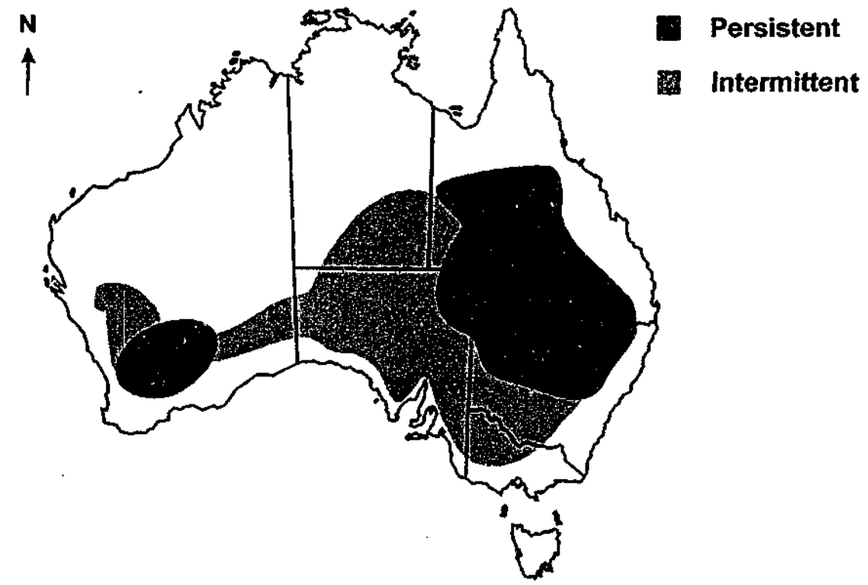


Fig. 1.2 Distribution of the Australian plague locust in Australia showing areas of persistence and areas that are invaded intermittently if conditions are favourable (Australian Plague Locust Commission 1986). The persistent area in eastern Australia corresponds to Mitchell grassland distribution.

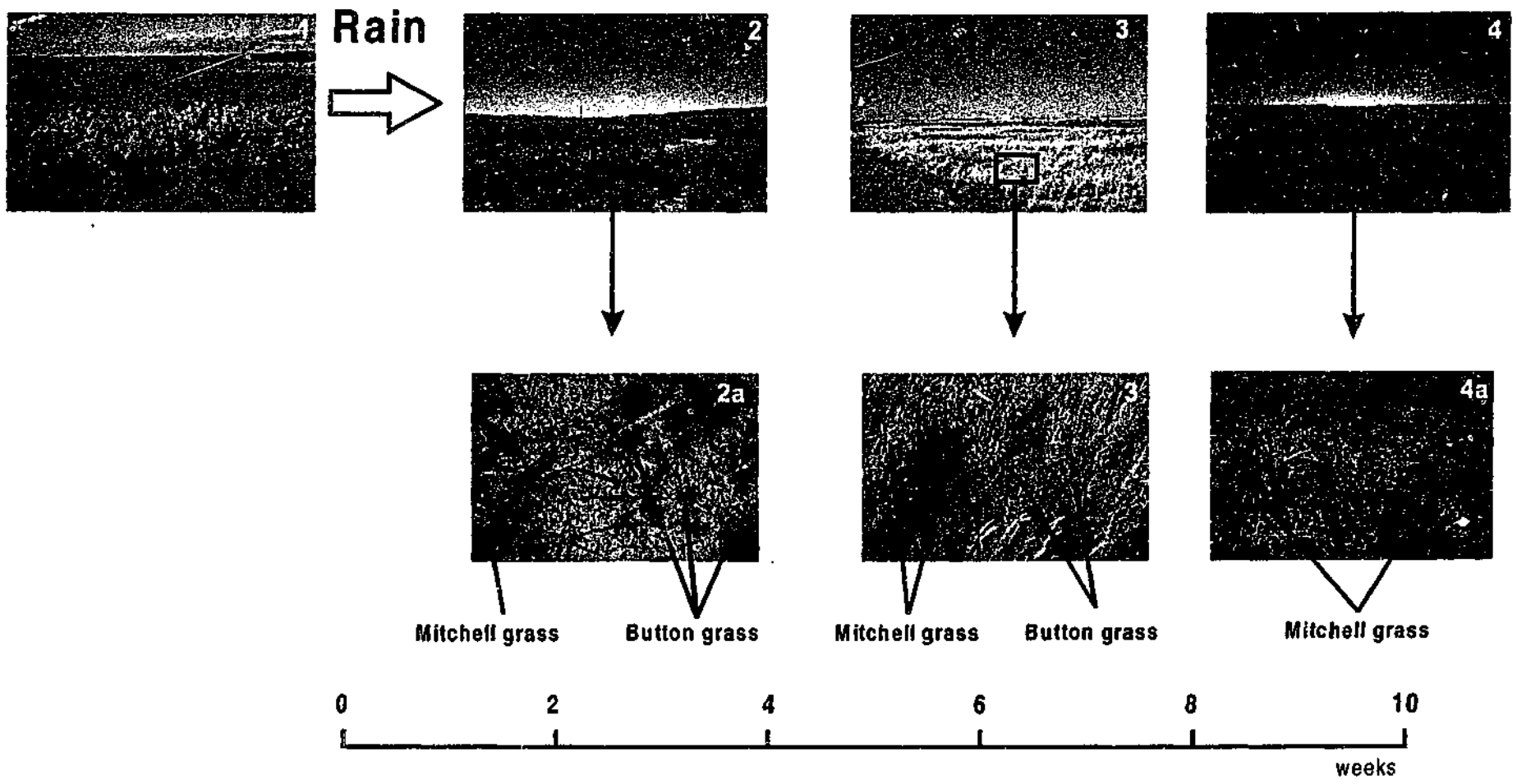


Figure 1.3 Mitchell grasslands shown (1) before a rainfall event and over 5 months since the last rain, and following rain at approximately (2) 3 weeks, (3) 6 weeks, and (4) 11 weeks. The inserts show the condition of the Button grass and Mitchell grass foliage. Following rain, both grasses have green foliage at 3 weeks (2a) but by 6 weeks only Mitchell grass has some green foliage with Button grass having already dried off (3a), and by 11 weeks Mitchell grass has also dried off (4a). Photos (1) and (2) were taken at the same location (Fowlers Gap, western N.S.W.), photo (3) south of Quilpie (southern western Qld,) and photo (4) was taken near Mitchell (southern Qld,).

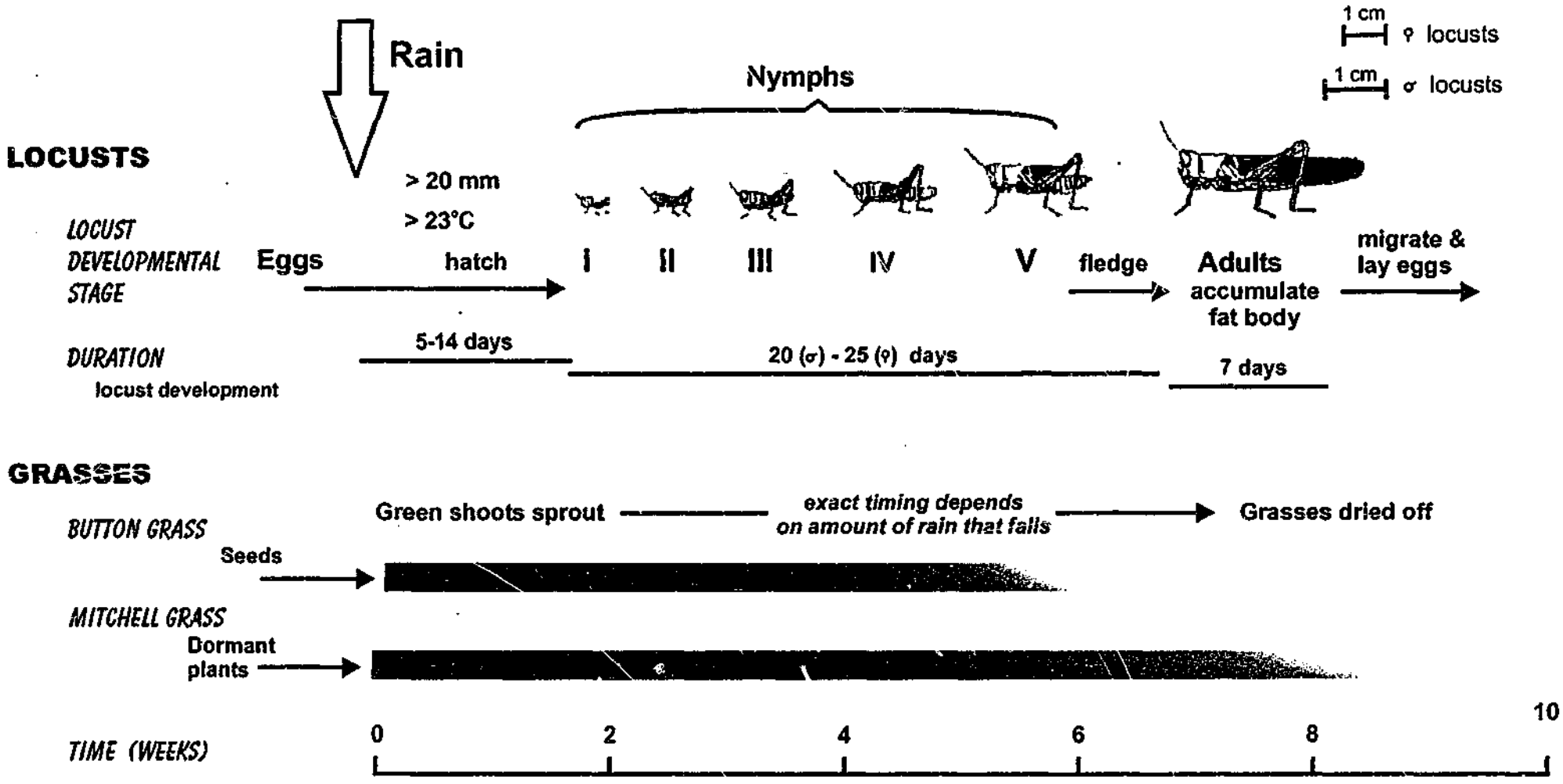


Figure 1.4 Typical life cycle of Australian plague locusts in Mitchell grasslands. Following a rainfall event of greater than 20 mm and with temperatures greater than 23°C, Australian plague locust eggs hatch and green shoots are produced rapidly from Button grass seeds and dormant Mitchell grass plants. The length of time that both grasses retain green leaves, depends on the amount of rain that falls. Insect stage durations given are for optimal conditions.

CHAPTER 2. INSECT HUSBANDRY AND PRESENTATION OF EXPERIMENTAL DIETS

SUMMARY

1. Quantification of diet utilization and utilization efficiency by insects requires consumption and growth to be derived from other parameters. Small errors in these estimations, can significantly affect conclusions regarding derived response variables. Plant metabolism during the course of an experiment not only affects the quality of the diet but also the quantity, which may in turn alter an insect's feeding behaviour.

2. Experiments were undertaken to determine a technique that minimized changes in plant chemistry during the experimental period.

3. Where plant chemistry (water loss and metabolic factors) changed during the course of an experiment correction factors were determined for both grasses.

4. Experiment duration, locust handling and numbers of replicates were examined.

5. Locust husbandry is described.

INTRODUCTION

Insect performance on a food resource can be quantified at different scales and in various ways, e.g. life history parameters (survival, time taken to reach maturity), measurements of dietary utilization (intake, assimilation) or utilization efficiency (insect metabolic rate on different diets). In this thesis, I wanted not only to determine the effect of two grasses on the life history of Australian plague locusts, but also to identify the patterns, trade-offs or compensatory responses made by locusts to achieve the measured outcome. This information will enable identification of the mechanisms that may be limiting nutrient assimilation and thus affecting population survival. Specifically, I wished to quantify the effect of two diets on Australian plague locusts in terms of:

(1) Performance, e.g. as measured by survival rates, growth (attainment of biomass), instar duration;

(2) Dietary utilization, including amounts and rates of consumption, digestibility and processing efficiencies.

Diet utilization has been measured by gravimetry (Waldbauer 1968), respirometry (van Loon 1991) and the use of gravimetry with an indirect estimate of consumption e.g. markers (Barbehenn 1993; Parra and Kogan 1981). Intake is estimated with all techniques. Gravimetry estimates intake from the ratio of fresh to dry weight derived from a subset of the diet. Respirometry derives intake by measuring respiration, frass production and growth, where

consumption (C) = respiration (R) + growth (G) + frass (FU).

When all four parameters are measured separately the energy budget rarely balances (Axelsson and Agren 1979; McEvoy 1985; van Loon 1991). There are some technical difficulties (discussed in this chapter) with the gravimetric technique, mostly with the estimation of C and G. The effect of errors in these measurements on subsequent calculations can be minimized (Bowers *et al.* 1991; Schmidt and Reese 1986; van Loon 1991; Wightman 1981). The choice of the method used for statistical analysis is critical (Horton and Redak 1993; Raubenheimer and Simpson 1992) and this is discussed further in Chapter 4. To be able to determine food utilization, four

factors need to be measured from which other indices of performance can be derived. These four factors are:

(1) Consumption of the diet,

(2) Frass produced,

(3) Growth or biomass gained/lost,

(4) Duration of the experiment.

The problems associated with these measurements have been extensively reviewed (Bowers *et al.* 1991; Schmidt and Reese 1986; van Loon 1991). However, a brief summary of these problems and others that effect of the calculation of derived nutritional performance parameters is included as a background to the decisions made when designing the techniques used here.

Determination of consumption

Consumption is derived from

$$C = F_i - F_e$$

where F_i is food dry weight offered at t_i, the time at which the experiment began, and F_e is dry weight of the remaining food (orts) at t_e, the time when the experiment ended. Consumption is expressed in dry weight units, so F_i must be estimated when using diets that contain any water. Hence,

$$F_i = FW_i \times DM$$

and

$$DM = [(DW_{i1}/FW_{i1} + DW_{i2}/FW_{i2} + \ + DW_{in}/FW_{in})/n]$$

where FW_i is fresh weight of the food offered at t_i, DW_i is dry weight of a matched sample of fresh food, and n = number of replicates. This 'ratio' is only an estimate of the initial dry weight offered, as plants or plant parts are not homogenous. The method that minimizes the effect of variation between leaves in DM is where a leaf is split in half and half is given to test insects and the other half is analyzed (Waldbauer 1968). It was not possible to use this method with my experiments as (1) it was logistically impossible with the number of insects I needed to feed each day;

(2) locusts consumed up to six whole leaf blades per day and individual leaves could not be marked to ascertain which orts belonged to which leaf; (3) it was not possible to determine chemical constituents of many extremely small samples of leaves. F_i has been measured with other methods including using indigestible markers to determine intake from the faecal matter produced, e.g. silicon (Barbehenn 1993) or wax alkanes (Dove and Mayes 1991) or converting surface area eaten to dry weight. However, these techniques have the same problem as that based on a fresh weight to dry weight ratio, as plants are not homogenous so ratios based on any factor have an inherent error.

F_e can be directly measured, but the relationship between F_e as measured at t = 24 h and F_e if it had been weighed at t = 0 h, is dependent on how much the food material has varied during the experiment (Bowers *et al.* 1991; Schmidt and Reese 1986; Slansky Jr. and Scriber 1985). Consumption can be over-estimated if the diet loses weight due to respiration (Axelsson and Agren 1979), or underestimated if it gains weight due to photosynthesis (Wightman 1981). This error increases the higher the proportion of food remaining compared with that offered (Schmidt and Reese 1986; van Loon 1991). Modelling of errors in the estimation of food consumption has shown that they can have a substantial effect on the values of other derived nutritional indices. For example, a 0.2% error in the estimation of dry matter can lead to a 20-25% error in the efficiency of conversion of digested matter (ECD) estimation (Schmidt and Reese 1986). However, when Bowers *et al.* (1991) accounted for actual plant metabolism, they only found a 0.75% difference[1] in AD (approximate digestibility) between that calculated without correction for plant metabolism and when calculated corrected for plant metabolism. Intake derived from other methods also is affected by plant metabolism occurring throughout the experiment's duration.

[1] A 7.5% difference is stated in the paper, however when the actual results (given) are recalculated only a 0.75% difference is found. All other results in Table 6B agree with the results given in Table 6A when recalculated.

Presentation of the diet

The aim was to provide the locusts with a food source that resembled that of the living plant as closely as possible and have it remain that way throughout the duration of the feeding period, so that (1) problems involved with the measurement of consumption could be avoided, and (2) that the food utilization measured could be related to the biology and ecology of the plants and locusts in the field (in terms of indicating susceptibilities (Grime 1965)). Only the leaf blades were used in order to reduce the effect of heterogeneity between plant parts (Albrecht *et al.* 1987; Borrell *et al.* 1989; Watson and Casper 1984), thus minimizing any effects of dietary selection by the locusts on plant parts. While *ad lib.* food was provided, only enough was supplied to ensure that only the locust's responses under no-choice conditions were measured. Detached leaves were used, as it was not possible to confine the locust to the leaf blade on an intact plant and estimate consumption from removed surface area. This introduced another set of potential errors that could affect the calculation of consumption in terms of actual dry weight intake but also 'diet quality' (McCaffery 1982; Olckers and Hulley 1994; Risch 1985) which affects insect performance and utilization. Factors causing potential errors include moisture loss from the leaves, the loss/gain of dry matter due to respiration/photosynthesis, i.e. change in the ratio of DW:FW, metabolism of compounds e.g. proteins and carbohydrates, and triggering of secondary metabolites (factors that may affect ingestion and digestion of the diet).

While some of these factors are more likely to be altered in excised leaves, all the above factors need to be considered whether or not the leaves are attached to the plant (Baldwin and Ohnmeiss 1994; Detling *et al.* 1979; Karban and Myers 1989; Wallace 1990).

Experiments were performed to (1) determine a technique that minimized changes to the grasses; (2) determine the time they could be offered to the locusts; and (3) quantify any changes in terms of metabolic losses/gains, water content, total protein and total non-structural carbohydrates in the grasses during the length of time it was offered to the locusts.

Measurement of frass

Frass (FU) produced can be underestimated due to coprophagy, respiration, microbial bacterial activity, and loss of volatiles (Edwards and Wightman 1984; van Loon 1991; Wightman 1981). Overestimation of FU results from contamination by endogenous material, such as uric acid and other nitrogenous wastes, and the peritrophic membrane. Underestimation of FU produced will lead to an overestimation of approximate digestibility while the reverse is true if FU is overestimated. However, Waldbauer's (1968) nutritional indices were developed knowing that the peritrophic membrane and endogenous wastes were part of the frass, and hence the term 'approximate digestibility'. The other dilemma is what to categorize partially to fully digested food remaining in the gut. This food is by definition neither frass nor orts but can affect the measurement of diet digestibility if this is being determined over a short time frame (Barbehenn and Keddie 1992; Bowers *et al.* 1991).

Measurement of growth

Growth is measured as

$$G = B_e - B_i$$

where B_e is the dry weight of the insect at t_e and B_i the initial dry weight of the insect. B_i is estimated by the determination of the dry weight from a group of sacrificed insects at t_i. This is affected by varying amounts of water in an individual (Yang and Joern 1994c), and variations in gut contents (Barbehenn and Keddie 1992; Edwards and Wightman 1984). B_e is measured directly but can be underestimated because not all exuviae and secretions e.g. digestive enzymes, peritrophic membrane, can be collected. Failure to remove or account for plant material consumed but not excreted resulted in an over estimation of dry weight by 18-19% and it significantly affected determination of relative growth rate for final instar larvae of *Paratrytone melane* (Lepidoptera) (Barbehenn and Keddie 1992). Edwards and Wightman (1984) working on the coleopteran, *Paropsis charybdis*, found that the guts and their contents contributed up to 50% of total larvae dry matter and 15% of adult dry matter.

Duration of experiment

Intake varies within an instar (Simpson and Raubenheimer 1993b) and among instars (Simpson and Simpson 1990). It is difficult to compare diet utilization between instars because instar duration varies between instars when fed identical diets, and the diet consumed can affect instar duration. To compare the utilization of two diets by the locusts, it was decided to use an entire instar, as moulting is essential for survival. This was not possible for adults. The adult phase consists of a somatic growth phase during which lipid reserves of fat body are built up (Walker *et al.* 1970) similar to what occurs during an instar (Hill and Goldsworthy 1968). I decided to investigate food utilization during the adult male somatic growth phase only.

The use of a physiological time period (an entire instar) also avoids other problems, i.e. (1) the gut in most species is relatively empty when moulting (Waldbauer, 1968); and (2) attempting to starve an insect to clear the gut pre-and post- diet is often unsuccessful (the gut does not empty) (Waldbauer 1968) and starvation prior to measuring food utilization can alter the digestive strategy (Bernays and Weiss 1996), which masks the effects of the treatment diets (Grabstein and Scriber 1982).

Effect of handling

Bowers *et al.* (1991) showed that disturbance stress had a significant effect by increasing instar duration but not on the biomass gained. Regardless of the method used to change the diets and remove the orts, stress to every locust should be the same and equivalent across treatments, i.e. whilst stress could affect the locusts it should not affect the direction of dietary effect (Grime 1965). An experiment was performed to investigate different methods of handling the locusts when changing the diets. The method that resulted in the faster growth and greater intake was chosen as this was deemed to be the less stressful of the two methods investigated.

MATERIALS AND METHODS

Locust husbandry

Australian plague locusts were cultured to provide sufficient numbers for experimentation when required. It is acknowledged that laboratory cultures often perform differently from wild stock. However, it would have been impossible to conduct these experiments on field-caught grasshoppers, because of the unreliability of numbers and the chronic diseases prevalent in wild populations.

Australian Plague Locust Commission field workers collected fledging adults or late Instar V nymphs in the field at various times over the locust distribution range in eastern Australia, which were cultured.

Stock cultures were reared in a constant temperature room maintained at 32 ± 0.5°C (mean ± se) with a 16:8 h L:D photoperiod (lights on at 0600 hrs E.S.T.). These conditions prevent nymphal diapause (Wardhaugh 1979). Nymphs were raised in cages constructed of fine terylene netting sewn to fit over a 25 x 25 x 25 cm metal frame. Newly fledged nymphs were transferred to metal cages (L x W x H; 52 x 44 x 47 cm) similar to those described by Dudley *et al.* (1962) as modified by Gregg (1981). These cages had false bottoms covered with perforated metal to allow the frass to pass through and recesses to hold the sand containers in which the females oviposit.

A fluctuating temperature regime was chosen, as this optimizes both development time and survival rates (Gregg, 1981). The fluctuating temperature regime was maintained by placing a 100 W incandescent bulb either in the metal cages or just to the rear of the cloth cages. A 16 h light photoperiod was chosen to ensure only non-diapausing eggs were laid (Wardhaugh, 1980). Under these conditions, temperatures in all cages were 31.9 ± 0.1°C during the dark phase and 37.0 ± 0.1°C on the floor and 43.9 ± 0.1°C within 12 cm of the light bulb in the metal cages and 41.7 ± 0.2°C in front of the light bulb in the cloth cages during the light phase. Strips of wire mesh, 8 cm x 40 cm, were placed diagonally at the rear of the metal cages as a perch. This allowed the locusts to bask and regulate body temperature. Locusts are able to maintain body temperatures above ambient temperatures (Hunter, 1981) which is essential in the control of internal parasites (Carruthers *et al.* 1992; Gregg *pers. comm.*). High humidity has been linked

with the increase of many pathogens (Hinks and Erlandson, 1994; Simpson *pers. comm.*). Humidity was not controlled but remained relatively constant between 10-40% RH. Under these conditions a new generation was produced every 6.5-7 weeks.

Sand was provided for oviposition in containers 9.5 cm deep x 10.5 cm diameter (Brand Pak). Triple-washed builders sand was mixed with unwashed builders sand (4:1, v:v) to provide the clay levels necessary for oviposition (Gregg 1981). The sand was sterilized (autoclaved 90 mins) and then moistened with 12 ml of sterile water containing 0.5 µg/ml fungizone® (Bristol-Myers Squibb Company) per 100 g sand mix to prevent fungus growth. The sand containers were stored at 4°C until used. The sand containers were changed twice weekly and those containing eggs were moistened if dry, sealed and incubated at 32°C.

As the previous dietary history can influence subsequent digestion of food (Stockhoff 1992; Yang and Joern 1994b), it was necessary to ensure that all locusts were reared on the same diet. Therefore, only seedling wheat blades were provided to the instars. Initially, the locusts were provided daily with 14 day-old seedling wheat grown in a glasshouse in 'organic mix' soil (nursery supplier; *c.* 50% mushroom compost, 40% top soil, 10% clay). The seedling wheat was harvested and the leaf blades were placed in small beakers in water. Cotton wool was placed around the wheat to prevent the locusts drowning in the water. Later, wheat was grown for 12 days in small plastic containers that were placed directly in the cages. After 12 days the wheat had grown to stage 11 (1 leaf unfolded (Tottman and Makepeace, 1979)). Wheat was harvested every Monday, Wednesday and Friday and stored at 4°C until used the following day(s). Adults were also provided with fresh bran.

A rigorous cleaning procedure was followed to prevent the build up of pathogens. The metal cages were swept daily to remove any frass or dead locusts and sanitized at the end of each generation (*c.* 4 weeks). Until April 1988 a strong iodine disinfectant, Iophos (Dasco) was used to clean the metal cages and floors. After April 1988, due to the unavailability of Iophos, a chlorine based solution 'White King' was used instead. Food containers were sanitized by soaking in a solution of 'White King'. On completion of the nymphal stage the white terylene cages were soaked in 'Sard Wonder Laundry Soaker' to clean and sterilize them. The floor of the insectary was swept daily and sanitized monthly.

Towards the end of 1997 the locusts had low fecundity and poor survival. Dead locusts were turning pink-red when dying, while live locusts were producing red-tinged faecal pellets. Bacterial infection by *Serratia marcescens* and *Pseudomonas aeruginosa* was ruled out by swabbing (Department of Microbiology, Monash University). *Malaomeba locustae* has previously been identified in *C. terminifera* cultures (Davies, 1973) and is almost inevitable in any acridid culture (A. K. Charnley *pers. comm.*). It is a parasitic amoeba, which attacks the malpighian tubules where they obstruct physiological functions. Locusts were treated with a 10% solution of 'Sulpha 3' (Inca (Flight) Company Pty. Ltd.), in sterilized water sprayed onto the grasses with a mister. 'Sulpha 3' is a triple sulphur drug containing 120g/L sulfathiazole, 40g/L sulfadimidne, 40g/L sulfamerazine. New locusts caught in the field (January 1998) were added to the colony to boost numbers. The offspring from these locusts all had extremely low survival and the colony 'crashed' in March 1998. Growth of the population was exponential following this crash. It was presumed that either the 'Sulpha 3' controlled the *M. locustae* or that the remaining locusts were disease free. Subsequently all grass and bran was treated with 'Sulpha 3' as a prophylactic measure and field-caught locusts were not added to the main colony until they had passed through two generations and appeared 'healthy'.

Diet presentation and determination of factors used to correct consumption measurement

The leaf blades were monitored for changes over a 24 h period in the ratio of water to dry weight, dry leaf weight itself, protein, and non-structural carbohydrate per gram dry matter. All leaf blades were weighed at the start of the experiment (FW = fresh weight) and then at selected intervals (WW = wet weight) before being freeze-dried to a constant weight (DW = dry weight). To determine if there were metabolic changes in dry weight over time (MCF), dry weight measured at t_n was compared to the dry weight predicted from the ratio of FW to DW measured at t = 0 (Table 2.1). Water content was measured as the ratio of water to DW (Table 2.1).

In 1999 two trials were performed, one prior to and the other after the experiments described in Chapters 3, 4 and 5. Conditions in the controlled temperature room were the same as those under which the locusts were reared. Results from these two trials were combined as no significant difference was found between the two trials in terms of

MCF due to respiration/photosynthesis for each grass (Button grass experiment $P = 0.459$, time $P = 0.176$, experiment x time $P = 0.316$: Mitchell grass experiment $P = 0.841$, time $P = 0.440$, experiment x time $P = 0.224$). A third trial was performed in March 2000 prior to any further experiments being performed (those described in Chapters 6 & 7) as the controlled temperature rooms I had been using previously were rebuilt. These new rooms were approximately the same size and heated identically to the old rooms except the minimum humidity level could be controlled. A minimum humidity level was set at 30% RH.

Initially two methods of presenting the diets were trialled using 14 day-old wheat blades. A 10 ml plastic vial with a rubber lid (as used in the cut flower industry) was used. A cross was cut into the rubber lid and the bases of the grass blades were pushed through about 1.5 cm. The grass blades were then either placed in water, or wrapped in moist cotton wool. The containers were set up as for a feeding trial but the locusts were omitted and left for 48 h. The wheat blades wrapped in moist cotton wool were almost dry after 24 h but still retained their green appearance whilst those in the water were still turgid but had discoloured considerably. It appeared they had begun to decompose, with yellowing at the base, which extended up the blades, the paradox being the drier the plant the closer the MCF was to 1 (i.e. no change). However, the proportion of water needed to be maintained, as this factor is known to affect insect nutrition (e.g. Scriber 1977; Slansky and Wheeler 1991). A compromise was found between these two methods, which prevented decomposition and water loss. It was found that a plug of cotton wool inserted 1 cm into a water filled vial (i.e. water above and below the cotton wool) with the base of the blades inserted into the cotton wool (Fig. 2.1) prevented excessive water loss but also minimized dry matter changes (Table 2.2). Food could not be left longer than 24 h before significant drying occurred.

This last method of grass blade arrangement was then used to investigate the behaviour of the treatment grasses under experimental conditions (i.e. when presented to the locusts). Whole grass plants were harvested and the grass blades prepared identically to the way they were presented to the locust, except that the tips of the blades were removed to mimic the effect of grasshopper feeding. The grass blades were weighed into aliquots of approximately 250 mg of Mitchell grass and 450 mg of Button grass. This corresponded to the amounts required to feed an adult locust. Each weighed (FW) aliquot was placed into a vial. At 0, 6, 12, 20, and 24 h after placing the grass in the

experimental containers (t = 0 h corresponded to the same time the locusts were fed daily (1000 E.S.T.)), eight randomly selected containers for each grass species were removed. The grass blades were removed from the vials, any moisture from the blades was absorbed onto tissue and the grass weighed (WW). Immediately after being weighed the grass blades were frozen before being freeze-dried to a constant weight (DW). As the lights were off for 8 h the grass blades were sampled 20 h rather than 18 h after the experiment commenced. The MCF and ratio of water to dry matter was calculated for each replicate.

Chemical Analysis

Due to the small amount (*c.* 50 mg) of plant material, not all eight replicates could be analyzed for each chemical constituent. Four replicates of plant tissue for each species at each time were analyzed for cell wall material, and the remaining four for total protein and non-structural carbohydrate content.

Cell wall material

Total non-pectin plant cell wall material was estimated using the Van Soest method omitting sodium sulphite (Van Soest 1994a) (outlined in Appendix 1.).

Protein

Protein was extracted from approximately 25 mg lyophilized plant material with 500 µl 0.1 M NaOH by sonicating the solution for 30 min and then heating at 90°C for 15 mins. The solution was centrifuged (11,000 rpm 10 min) and the supernatant collected. The pellet was washed once with 300 µl 0.1 M NaOH. The combined supernatants were neutralized with 5.8 M HCl and then TCA was added to obtain a final concentration of 10%. The solution was incubated on ice for 30 min to precipitate the proteins which were collected by centrifugation (11,000 rpm 10 min) and resuspended in 1 ml 0.1 M NaOH. Appropriate dilutions were made that ensured the concentration of NaOH was less than 0.01 M so that it did not interfere with the coomassie blue solution (*unpub. data*). The Bio-Rad micro assay (0-8 µg IgG (bovine gamma globulin) protein) was used, with duplicate samples read in triplicate. The 'Bio-Rad' micro assay is based on the Bradford assay and is sensitive to less than 1 µg of protein.

Total non-structural carbohydrates

Duplicate samples of approximately 20 mg of finely ground plant sample were placed for 1 h in a boiling water bath with 1 ml 0.1 M H_2SO_4 (Smith *et al.* 1964). After cooling, the solution was centrifuged and the supernatant removed. Following appropriate dilutions, total non-structural carbohydrates were measured as (D+) glucose equivalents determined colorimetrically using the phenol-sulphuric acid assay (Dubois *et al.* 1956). Duplicate readings were made of each duplicate sample, with the standard curve generated in triplicate (0-75 mg (D+) glucose).

Net carbon assimilation by the two grasses

Infra-red gas analysis (IRGA) was used to confirm if there was actually a net carbon gain or that the increase in biomass recorded for Button grass was the result of a Type II error, because no significant corresponding increase was found in the amount of the chemical constituents measured (refer Results and Discussion). For each species of grass, five grass vials were made up as previously stated and randomly allocated to positions on the feeding racks. One of these was randomly allocated to the IRGA. An open-system ADC IRGA was used to measure net carbon assimilation. The IRGA chamber enclosed as much as was possible of the grass blades that protruded above the rubber lid. The inlet and outlet air hoses of the IRGA were placed into an empty setup feeding container to mimic the conditions of the chamber. A light was placed at a distance that provided the same amount of light that the plant would have received at that position on the feeding rack (photon flux density of 109-116 µmol m^{-2} s^{-1}). Net CO_2 assimilation values (µmol m^{-2} s^{-1}) were recorded after 0.5, 6, 11.5, 12.5, 19.5, 20.5 and 24 h after the grass blades were placed in the IRGA (1000 E.S.T.) i.e. five measures were taken during the light phase and two in the dark phase. After 24 h all the grasses were removed from the vials, any excess water was wiped from the leaf blades and the weight was recorded before being frozen and then freeze-dried to a constant weight. The parts of the leaf blades that had been in the IRGA chamber were severed from the rest of the grass blades and their surface area and dry weight was determined. Three replicates of each species were used. From the net assimilation values, mg CO_2 assimilated per g dry weight grass per day was estimated. The MCF and water content at t_{24} was determined for all five grass replicates per day, to

ensure that the plant in the IRGA chamber was behaving in a similar manner to grasses in the feeding containers.

Measurement of Frass and Growth

Frass was removed every 24 h and immediately frozen (-20°C) until freeze-dried to a constant weight. Two cases of coprophagy were observed when the diet was not fed in excess and both of these locusts were discarded. Regardless of whether coprophagy occurred or not, any locust where the diet was not in excess was removed from that experiment. Frass respiration was not measured, nor was it corrected for nitrogenous wastes. Studies that have attempted to measure nitrogenous outputs have been unable to account for 45% (Zanotto *et al.* 1993) and 78% (Ferreira *et al.* 1992a) of nitrogen frass and the type of nitrogenous wastes excreted are diet dependent (Bhattacharya and Waldbauer 1972). The proportion of uric acid varied from 1-60% and with no apparent pattern in a beetle species fed four different wheat diets (Bhattacharya and Waldbauer 1972).

Once the duration of the adult stage was decided as seven days, locusts were starved for 1, 2, 4, 6, 8, 12, and 24 h to investigate how quickly and how much of the food in the gut cleared.

Duration of Adult Feeding Bout

To determine the growth phase of the adult lifecycle, adult locusts were grown on wheat under the conditions described in Chapter 4. Intake, faecal production and weight gain was measured daily on 20 adults until they died (median 26 days, maximum 48 days).

Effect of Handling

As the conditions of the constant temperature rooms meant that the diet needed to be changed every 24 h, daily disturbance of the locusts was unavoidable. Two methods were trialled to minimize the effects of stress when changing the diet: (1) adult locusts were anaesthetized using CO_2 gas while the diet was changed and orts removed, and (2) diet changed and orts removed while the adult locust remained in the container. The latter was achieved by lifting the entire experimental chamber from the rack, gently shaking so that the locust attached itself to the side of the container and then removing the base with the frass and orts.

Pilot study to determine the number of replicate locusts required for the main study

A pilot study was undertaken to determine the amount of variation for the parameters being measured using Instar V nymphs feeding on both grasses. Fifteen freshly moulted (timed to within 4 h) Instar V nymphs were randomly allocated to each diet. The diet was offered to the locusts as stated in this chapter, and every 24 h until moulting fresh diet was offered and the orts and frass removed. A subset of the grasses harvested daily for locust consumption, the orts and the frass were all freeze-dried to a constant weight and weighed to the nearest 0.1 mg. Consumption, growth and digestibility were determined.

Data Analysis

ANOVA with Tukey's *post hoc* test was used to determine for each grass whether over time the ratio of water to dry weight, MCF, % protein and % non-structural carbohydrates varied with respect to (1) the dry weight of the grass at the time sampled, and (2) the predicted initial (t = 0) dry weight of the grass. Differences with the MCF over time indicated the leaf blades had lost or gained weight. This could have been due to a proportionate increase in all the constituents or a disproportionate increase in one. Box plots and plots of residuals versus means were used to check that each factor was normally distributed and variances were equal. Log_{10} transformations were used where necessary. For the MCF only, the difference between the food offered and that remaining i.e. the orts, was determined using a *post hoc* planned comparison performed between the t = 0 and t = 24 h values. Trend analysis was performed where appropriate.

Power analyses (GPOWER) were performed on the growth, consumption, frass production and AD (approximate digestibility (assimilation/intake)) data obtained from the pilot study to determine the number of replicates that would minimize the chances of making a Type II error (Quinn and Keough 2002). As it is not known what is a biologically meaningful effect size, the variance of the response variables was used with a significance level of 0.05, and $1 - \beta$ set at 0.8 by convention (Quinn and Keough 2002), i.e. there was an 80% chance of detecting a difference if there was one between locusts feeding on each diet.

RESULTS

Dry weight changes of grasses over 24 h

Except for Button grass in the 'new' room, plant metabolism did not significantly alter the expected amount of dry matter at the four times it was measured (Fig. 2.2). However, trend analysis found that for Button grass in both rooms there was a significant linear upward trend ('old' room: $P = 0.046$; 'new' room: $P = 0.017$). After 24 h, Button grass had increased 5.5 ± 1.9% in the 'old' constant temperature room ($F_{1,75} = 3.95$ $P = 0.051$) and 6.5 ± 2.5% in the 'new' constant temperature room ($F_{1,35} = 2.88$, $P = 0.099$). For Mitchell grass after 24 h there was not a significant increase in either room ('old' room: 1.85 ± 2.08%, $F_{1,74} = 0.36$, $P = 0.552$, 'new' room: 0.45 ± 1.04%, $F_{1,35} = 0.04$, $P = 0.847$). Results are given as the mean ± se and the P values for the planned comparison analysis between t = 0 and t = 24.

Changes in cell wall, total protein and non-structural carbohydrates

Neither the ratio of cell wall to dry matter, or that predicted from the initial fresh weight, varied for either diet in either room over 24 h (Table 2.3). This same pattern was found for non-structural carbohydrates (Table 2.4). The ratio of protein per unit dry matter decreased significantly (26.6 ± 6.4%) after 24 h for Button grass in the 'old' room ($F_{1,35} = 6.45$, $P = 0.048$), but not in the 'new' room ($F_{1,15} = 0.004$, $P = 0.951$). However, for Mitchell grass after 24 h, no change in the amount of protein per unit dry weight was found in the 'old' room ($F_{1,14} = 3.37$, $P = 0.088$) but a significant decrease (19.8 ± 6.4%) was found in the 'new' room ($F_{1,15} = 3.2$, $P = 0.044$). However, when protein was expressed relative to the initial mass only Mitchell grass in the 'new' room 'lost' protein ($F_{1,15} = 3.2$, $P = 0.044$).

Changes in the ratio of water to dry matter

In the 'old' controlled temperature room, the ratio of water to dry matter for Button grass decreased significantly (34.9 ± 6.2%) compared to that of Mitchell grass (7.1 ± 0.7%) in a 24 h period (Button grass: $F_{4,75} = 7.4$, $P < 0.001$; Mitchell grass; $F_{4,75} = 1.6$, $P = 0.176$) (Fig. 2.3a). However, after 24 h Button grass still had more water per unit dry matter than Mitchell grass (3.36 ± 0.24 g compared with

2.07 ± 0.06 g water per g dry matter). When the changes in dry matter were accounted for the pattern was the same (Fig. 2.3b).

In the 'new' controlled temperature room where minimum humidity was controlled, in a 24 h period, Button grass lost three times less water relative to dry weight 13.8 ± 5.5% than in the 'old' room. Mitchell grass, in contrast, lost twice as much water, 14.9 ± 3.9% compared to when in the 'old' room, even when changes to dry matter were accounted for (Fig. 2.3a & b). Both grasses in the 'new' room remained at the same hydration levels for longer before beginning to dry. For Button grass the ratio of water to dry matter decreased significant over the 24 h ($F_{4,35} = 3.8$, $P = 0.012$) but when the increase in dry matter was accounted for the actual amount of water did not alter ($F_{4,35} = 1.6$, $P = 0.197$). The ratio of water to dry matter in Mitchell grass decreased significantly over the 24 h ($F_{4,35} = 5.1$, $P = 0.002$) and when dry matter changes were accounted for ($F_{4,35} = 7.8$, $P < 0.001$).

Net carbon assimilation by the two grasses

IRGA of the two grasses showed that the assimilation rate for Button grass was over seven times higher, 47.5 ± 25.2 mg g^{-1} dry leaf matter per day than for Mitchell grass, 6.5 ± 15.9 mg g^{-1} dry leaf matter per day. In the IRGA chamber both grasses lost less water than the grass blades in the feeding vials. The MCF and water loss values from the other vials showed the grasses behaved as previously recorded.

Measurement of frass and growth

Locusts timed to within 4 h of moulting from Instar II to adult were frozen and then their gut contents removed and examined for the presence of food remaining from the previous instar. Approximately 80% had no food remaining, 10% had some food remaining in the foregut only and less than 10% had a very small amount of food in both the fore- and mid-gut. This food was removed and dried but the amount was so small it was unable to be measured with the available balance (0.1 mg). The adults were starved for 4 h at the end of the experiment as it was found that the majority of food was excreted by this time in over 60% of the locusts with no food remaining in the gut. Shorter starvation periods led to increased locust numbers with increased food remaining in the gut, whilst starvation for longer periods did not result in any more locusts with food-free guts. The majority of any remaining food was located in

the foregut and this and any food in the midgut was extracted and included in the frass.

Duration of adult feeding bout

Feeding on wheat, adults reached a constant weight, with a constant food intake and frass production, five-six days after moulting. The pilot study indicated that the locusts feeding on Mitchell grass took seven days, almost two days longer than those feeding on Button grass, to stabilize their weight.

Effect of handling

There was a significant difference in the weight gain over the first four days ($t = 3.2$, $P = 0.029$) between the two treatments; the locusts gassed gained weight more slowly than those not anaesthetized.

Number of replicates

Power analysis suggested that 12 replicates per diet were required for the determination of growth, 18 for consumption, 14 for frass and 11,000 for approximate digestibility to have an 80% chance of not making a Type II error.

DISCUSSION AND CONCLUSIONS

A method was devised that minimized water loss and metabolic changes when presenting detached grass leaf blades to the locusts so that dietary utilization of the grasses could be accurately determined. Dry matter of Button grass blades increased significantly over the 24 h period it was available for the locust to eat. This net gain was higher than the inherent error from estimating dry weight from the initial DW to FW ratio. The carbon assimilation data and MCF suggested that Mitchell grass had a very slight net carbon gain over 24 h but this would not significantly alter the estimation of consumption. Hence, the calculation of Button grass consumption required the net weight gain to be accounted for, since a small error in the consumption value will have a significant effect on subsequent derived values (van Loon 1991). Hence, when determining Button grass consumption, the orts (F_e) were corrected for their increase in dry matter (MCF_{24}) and then the consumption value was corrected for weight gain (average MCF over the 24 h period) (Fig. 2.2 & Table 2.6). The patterns observed for each species did not change between the constant temperature rooms.

Concentrations of non-structural carbohydrates and cell wall in Button grass did not differ significantly over the 24 h. However, their concentrations mirrored that of the MCF values suggesting their additive effect may have been responsible for the increase measured. The absolute amount of protein did not vary, except for Mitchell grass in the 'new' room, but the ratio of protein to dry matter decreased over the 24 h. As Button grass dry matter consumption was adjusted, the amount of protein ingested needed to be corrected in the 'old' controlled temperature room (Table 2.7). Protein intake of Mitchell grass was corrected in the 'new' controlled temperature room (Table 2.7).

Values were calculated to adjust water consumption for Button grass blades in the 'old' room and Mitchell grass in the 'new' room (Table 2.8). Although Button grass lost more water, it still retained a higher ratio of water to dry matter than Mitchell grass. Water consumption by the locusts was calculated as the water correction factor (Table 2.8) multiplied by the mean ratio of water to dry matter of the grass species on a particular day multiplied by the consumption of dry matter for that day, where the water correction factor was the average ratio of water to dry matter ratio.

After correcting for changes in chemistry of the grasses, the biggest error in the calculation of consumption was the initial estimation of dry weight from fresh weight. This value is derived from the ratio of a subset of the grass blades available each day. A minimum of five controls daily was used to derive the FW to DW ratio. This resulted in an average standard error of the mean of less than 2% for both grass species.

The more wet weight offered, the smaller the effect of this error on the calculated intake value. Age and the grass species affected the amount of wet weight of grass blades offered, thus adults feeding on Button grass were fed the greatest amount. This is reflected in the intake values where the standard error of the mean is largest as a percent of the mean consumption of Mitchell grass by Instar II nymphs.

The correction values applied to parameters assumed that the locust consumed the diet at a consistent rate over the 24 h. Field data suggests that locusts consume the majority of their food immediately after sunrise and just prior to sunset (Bernays and Chapman 1973b; Gregg 1981). Examination of the locusts in the feeding containers observed that they appeared to have a large meal immediately the diet was replaced and then ate steadily until 'lights out' when very little was consumed until 'lights on' after which a large meal was again consumed. This would suggest that most of the diet will be consumed before t = 12, so the correction factors may overestimate consumption.

In the following chapters locust consumption was calculated as outlined. Changes to dry matter, protein and water were corrected for where necessary. Fresh diet was provided daily and the orts and frass removed without gassing the locusts. It was decided to use 20 replicate locusts per age per diet to minimize the chance of making a Type II error in subsequent analyses. This number of replicates is slightly higher than that suggested is required for the locust response variables that are directly measured (growth, intake, and frass production) and very much less than that suggested for the derived response variable (AD). The number of replicates suggested to maximize the chance of not making a Type II error when analyzing diet digestibility suggests (1) that there is not a difference by the locusts on either grass and (2) would be impossible to carry out. Adult dietary utilization would be measured over a seven-day period with the locusts starved for 4 h at completion of the experiment.

TABLES AND FIGURES

Table 2.1 Calculation of water : dry weight ratio (g g^{-1}) and change in dry matter of the diet.

Ratio of water to dry weight (g g^{-1}) = (WW-DW)/DW.

Metabolic Correction Factor (MCF) = $(DW_n/FW_n)/(DW_C/FW_C)$

where n = replicate at time n

c = control; mean of replicates of DW/FW at t = 0 h

FW = fresh weight (weight of leaves when removed from plant)

WW = wet weight (weight of leaves at time n)

DW = dry weight (weight of leaves after being freeze-dried to a constant weight)

If the MCF is greater than 1 then the leaf blade has increased dry matter due to net photosynthetic gain, alternatively if the MCF is less than 1 then the plant has lost dry matter due to respiration or potential losses into the water in the vial (Bowers *et al.* 1991).

Table 2.2 Percentage change in dry weight after 24 h from that predicted from the initial fresh weight for blades of wheat under three different conditions, water only, moist cotton wool and water with a cotton wool plug, $n = 4$.

	% change in dry weight relative to initial predicted dry weight	Turgidity estimate
Water	96. 9 ± 0.7	Moist
Moist cotton wool	101.5 ± 1.3	Dry
Water and cotton wool	101.2 ± 0.3	Drier but still moist

Table 2.3 Statistical results (F and P values) for the changes in cell wall expressed relative to the dry matter present at each time and as predicted from the initial fresh weight over the 24 h trial.

	Ratio of cell wall to dry matter	Predicted amount of cell wall
'Old' room		
Button grass	$F_{4, 35} = 1.93$, $P = 0.214$	$F_{4, 35} = 9.76$, $P = 0.089$
Mitchell grass	$F_{4, 35} = 0.01$, $P = 0.949$	$F_{4, 35} = 4.76$, $P = 0.161$
'New' room		
Button grass	$F_{4, 15} = 1.93$, $P = 0.214$	$F_{4, 15} = 0.089$, $P = 0.382$
Mitchell grass	$F_{4, 15} = 0.13$, $P = 0.733$	$F_{4, 15} = 1.15$, $P = 0.325$

Table 2.4 Statistical results (F and P values) for the changes in non-structural carbohydrates expressed relative to the dry matter present at each time and as predicted from the initial fresh weight over the 24 h trial.

	Ratio of non-structural carbohydrates to dry matter	Predicted amount of non-structural carbohydrates
'Old' room		
Button grass	$F_{4,35} = 0.15$, $P = 0.961$	$F_{4,35} = 1.74$, $P = 0.163$
Mitchell grass	$F_{4,35} = 0.66$, $P = 0.626$	$F_{4,35} = 1.07$, $P = 0.407$
'New' room		
Button grass	$F_{4,15} = 1.56$, $P = 0.207$	$F_{4,15} = 0.54$, $P = 0.709$
Mitchell grass	$F_{4,15} = 0.872$, $P = 0.491$	$F_{4,15} = 0.629$, $P = 0.649$

Table 2.5 Calculations used to measure consumption of Button grass and Mitchell grass by the locusts.

Button grass consumption was corrected by

(i) correcting the orts (F_e) for their increase in dry matter and then

(ii) correcting the consumption value for the average MCF over the 24 h period (Table 2.6).

Button grass consumption $C = MCF*(F_i - (F_e/MCF_{24}))$

MCF_{24} = MCF calculated at t = 24 h.

$= (DW_{24}/FW_{24})/(DW_C/FW_C)$

Mitchell grass consumption; $C = F_i - F_e$

where F_i is the dry weight of the food offered at t(0), the time at which the experiment begins and F_e is the dry weight of the remaining food at t(24), the time when the experiment ends.

Table 2.6 Values used to correct for metabolic changes to Button grass.

	'Old' Room	'New' Room
MCF	1.014	1.014
MCF_{24}	1.055	1.064

Table 2.7 Values used to correct protein consumption of Button grass and Mitchell grass in both rooms.

	'Old' Room	'New' Room
Button grass	0.864	1*
Mitchell grass	1*	0.924

* Tukey's analysis: no difference between any of the times.

Table 2.8 Water correction values used for each species in each room.

	'Old' Room	'New' Room
Button grass	0.834	1*
Mitchell grass	1*	0.941

* Tukey's analysis: no difference between any of the times.

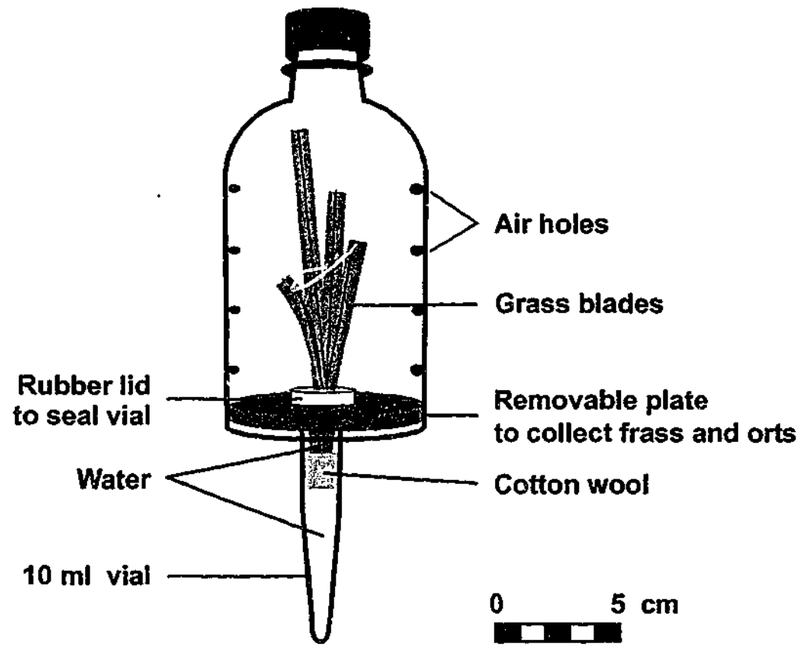


Fig. 2.1 Experimental chamber used for the digestibility experiments showing the setup of the grass blades.

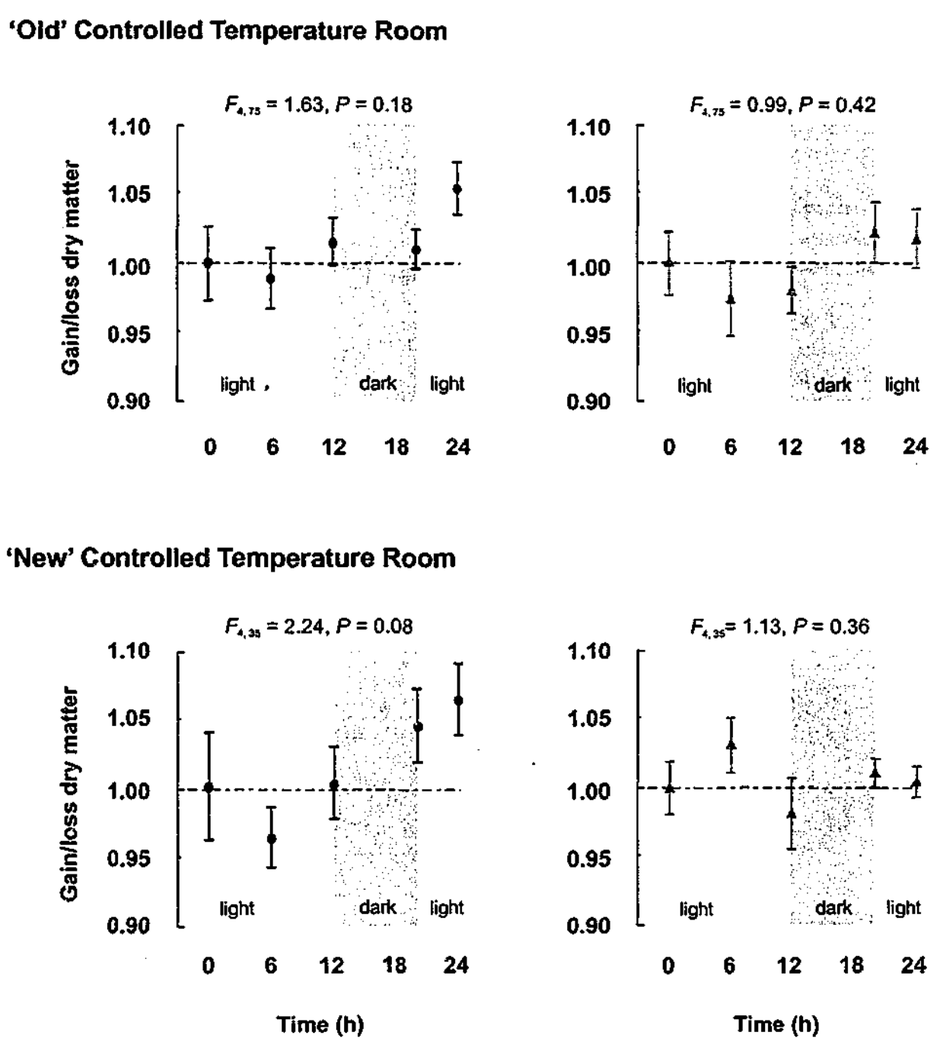
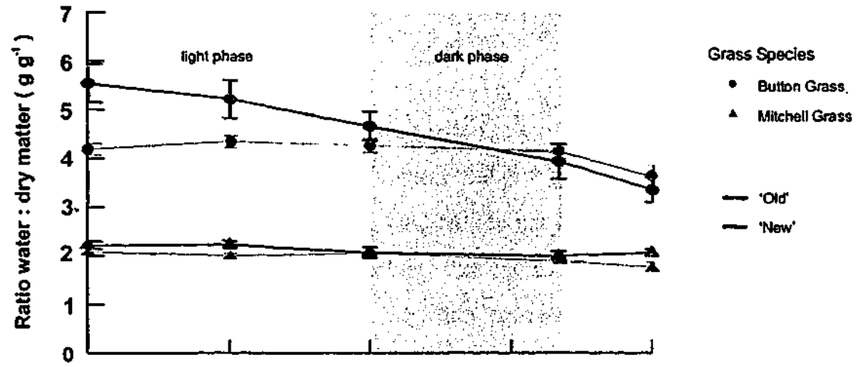


Fig. 2.2 Changes in dry matter of the leaf tissue during the 24 h period the food was offered to the locusts in both the 'old' and the 'new' controlled temperature room (means ± se). The values are expressed relative to that predicted from the initial (t = 0) ratio of fresh wt to dry wt. A value greater than 1 indicates the leaf material has gained dry weight compared to that initially predicted, and values less than 1 that there has been a loss of dry weight. The F and P values given are for ANOVA, n = 16 ('old' controlled temperature room), n = 8 ('new' controlled temperature room).

(a)



(b)

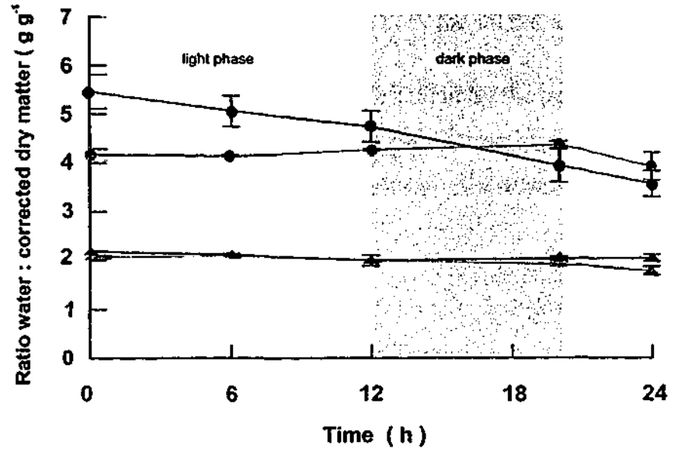


Fig. 2.3 (a) The ratio of water per gram dry matter and (b) the ratio of water per gram corrected dry weight, (mean ± se) for Button grass and Mitchell grass in the 'old' and 'new' controlled temperature rooms. $n = 16$, 'old' room and $n = 8$ 'new' room.

CHAPTER 3. DIGESTIBILITY OF THE CELL WALL BY THE AUSTRALIAN PLAGUE LOCUST

SUMMARY

1. A range of hypotheses has been suggested to explain how plants dominate terrestrial ecosystems given the variety and potential abundance of herbivores. Limited herbivory on plants and host choice has been explained by various plant properties ('bottom-up' factors) through to predators ('top-down' factors). Plant cell wall may play a more important role in herbivore defence than has been previously attributed (Abe and Higashi 1991; Hochuli 1996; Martin 1991). However, evidence to date is equivocal.

2. Determination of plant cell wall digestion by locusts required a precise methodological procedure to determine both the exact intake of dry matter and the concentration of cell wall in both the intake and frass.

3. Plant cell wall quantification is affected by the particle size distribution of the dried plant material. To prevent overestimation of cell wall in the diet compared to that of the faeces, all samples should be ground so that they pass through a 0.2 mm sieve.

4. The Australian plague locust was unable to digest the cell wall of three grasses (wheat, Button grass and Mitchell grass).

5. Plant cell walls appear to be a mechanical barrier preventing locusts assimilating nutrients. The arrangement of cells and constitution of their walls may be limiting access to nutrients rather than the amount of nutrients *per se*.

INTRODUCTION

Several hypotheses regarding factors that limit herbivory in general and that define host choice have been suggested (Behmer and Elias 2000; Bernays and Graham 1988; Feeny 1976; Hairston *et al.* 1960). While no particular 'degree of importance' can be applied to these factors, plant cell wall may play a more important role in herbivore defence than has previously been suggested (Abe and Higashi 1991; Hochuli 1996; Martin 1991). Plant cell wall may be a 'key substance' maintaining 'green' plant communities under continuous predation pressure (e.g. Murdoch 1966, Abe and Higashi 1991; Martin 1991), and if so, would support Murdoch's (1966) contention that 'all that is green is not edible'. It has been inferred from studies on digestion of artificial diets diluted with cellulose (e.g. McGinnis and Kasting 1967) and assays on digestive enzymes (e.g. Morgan 1976) that the cell wall is indigestible to most insects (Martin 1991). However, the results of direct measurement in two studies are conflicting (Ferreira *et al.* 1992b; Hochuli *et al.* 1993).

Plant constituents can be divided into two main categories, the cell wall and the cell contents. The cell contents are usually easily digested by the enzymes found in most animals, whilst the majority of the cell wall is not (Bacic *et al.* 1988). Cell walls of higher plants are constructed from long fibres (microfibrils of cellulose) embedded in a matrix of polysaccharides, proteins and phenolics such as lignin. The ratio of cellulose to matrix components and the composition of matrix components varies with the stage of growth, type of cell and plant species (Brett and Waldron 1996; Wilson 1993). The matrix polysaccharides can be divided into two groups based on their solubility, (i) the hemicelluloses, which are soluble in acid or alkali, and (ii) the pectins, which are soluble in hot neutral solutions of ammonium oxalate or diaminoethanetetra-acetic acid disodium salt (EDTA) (Brett and Waldron, 1990; Wilson, 1993; Reid, 1997).

It is thought that the capacity to extract the energy from cellulose has evolved in very few insects because growth and reproduction in herbivorous insects is limited by nitrogen and/or water. Sufficient carbon or energy is assimilated when exploiting the more easily digestible cell contents to obtain nitrogen (Martin 1991).

Larval lepidopterans appear to be able to access the cell contents without rupturing the cell wall. Their mid-gut has a high pH which appears to promote leakage of cell contents through the plasmodesmata (Barbehenn 1992). In contrast, orthopterans have neutral pH conditions in their digestive tract (Evans and Payne 1964; Ferreira *et al.* 1990; Uvarov 1966) that do not encourage the dissociation of proteins. This suggests that for orthopterans to liberate nutrients from plant cells they must mechanically fracture the cell wall with the mandibles. However, it has also been suggested that cell wall-degrading enzymes may exist and assist cell rupture (Ferreira *et al.* 1992b).

The aim of this study was to measure the digestibility of the non-pectin cell wall components in Australian plague locusts. This was investigated on three different grasses, mature Button grass, mature Mitchell grass and seedling wheat (*Triticum aestivum* L.). Button grass and Mitchell grass were included to enable interpretation of subsequent components of this research project. Wheat was also included so more general conclusions could be drawn. Both Button grass and Mitchell grass are C_4 grasses while wheat is a C_3 grass. It was thus predicted that Button grass and Mitchell grass would be more similar in cell wall structure than wheat. Very early growth stage wheat was used as it has less cellulose and lignified tissue and it is predicted that the cell wall may be more digestible than the two grasses on which Australian plague locusts normally feed.

There is no one method, or combination of methods, that can unambiguously provide complete quantitative analyses of all the chemical components of plant cell walls (Obst 1993; Selvendran 1975). Quantification of the monosaccharides after acid hydrolysis determines their relative composition but reveals nothing about their linkages that contribute to the physical properties of the wall. Methods that quantify the cell wall based on solubility do not provide information on the chemical structures of polymers solubilized and what is solubilized is dependent on the development stage of the cell wall (Bacic *et al.* 1988). For this research, the Van Soest method (Van Soest *et al.* 1991) based on solubility of the cell wall components was chosen to isolate and fractionate the cell wall because it gives more information about the physical structure and thus biological function of the cell wall. This method fractionates the cell wall into cellulose, and the major components of the matrix polysaccharides, acid-detergent sulfuric lignin and hemicellulose. Pectins, the other

polysaccharide component of the hemicellulose, are solubilized with the cell contents in this method. However, they generally comprise less than 4% of the cell wall from grass leaf blades (Aman 1993) and are unlikely to contribute to locust nutrition (Morgan 1975).

The frass is enclosed in a peritrophic membrane, which is nearly always chitin, set in a protein-polysaccharide matrix (Chapman 1985a). Chitin behaves like lignin (Acid-detergent sulfuric lignin) and the other polysaccharides will most likely be solubilized together with the hemicelluloses, regardless of the method used (Merck 1989), which will lead to overestimation of the cell wall components remaining in the frass.

MATERIALS AND METHODS

Fifteen freshly moulted (timed to within 4 h) Instar V locust nymphs, reared as described in Chapter 2, were randomly assigned to one of three treatment diets, wheat, Button grass or Mitchell grass. The method of presenting the diet to the locusts and calculation of consumption was as described in Chapter 2. Neither wheat nor Mitchell grass intake by the locusts required correction for metabolic changes to the orts. The Button grass orts were corrected for changes in dry weight ($C = F_i - (F_e/1.055)$; Chapter 2). No corrections to the amount of cell wall consumed were necessary as the amount of cell wall did not vary from that found initially (Chapter 2 and assumed for wheat as there was no change in MCF). Button grass, Mitchell grass and wheat were grown as described in Chapter 4. All frass and orts were collected daily when the food was changed.

The non-pectin cell wall components of the diets and frass were determined by the Van Soest method omitting sodium sulphite (Van Soest *et al.* 1991). The samples of the grasses collected daily and frass were lyophilized before being finely ground in a Spex® freezer/mill. The importance of grinding both the diet and the frass to a similar very fine powder is discussed in Appendix I, with reference to experimental data. Approximately 100 mg duplicate grass and frass samples were fractionated into hemicellulose, cellulose and acid insoluble residue. Only *c.* 50 mg duplicate samples were used for the wheat diet, due to the higher digestibility of the wheat diet by the locusts that resulted in less frass being produced. The cell wall components were isolated from the plant material by refluxing for 1 h in neutral detergent solution and then transferring the entire sample to a sintered glass crucible (porosity 2) where the sample was thoroughly rinsed with 80-90°C distilled water and then acetone. The crucible and cell wall material was then dried at 100°C for a minimum of 8 h, before being cooled to room temperature in a desiccator and then weighed. The cell wall material was calculated as the weight of the sample remaining in the crucible minus the weight of the crucible divided by the original weight of the sample. To further fractionate the isolated cell wall, it was refluxed for 1 h in acid detergent solution, solubilizing the hemicelluloses. The remaining cell wall components were collected in a sintered glass crucible (porosity 2) rinsed and dried as before prior to being weighed. The remaining residue was incubated at room temperature in 72%

sulphuric acid for 3 h to remove the cellulose component, and rinsed, dried and weighed as before. The remaining material was acid insoluble residue. Hemicellulose was calculated as the weight of the sample after neutral detergent extraction minus the weight of the sample after acid detergent extraction. The amount of cellulose was calculated as the weight of the residue after acid detergent extraction minus the residue remaining after 72% sulphuric acid hydrolysis (Fig. 3.1).

Data analysis

The amount of cell wall in each grass species was expressed as a percentage of the dry matter and values were arcsin square-root transformed prior to ANOVA. *Post hoc* Tukey's tests were performed to determine where the differences lay among grass species.

The consumption of cell wall was determined from the daily intake for each insect and the known percentage of cell wall from the control grass from that day. Cell wall digestion was calculated as

(cell wall intake /frass cell wall) x 100

Values less than 100 suggest that cell wall was digested by the locust, while those greater than 100 suggest that there was more cell wall in the frass than that predicted from the intake. Values obtained were averaged for each diet and a one-sample 2-tailed *t*-test was used to determine if the means differed significantly from 100, i.e. no cell wall was digested. A one-factor ANOVA (diet) was performed for total cell wall and each cell wall component. Other endogenous wastes in the frass (e.g. uric acid) cannot be accounted for, but these will not affect the calculation of cell wall digestibility. All analysis were performed using Systat® 10.

RESULTS

Percentage of cell wall in the dry matter differed significantly for each species, with wheat having the least and Mitchell grass the most (Table 3.1). The various components when expressed as a percentage of the cell wall fraction also differed significantly among species. The cellulose content was significantly lower and the hemicellulose content was significantly higher in Mitchell grass than in wheat and Button grass. Cellulose was the major component of the cell wall in all three grasses.

There was no digestion of total cell wall material or cellulose by the locusts consuming any of the grasses (Table 3.2). Locusts were unable to digest hemicellulose from wheat and Button grass, but for Mitchell grass there was significantly ($t_{1,14}$ =6.3, $P < 0.001$) more hemicellulose measured in the frass than predicted from the intake. For locusts feeding on the wheat and Button grass diets, more acid insoluble residue was found in the frass than predicted (Table 3.2) from their intakes. These high values correlated negatively with the amount of frass available for analysis due to the higher digestibility of wheat and Button grass than Mitchell grass (*unpub. data*), and positively with the percentage of acid insoluble residue in the dry matter of each species. As the cell wall components were determined gravimetrically, the less frass there was initially, the larger any error would have been when weighing the extremely small amounts remaining at the end of the process. A 0.1 mg error when weighing the acid insoluble fraction in the frass translated to *c.* 15% change in the estimation of acid insoluble residue digested. Even if the values of acid insoluble residue remaining in the frass were not overestimated for the wheat and Button grass frass, this fraction consisted of less than *c.* 2.5% of the total dry matter and did not significantly influence the values obtained for the total cell wall. Only the acid insoluble residue was significantly different from 100 when the results from all three diets were combined ($t_{1,44}$ = 4.6, $P < 0.001$).

DISCUSSION

This study demonstrated that the Australian plague locust does not digest non-pectin cell wall. The ratio of cell wall to cell contents, and ratio of hemicellulose:cellulose:acid insoluble residue within the cell wall fraction differed significantly between the three grass species. Therefore, if cell wall digestion depends on the type of cell wall construction or the relative amounts of components within the cell wall it should be apparent from this experiment. Although more hemicellulose was found in the Mitchell grass frass from that predicted from the intake, it is still likely that hemicellulose is not being digested and that this is a result of experimental error when attempting to quantify extremely small intakes and amounts of frass produced. An error as small as 1% in the weighing of the fractionated cell wall will lead to an overestimation of hemicellulose and a corresponding 3% underestimation of cellulose. The experiments performed for this study are the first to correct the intake for any metabolic changes when investigating digestibility of cell wall components and to quantify the various components of the cell wall.

It was hypothesized that more acid-insoluble residue should be found in the frass than what was ingested, as the majority of the peritrophic membrane will behave as lignin. Transmission electron microscopy of a thin section of frass revealed that the width of the peritrophic membrane (*c.* 1.5 µm) was less than the width of the cuticle (*c.* 5.5 µm) of either Button grass or Mitchell grass blades (*unpub. results*). Therefore, it was thought that the peritrophic membrane contribution would be negligible. Reanalysis of the data, ignoring the acid-insoluble fraction (i.e. combining hemicellulose and cellulose fractions) for all three diets showed that there was no digestion of the combined hemicellulose and cellulose fractions.

Of the two previous published studies investigating digestibility of cell wall by grasshoppers, one study (Hochuli *et al.* 1993) of two graminivorous grasshoppers, reported that 4.1 ± 2.6% and 7.0 ± 4.0% of the cell wall was digested, while the other (Ferreira *et al.* 1992b) found significant digestion (*c.* 30%) of both cellulose and pectin but not of hemicellulose by their grasshopper species. Neither study corrected the orts for metabolic changes (Chapter 2). Hochuli *et al.* (1993) used the Van Soest method to determine total cell wall but did not grind samples to a very fine particle

size (Appendix I) and calculation of the 95% confidence interval for their results suggested that no digestion of cell wall occurred. Ferreira *et al.* (1992b) used a different method to quantify the cell wall components that appears to greatly underestimate the amount of polysaccharides in the dietary cell wall (cellulose 5.7% and hemicellulose 2.1%; whereas 20-45% cellulose and 10–30% hemicellulose is expected in terrestrial plants (Van Soest 1994b)) and also used an imperfect fractionation procedure. Since cellulose is regarded as being indigestible to locusts, their findings should be regarded with caution (McGinnis and Kasting 1967).

There is little or no reported activity of enzymes that degrade cell wall in locusts (Evans and Payne 1964; Morgan 1975; Morgan 1976). Cellulase activity has been recorded in three out of nine species tested. Xylanase, the major hemicellulose polysaccharide, was found in the only grasshopper species investigated for this enzyme. Furthermore, I would argue that the contribution of enzymes to cell wall degradation would be extremely minor due to the extremely short time the food is resident in the gut (Cooper *pers.comm.*; my observations (Chapter 5); Baines *et al.* 1973; Uvarov 1966; Yang and Joern 1994c). Even if the hemicellulose component is degraded to its sugar monomers, it appears that locusts are unable to utilize pentoses, e.g. xylose and arabinose (Dadd 1960), which constitute the majority of hemicellulose polysaccharides in Poaceae (Wilkie 1979).

Digestibility of pectins was not measured in this study, but pectins are not predicted to be a major source of energy due to their very low concentration in the cell wall of grasses (< 4%) (Aman 1993). In addition, pectins are not predicted to be utilized, as no pectinase activity has been recorded in any locusts (Morgan 1975). Even if pectins are digested they are not expected to alter the cell wall structure significantly as they are largely independent of the cell wall (Brett and Waldron 1996).

Many herbivorous mammals are able to utilize symbiotic bacteria to digest the cell wall (e.g. Foley and Cork 1992; Van Soest 1994b), and the contribution to insect nutrition by symbiotic gut flora producing cellulases may be more widespread than currently assumed (Slaytor 1992). Although locusts appear to have an abundant gut bacterial flora (Hunt and Charnley 1981; Stevenson 1966) they do not appear to contribute to locust nutrition (Charnley *et al.* 1985).

Grasshoppers have mandibles with both biting and chewing regions arguably comparable to the incisors and molar teeth of mammalian herbivores. Mandibular morphology is correlated with the predominant food type in the diet, i.e. grasses or forbs (Chapman 1964; Isely 1944; Patterson 1984). However, little is known about how the differing morphologies may optimise cell rupture, although Boys (1981) found that the graminivorous mandibles were able to shear the adaxial and abaxial surface of a grass leaf exposing the parenchyma, whereas the forbivorous mandibles could not. Locusts with mandibles blunted by the ingestion of carborundum powder took longer to eat an equivalent size piece of grass than locusts with sharp mandibles (Chapman 1964).

Therefore, the cell wall appears to be a barrier that locusts must rupture mechanically to assimilate nutrients. It will also act as a dilutant of these more easily digested cell contents. Many studies have investigated the effect of low nutrients on locusts from life history to digestive strategies (e.g. Joern and Behmer 1997; Simpson and Raubenheimer 1993b; Yang and Joern 1994c). These studies suggest that locusts are able to compensate by increasing consumption for a diet consisting of up to 80% of an indigestible component (McGinnis and Kasting 1967; Raubenheimer and Simpson 1993; Timmins *et al.* 1988). This is higher than that typically found for the ratio of cell wall to cell contents in a fresh plant (Van Soest 1994b). These studies used cellulose as an indigestible nutrient diluter, but if the effect of the cell wall is that of a barrier to the easily digested contents, then the arrangement of cells and the walls of these cells may be more important than the absolute amount of cellulose, hemicellulose, etc. That is, it does not matter how much nitrogen the plant contains if it is inaccessible. Conclusions drawn regarding the potential nutritive value of dried plant material will fail to realize the actual nutritive value for an insect.

Leaf anatomy was used to explain the observed preference of a generalist grasshopper feeding on a range of C_3 and C_4 grasses as no correlation with leaf chemistry was found (Heidorn and Joern 1984). Plants that use the C_4 photosynthetic pathway are characterized by having thick-walled bundle sheath cells, which are the most nutrient-rich tissues, however, digestibility of the grasses was not determined. These appear to pass through the digestive system intact (Caswell and Reed 1976), the inference being that the thick cell walls resist crushing. This was also observed by Hehn and Grafius (1949), who reported a strong negative correlation between the amount of

mechanical tissue in the peduncle of spring wheat varieties, and the percentage of heads clipped by a locust. For orthopteran herbivores, the cell wall, might be considered a mechanical defence, and may be more important than previously thought.

Table 3.1 Percentage of cell wall in the three grasses expressed as a total and fractionated into components (mean ± s.e.). Values in brackets represent the percentage of each fraction in the total cell wall. All values were arcsin square root transformed prior to analysis. Shared alphabet letters indicate no significant difference ($P > 0.05$) using Tukey's *post hoc* comparisons.

DRY MATTER	Wheat n = 15	Button grass n = 14	Mitchell grass n = 14	
Total Cell wall material	38.1 ± 0.5	49.0 ± 0.3	51.6 ± 0.9	$F_{2,40}$=127.8, P<0.001
Hemicellulose	15.2 ± 0.2 (40.0 ± 0.4[a])	20.0 ± 0.4 (40.8 ± 0.8[a])	23.1 ± 0.6 (44.7 ± 0.5)	$F_{2,40}$=95.6, P<0.001 ($F_{2,40}$=19.1 P<0.001)
Cellulose	21.8 ± 0.4 (57.1 ± 0.3[a])	26.8 ± 0.4[a] (56.2 ± 0.7[a])	27.9 ± 0.5[a] (52.2 ± 0.5)	$F_{2,40}$=64.8 P<0.001 ($F_{2,40}$=27.6 P<0.001)
Acid Insoluble Residue	1.5 ± 0.1 (3.8±0.3)	2.6 ± 0.2 (5.3 ± 0.3)	3.6 ± 0.2 (6.9 ± 0.4)	$F_{2,40}$=35.6 P<0.001 ($F_{2,40}$=18.2 P<0.001)

Table 3.2 Percentage of cell wall components remaining in the frass after digestion. Results are the mean ± s.e. * denotes those values that are significantly different from 100. The *P* values are for the ANOVA results between diets.

Cell wall component	Wheat n = 15	Button grass n = 15	Mitchell grass n = 15	
Total cell wall	102.5 ± 3.3	100.0 ± 2.8	103.4 ± 4.3	0.327
Hemicellulose	95.3 ± 4.3	101.9 ± 2.6	115.0 ± 2.4*	0.069
Cellulose	102.3 ± 2.9	96.3 ± 4.0	95.4 ± 5.0	0.391
Acid insoluble residue	188.9 ± 14.4*	165.1 ± 23.4*	104.4 ± 14.3	< 0.001

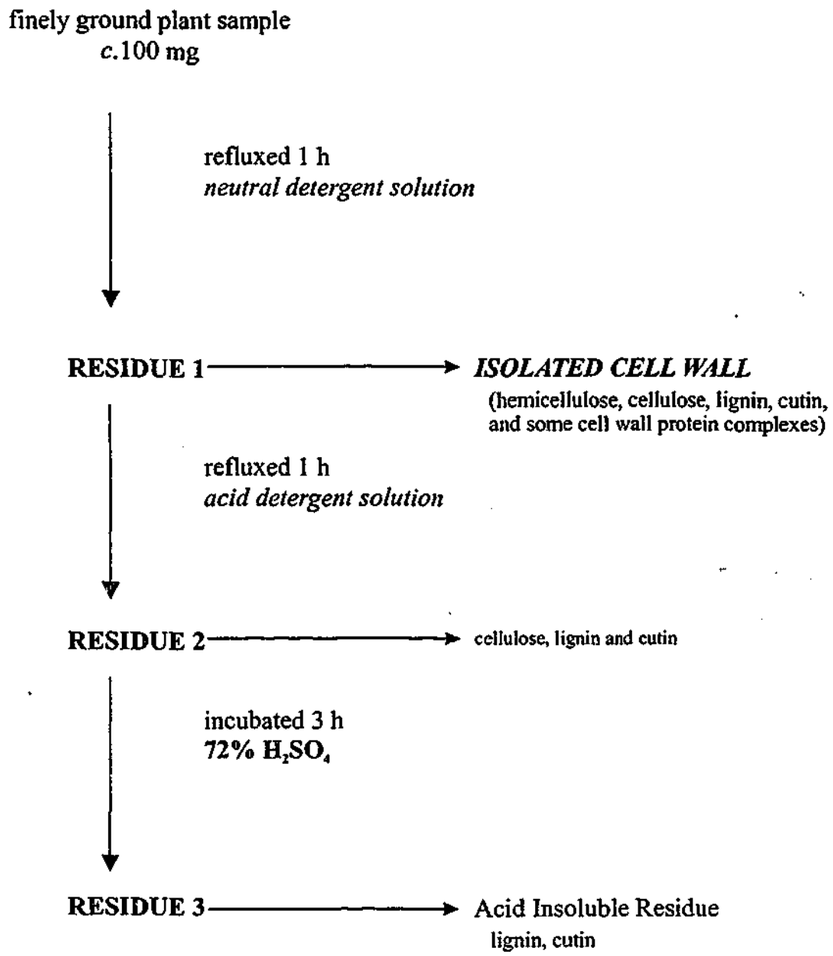


Fig. 3.1 Schematic diagram showing the steps required to isolate the cell wall and the various cell wall components from the grasses and frass. Hemicellulose was calculated as the weight of residue 1 minus the weight of residue 2. Cellulose was calculated as residue 2 minus residue 3. The remaining cell wall components are the acid insoluble residue.

CHAPTER 4. EFFECTS OF DIET ON THE LIFE HISTORY OF THE AUSTRALIAN PLAGUE LOCUST

SUMMARY

1 The performance of Australian plague locusts was investigated on the two grasses of contrasting life history strategies that predominate in areas where plagues develop.

2 Both grasses (diets) were chemically very similar in terms of the amount of cell wall, protein and non-structural carbohydrates in the dry matter. They differed structurally, with Button grass having about twice the water per gram dry matter and specific leaf area (SLA) of Mitchell grass.

3 There was a significant interaction between diet and age, with the younger nymphs performing similarly on each diet, but the older nymphs performing differently. The later instar nymphs consuming Mitchell grass gained less weight and their instar duration was longer compared to those feeding on Button grass. This appeared to be due to increased consumption (pre-ingestive mechanisms) rather than differences in diet digestibility or metabolic costs associated with processing (post-ingestive mechanisms).

4 Button grass was more digestible than Mitchell grass, and this could be explained by differences in the amount of cell wall. There was no difference in the percentage of available nutrients digested from either grass.

5 As the locusts increased in age (and mass), growth and growth rate increased per unit body weight. Although diet digestibility decreased, consumption per unit body mass increased, which resulted in dry matter assimilation per unit body mass remaining the same. With increasing age the rate of growth, consumption and assimilation increased significantly.

INTRODUCTION

Foraging strategies, shaped by natural selection, aim to minimize the differences between the nutrient requirements and current nutritional state of an organism. Nutrient requirements vary both within an instar and across instars. Laboratory studies have demonstrated that locusts unable to achieve an optimal intake of nutrients incur fitness costs (e.g. Joern and Behmer 1997; Joern and Behmer 1998; McCaffery 1975; Simpson and Raubenheimer 1993b).

All organisms have an optimal intake of nutrients (Simpson and Raubenheimer 1995) however, natural environments are continually changing. To reach the growth target (Fig. 1.1) in a continually changing environment an insect needs to compensate behaviourally and/or physiologically when nutrients are limiting (Slansky 1982). Compensatory mechanisms fall into two interdependent categories, pre-ingestive and post-ingestive. Pre-ingestive mechanisms include switching diets (e.g. Abisgold *et al.* 1994; Chambers *et al.* 1995) and altering the amount consumed (e.g. Simpson and Raubenheimer 1993b; Yang and Joern 1994a). Post-ingestive mechanisms can be either pre-digestive (digestive efficiency) or post- digestive (metabolic efficiency). This is achieved by mechanisms such as altered food retention time (Yang and Joern 1994c), selective absorption (Simpson 1982a; Zudaire *et al.* 1998), selective egestion (Zanotto *et al.* 1994), and in the longer term, by altered allocation to body parts (Yang and Joern 1994b). If the nutrient target cannot be reached, growth is reduced and growth rate may also be reduced (Raubenheimer and Simpson 1997). *Locusta migratoria* was generally able to reach the growth target even with highly unbalanced protein:carbohydrate diets (Raubenheimer and Simpson 1993; Zanotto *et al.* 1993; Zanotto *et al.* 1997). However, studies on multiple grasshopper species found that it was not possible to draw generalizations about diet quality on adult performance (Joern and Behmer 1997; Joern and Behmer 1998).

Many factors influence diet quality, such as the balance of nutrients (including water) in the diet (e.g. Bernays 1990; Raubenheimer and Simpson 1990; Roessingh *et al.* 1985; Simpson and Simpson 1990; Simpson and Raubenheimer 1993b), the amount of nutrients obtained per meal (dilution factors) (e.g. McGinnis and Kasting 1967; Slansky Jr. and Wheeler 1991; Timmins *et al.* 1988; Yang and Joern 1994c), the accessibility of nutrients (ability to fracture cells and the presence of digestibility

reducers e.g. tannins) (e.g. Behmer *et al.* 2002; Bernays and Chapman 1977; Caswell and Reed 1976; Hehn and Grafius 1949) and the presence/absence of deterrents or phagostimulants (e.g. Chapman 1995; Hinks *et al.* 1993).

Very little is known about nutrient requirements across instars as the majority of insect nutritional studies have concentrated on one instar (usually the last), (Barton Browne 1995; Slansky 1993). General trends found for Orthoptera with increasing age are increased total consumption and rate of consumption, increased efficiency of conversion of digested material to biomass, and decreased approximate digestibility and consumption per gram of body weight, (Hill and Goldsworthy 1968; Slansky Jr. and Scriber 1985; Woodring *et al.* 1979). It also appears that relatively more carbohydrate is required with increasing age (Dadd 1960). Based on these observations it has been hypothesized that variations in diet will have the greatest impact on the earlier instars (Bernays and Simpson 1990; Slansky Jr. and Scriber 1985).

Grasses largely consist of structural and non-structural carbohydrates, proteins and water, thereby providing all the essential nutrients for growth, and are generally deficient in secondary compounds (Bernays and Barbehenn 1987). Grass-feeding locusts have characteristic morphological traits. Graminivores typically have larger head/body ratios than comparable sized forb feeders (Bernays and Hamai 1987), very specific mandible morphology (Chapman 1964; Isely 1944) and posterior caeca are reduced or absent (Chapman 1988a).

Organisms can respond to stress (any environmental condition unfavourable to growth) using either escape or resistance strategies (Levitt 1980). Escape strategies allow the organism to avoid experiencing the external stress and include diapause and migration. Resistance strategies are those that allow the organism to survive the external stress. This can be achieved in two ways, by avoiding internal stress or by tolerating the stress. For example in areas of low rainfall, plants can have long roots to reach the water table, thereby avoiding internal water deficits. Some plants can tolerate internal water deficits e.g. resurrection grasses. Australian plague locusts both escape and resist the stress imposed by low water availability (reduced nutritional resources). Locusts can escape low nutrient conditions by either the adults migrating to areas with favourable nutritional resources, or as eggs, in diapause or

quiescence. Instar III nymphs may experience environmental nutrient deficits but avoid internal stress by modifying activity levels.

Unpredictable rainfall in the Mitchell grasslands provides a highly variable 'window of opportunity' for growth. Both the plants and animals that inhabit these areas have developed mechanisms to escape or resist the stress of water scarcity. Australian plague locusts develop in Mitchell grasslands following at least 20 mm of rain. This amount of rain allows the two components of Mitchell grasslands, long-lived perennials, mostly Mitchell grass, and a suite of ephemerals of which Button grass is the major component, to grow and produce seeds. Australian plague locusts appear to preferentially feed on Button grass, but as this grass mostly completes its lifecycle before the locust, the later instars and adults rely on Mitchell grass, which remains greener for longer, to complete development and migrate. It was hypothesized that Button grass would be a better resource for the locusts because annual plants tend to be higher in nitrogen and are softer (Chapin III 1991; Garnier 1992).

I investigated the 'quality' of Button grass and Mitchell grass by (1) direct analysis of the major chemical constituents of the grasses, i.e. water, protein, carbohydrate and structural carbohydrates (cell wall), and (2) indirectly by their effect on the life history of the Australian plague locust. Where locust performance differed between the two diets I used ANCOVA (Raubenheimer and Simpson 1994) to determine whether these differences were due to pre-ingestive (amount or rate of consumption) or post-ingestive (digestive and utilization efficiency) or a combination of both. Where there was a post-ingestive difference, I investigated whether this was due to differences in digestibility of the two grasses or metabolic utilization. Specifically, did the two diets differ in terms of their major nutrients (water, protein and non-structural carbohydrates), and were the locusts able to access these nutrients equally across diets and with locust age? If not, what was the effect on the life history of the locust? As each instar was restricted to a single diet, only pre-ingestive mechanisms involving consumption were investigated.

MATERIALS AND METHODS

Growing of plant material

In the field, the different instars would potentially encounter grasses of varying nutrient status, both between grass species and within a grass plant. Time and resources prevented comparison of grass blades of various ages. In this experiment I used a standard sample of fully expanded leaf blades from both species. Mitchell grass blades were *c.* 15-25 days old. Since Button grass blades grew faster, mature leaves were slightly younger, *c.* 10-15 days old. Chemical analysis of similar samples indicated little variation in dietary properties within this range of leaf ages (Chapter 2).

Button grass spikelets were harvested either from field-grown plants or plants transplanted from Fowlers Gap, western New South Wales, to Monash University, or from plants grown from seeds at Monash University. Spikelets were air-dried and stored in paper bags at room temperature until needed. Mitchell grass spikelets were collected from the field and stored at room temperature until required. Seeds collected from the field were collected over the range where the locust is found. For each species, collected seeds were mixed together to ensure a random mix of localities.

Button grass seeds were germinated in 5 mM gibberellic acid and Mitchell grass seeds in water on paper five days before they were transplanted (Refer Appendix II) to 'organic mix' (*c.* 50% mushroom compost, 40% top soil, 10% clay) in tubs *c.* 60 x 40 x 30 cm (length x width x depth). All grasses were raised in glasshouses with a minimum temperature of 10°C and a maximum of 35°C. Planting of both grasses commenced at the end of September and ceased in February, which provided grass from January through to the end of May. From April to September the growth rate of the plants was too slow to provide sufficient grass for feeding experiments. New plants of both species were planted at 2-weekly intervals.

Experimental design

Two factors were investigated, diet and age. The two diets were Button and Mitchell grasses, while the ages used were instars II, III, IV, V and the first 7 days of the adult phase (the somatic growth stage as measured previously). Freshly moulted (timed to within 4 h) Instar II, III, IV and V locust nymphs and adults reared on wheat as previously described (Chapter 2), were randomly assigned to either diet. To minimize variation due to differences in initial body size, and as the species is sexually dimorphic, only males whose freshly moulted weight was within 1 standard deviation of the mean from a previously weighed population were used. Instar I nymphs were initially included but due to their extremely small size (*c.* 1 mg) they were easily injured, and many died as they appeared to be unable to 'find' the meal. Some success was achieved by using plastic sleeves to restrict the Instar I nymphs to an area of 1 cm diameter around the meal. However, due to the intricacy of the extra sleeves when changing the diet, an increase in mortality occurred due to injury. Therefore, the Instar I treatment was abandoned. Twenty replicate locusts per age per diet were used. Initial locust weight was estimated from those sacrificed randomly throughout the experiment.

Experimental conditions

Experiments were performed in the 'old' constant temperature room (Chapter 2) maintained with identical conditions to that of the stock culture; i.e. at 32 $\pm$0.5 °C with a 8:16 h D:L photoperiod (lights on 0600 E.S.T.). This experiment required 100 locusts and since only 60 locusts per day could be fed the experiment was fully randomized over time. Each locust was individually housed in a modified 500 ml 'soft drink bottle' (Cadbury-Schweppes) so that consumption and frass output could be determined as previously described (Chapter 2). Higher 'day' temperatures (36.8-38.1 °C) were maintained by six 100 W incandescent globes suspended above the containers (Fig. 4.1). Six-eight week old Button grass and 12-14 week old Mitchell grass (only if it had begun to produce spikelets, Appendix II) was harvested daily and presented to the locusts as described in Chapter 2. Food was provided *ad lib* and insects were allowed to self-regulate body temperatures, thus individuals had as much control over processing rate as was physiologically possible, so that nutrient intake was limited by food quality. While the diet was provided *ad lib* and although I

corrected the remaining orts for metabolic changes I still tried to minimize the amount remaining at the end of each feeding bout. The locust meals were replaced every 24 h and the orts and frass from the previous 24 h removed and stored at –20°C until being freeze-dried to a constant weight. The orts and frass were then separated and weighed. Consumption was calculated as stated in Chapter 2.

Plant analysis

To enable the calculation of total intake and intake of each of the major chemical constituents on a daily basis, control fractions of grass were collected daily and analyzed for water content, specific leaf area (SLA), protein, non-structural carbohydrates and cell wall material. The major chemical components were expressed per unit dry matter and for protein and non-structural carbohydrates as a percentage of the cell contents (% protoplasm). To calculate intake of total dry matter and the specific chemical constituents, where appropriate, corrections were made for metabolic changes to the grass blades over the 24 h they were offered to the locusts according to the procedure described in Chapter 2.

Preparation and storage of plant and frass material

Fresh grass blades, orts and frass were placed in paper 'seed' envelopes, frozen to -90 °C and then freeze-dried (lyophilized) to a constant weight (48-96 h). Dried material was finely ground (Spex® freezer/mill) and stored at –20 °C in airtight containers until analyzed. Freeze drying rather than oven drying was used to lyophilize material as oven drying can lead to changes in the plant material e.g. induction of a Maillard reaction if temperatures exceed 40 °C (Van Soest 1994b).

Water

Locust water consumption was calculated as described in Chapter 2 from five haphazardly selected grass-blades from each species. Detached leaf blades were placed in water until fully turgid, weighed and frozen (-20 °C) until being freeze-dried to a constant weight.

Chemical constituents

Plant material was analyzed for total protein using the 'Bio-Rad' Bradford assay, which is sensitive to less than 1 μg of protein. This assay was chosen as it only responds to protein and not amino acids or other nitrogenous waste products (Zanotto *et al.* 1997). Protein was extracted as described in Chapter 2. Nitrogen was measured on 10 randomly selected samples of each diet with a Leco CHN-2000 analyzer. Total non-structural carbohydrates were extracted and quantified as described in Chapter 2. Plant cell wall material was estimated using the Van Soest method (Van Soest 1994a) as outlined in Appendix I. Plant material was extracted with neutral detergent solution only and not fractionated further (Chapter 3).

Data analysis

The chemical constituents of Button grass and Mitchell grass were compared using ANOVA (using days as replicates with a single measure per day). Scatterplots were used to check if there was a trend in the level of each chemical constituent over the time frame of the experiment.

Analyses were also performed on the grasses offered to the locusts. Two-factor ANOVA (factors: age and diet) was used to check if, by chance, the chemistry of the two grasses offered to the different aged locusts differed. Where there was a significant interaction between the two factors, tests of simple main effects (Quinn and Keough 2002) between levels of one factor at each level of the other factor were performed.

Insect performance on the two diets (Button grass and Mitchell grass) was compared using ANOVA and ANCOVA where appropriate. Two-factor ANOVA was used to compare initial weight and instar duration. Traditionally, insect performance is evaluated using a set of ratios, the 'nutritional indices' developed by Waldbauer (1968). However, it has been demonstrated that ratio-based analyses can fail to recognize relationships between variables, identify spurious relationships that lead to incorrect biological interpretations or information is lost when variables are compounded into ratios (Packard and Boardman 1987; Raubenheimer 1995; Raubenheimer and Simpson 1992; Raubenheimer and Simpson 1994). Waldbauer's (1968) 'nutritional indices' scaled growth and consumption to body mass (mean) e.g.

relative growth rate (RGR). Studies have shown that ANCOVA using initial body mass (Raubenheimer and Simpson 1992) or gain in biomass (Horton and Redak 1993) as covariates when analyzing growth are more appropriate. ANCOVA allows discrimination of pre- versus post-ingestive effects on growth (Beaupre and Dunham 1995; Horton and Redak 1993; Raubenheimer 1995; Raubenheimer and Simpson 1992; Raubenheimer and Simpson 1994), while still maintaining the logic of Waldbauer's (1968) 'nutritional indices'. Pre-ingestive effects on growth are caused by proportionally lower or higher consumption, while post-ingestive effects on growth are independent of consumption, i.e. the efficiency of conversion of assimilated nutrients to body mass.

The ANCOVA that is equivalent to Waldbauer's (1968) 'efficiency with which ingested food is converted to body mass (ECI)' is the comparison of weight gain among treatments taking consumption as the covariate. Differences in diet digestibility (approximate digestibility, AD) are detected by comparing frass produced between treatments with consumption as the covariate. 'Utilization plots' of ANCOVA-adjusted means allows the differentiation of pre- and post-ingestive effects (Beaupre and Dunham 1995; Horton and Redak 1993; Raubenheimer and Simpson 1994).

ANCOVA was performed following the technique outlined in Quinn and Keough (2002). Scatterplots were used to ensure that the relationship between the covariate and the dependent variable was linear for each treatment. Box plots were used to check for normality and homogeneity of variances across the treatments and where appropriate data were $\log_{10}$-transformed. Where initial two-factor ANCOVA (factors: diet and age) resulted in complex heterogeneity of regression slopes, single factor ANCOVA comparing either diets or ages was used. If the test for homogeneity of slopes was significant (treatment x covariate interaction term), the regions of significance between regressions in the ANCOVA were determined using the Johnson-Neyman Technique (Wilcox 1987) with the WILCOX.exe programme (version 3.2, written by Andrew Constable, 1989). This test uses Dunnett's T3 test set at $P < 0.05$ to distinguish the regions where the regression lines were significantly different. Where the homogeneity of slopes tests was not significant, *post hoc* testing of adjusted means was performed using pairwise comparisons with *P*-values

corrected for multiple testing with the sequential Holm method using the MULTI.exe programme (version 2, written by Barry W. Brown and Kathy Russell, 1996).

Analysis of frass and consumption and assimilation of total fresh and dry matter, non-cell wall dry matter, protein, non-structural carbohydrate and water values used initial body weight as a covariate (all variables were log_{10} transformed). As water is an essential nutrient, fresh and dry weight intakes and growth were analyzed where possible. Compensatory feeding due to water limitation can not be ascertained if only dry weight intake is measured (Slansky Jr. 1993). It was not possible to collect the frass as it was produced in order to measure fresh weight of frass.

Rates of growth and consumption for Instars II–V were compared by ANCOVA using instar duration (h) as the covariate. Both growth and consumption were log_{10}-transformed.

Utilization plots (*sensu* Hagele and Rowell-Rahier 1999; Horton and Redak 1993) were used to distinguish if effects on growth were caused ingestively or post-ingestively (using consumption or assimilation as covariate) and differences in digestibility of the two grasses were detected by comparing the amount of frass produced with consumption as the covariate. One common constraint on the use of ANCOVA is that the covariates should not be affected by the treatments (Quinn and Keough 2002). This is clearly not true in this study because covariates such as initial body weight, consumption, etc. are correlated with age. However, any experiment that involves comparing animals of different ages faces this difficulty and conclusions drawn assume that the relationship between each response variable and the covariate is consistent across the covariate values beyond the covariate range for that age. This point was highlighted by Horton and Redak (1993) who argued that comparing groups with different covariate values can still be a valuable tool in investigating diet-dependent variation in digestive strategies. The effect of age was only investigated further for rate of growth and consumption, final weight, consumption total dry matter and non-cell wall dry matter, frass 'AD', growth 'ECI', and growth 'ECD'. All analyses were undertaken with SYSTAT® 10 unless otherwise stated.

RESULTS

The grasses as a resource

Button grass had significantly higher SLA (larger area per gram of dry matter), ratio of water to dry matter and ratio of protein to carbohydrate than Mitchell grass (Table 4.1). Mitchell grass had significantly more cell wall material, non-structural carbohydrates in the dry matter and more protein and non-structural carbohydrates in the protoplasm than Button grass (Table 4.1). However, there was no difference in the amount of protein, or nitrogen in the dry matter or the ratio of either protein or non-structural carbohydrates to cell wall material (Table 4.1).

There was no significant difference in most properties of the grass samples offered to each instar. Where there were significant differences the magnitude was less than 1.8 %. These values are unlikely to be of biological significance, rather a result of the high precision of the tests used. It was expected that these differences would be reflected in slight variations in consumption of the grasses.

The analyses above are based on dry matter, however fresh weight may be more relevant to ingested volumes. On a fresh weight basis, locusts consuming Mitchell grass ingested *c.* one third more protein, carbohydrate and cell wall per meal than those ingesting Button grass because of the diluting effect of water in the Button grass diet (Table 4.2).

Locust performance

There was no significant difference between the initial dry weights (and hence wet weights) for locusts randomly assigned to either diet for each instar (Table 4.3). Significantly more nymphs survived on the Button grass diet than on the Mitchell grass diet ($F_{1,6} = 18.082$, $P = 0.005$) (Table 4.4) and Instar II, IV and V had a significantly shorter instar duration (Fig. 4.2). Feeding on Button grass, the final wet weight (Fig. 4.3a) of Instars IV and V and final dry weight (Fig. 4.3b) of Instar V and Adults was significantly higher compared to locusts feeding on Mitchell grass. The effect of diet on both final dry weight and instar duration was greater the older the instar. Initial dry weight significantly influenced the final dry weight obtained

(Appendix III, Table III.1). The initial ratio of water to dry matter of the locusts decreased with locust age ($P < 0.001$) (Table 4.5).

Nymphal development took 3.6 days longer ($P < 0.001$) on Mitchell grass (28.5 $\pm$ 1.2 days) compared to Button grass (24.9 $\pm$ 0.7 days). The duration of the first instar was estimated to be 4.0 days for nymphs irrespective of diet, which was 88% of the duration of Instar II (from Gregg 1981).

Digestive performance

Between Diets

Locusts feeding on Button grass consumed more fresh material (Fig. 4.4a) and dry matter (Fig. 4.4b) than those feeding on Mitchell grass. As the ratio of water to dry matter and within the dry matter the ratio of cell contents to cell wall was higher for Button grass, all ages of locusts feeding on Button grass consumed more water (Fig. 4.5a) and non-cell wall material (Fig. 4.5b). While total dry matter consumption was different for each diet, only Instar V nymphs feeding on Mitchell grass ingested less protein than their counterparts feeding on Button grass (Fig. 4.6a). Instars IV and V and Adults feeding on Button grass ingested more non-structural carbohydrates (Fig. 4.6b). Growth rate was the same on both diets and increased with age (Fig. 4.7). There was a significant interaction between diet and age, with Instar IV and Instar V nymphs feeding on Mitchell grass having the same growth rate. Consumption rate increased with age (Fig. 4.8) and was higher for nymphs consuming Button grass.

Instars II, V and adults produced more frass feeding on Button grass than Mitchell grass (Fig. 4.9). For a given intake, significantly more frass was produced for Instar II's consuming Button grass and Instar IV and adults consuming Mitchell grass. When the indigestible component of the dry matter was removed from both consumption and frass, there was no difference between diets for all ages (Fig. 4.10). However, the adults feeding on Mitchell grass had significantly more protein in the frass compared to those feeding on Button grass for a given intake of protein (Fig. 4.11).

Significantly more Button grass dry matter was assimilated by all ages except Instar II nymphs (Fig. 4.12). Dry matter was assimilated at a faster rate with increasing age (Fig. 4.13). Instar V and adults assimilated significantly more protein feeding on Button grass (Fig. 4.14). The protein digestibility values were higher than the non-cell wall digestibility but the same pattern existed between diets and ages.

For each age, growth on a wet and dry weight basis was the same for a given intake (Waldbauer's 'ECI') (Fig. 4.15, 4.16). There was no difference in growth for the amount of nutrients assimilated (Waldbauer's 'ECD') except for the adults where those feeding on Button grass were more efficient at converting assimilated food to body mass (Fig. 4.17). Growth was higher on Button grass than Mitchell grass because of the higher intake (and assimilation) of Button grass.

With Age

For the majority of the insect performance variables there was a significant interaction between diet and age (Table 4.6). With increasing body weight significantly more wet and dry biomass was gained (Table 4.7, Fig. 4.18, 4.19) and significantly more dry matter was consumed (Table 4.7, Fig. 4.20). More frass was produced for a given consumption of dry matter with increasing age, for both diets (Table 4.7, Fig. 4. 21). The differences between instars were more pronounced on a Mitchell grass diet. However, when the indigestible component was removed, there was no difference between Instars II-V for both diets (Fig. 4.22). This resulted in the amount of material assimilated being the same regardless of body weight (Fig. 4.23).

Instar V and the adults converted significantly less dry matter to body mass (Table 4.7, Fig. 4.24). However, except for the Adults, body weight did not affect the efficiency with which assimilated material was converted to body mass (Table 4.7, Fig. 4.25).

DISCUSSION

The two grasses differed as predicted (annual versus perennial) in terms of their structure (water and SLA) but not chemistry (ratio of cell wall, protein and non-structural carbohydrates in the dry matter). This resulted in nymphs consuming Button grass gaining more weight and developing faster with higher survival rates compared to those consuming Mitchell grass, as predicted. However, contrary to predictions, the later instars were more affected by diet quality than the earlier instars (Table 4.6, 4.7).

Although nymphs were able to consume and assimilate more Button grass than Mitchell grass, only Instar V nymphs and adults differed in the total amount of protein assimilated. As the instar duration was longer when feeding on Mitchell grass, nymphs took longer to assimilate the same amount of protein. Button grass was more digestible than Mitchell grass but this could be explained by differences in the amount of cell wall between the two grasses. The slower rate of development and lowered final biomass appeared to be due to the rate of accumulation of nutrients (pre-ingestive mechanisms) rather than differences in metabolic costs associated with processing either diet (post-ingestive mechanisms).

Differences between the two diets

Lower Mitchell grass consumption by the older nymphs did not appear to be due to gut volume limitations, as nymphs consuming Button grass had higher consumption and produced more frass. Although Mitchell grass had a slightly higher percentage of cell wall material in the dry matter the amount of protein and non-structural carbohydrates was also higher and the ratio of cell wall to protein and non-structural carbohydrates were similar (*c.* 0.26) for both grasses. It was not expected that the amount of cell wall *per se* (*c.* 53%) in both grasses would be enough to cause a reduction in consumption. Previous research using artificial diets diluted with indigestible cellulose suggests that locusts are able to compensate by increasing consumption for a diet with up to 80% of an indigestible component (McGinnis and Kasting 1967; Raubenheimer and Simpson 1993). However, if the cell wall is acting as a barrier not just a dilutant then the differences in SLA and water content suggest that the two grasses are structured differently.

Consumption

Total intake is a result of the compromises an insect makes to its nutrient target (Raubenheimer and Simpson 1997; Simpson and Raubenheimer 1993b). Both the type and amount of compromise has been shown to be dependent on the insect species, the animal age and the diet (Chambers *et al.* 1995; Raubenheimer and Simpson 1997; Trumper and Simpson 1994). For these two grasses, protein may be driving the dietary strategy as significantly lower protein assimilation correlated with a corresponding significant decrease in growth. The 2.4% N in both these diets is within the range that previous researchers (Yang and Joern 1994c) have found to be critical, leading to either decreased consumption and growth or increased consumption with growth maintained. Only protein levels in the grasses were measured but free amino acids are highly variable and can comprise up to 10% of the available nitrogen (Bernays and Barbehenn 1987). The types of amino acids influence food selection and growth (e.g. Bernays and Woodhead 1984). Imbalance of essential amino acids can reduce consumption through lowered sensitivity of taste receptors (Abisgold and Simpson 1988).

Nymphs consuming Mitchell grass ingested more carbohydrate per unit protein ingested as a consequence of the protein:carbohydrate ratio, but as there was no difference between diets in terms of metabolic costs it is not expected that this was the factor causing either decreased growth or increased instar duration. The optimum ratio of protein:carbohydrate for locust growth has been measured at 1:1.28 (Chambers *et al.* 1995) to approximately 1:1.12 (Simpson and Raubenheimer 1993b). If Australian plague locusts require a similar ratio of protein:carbohydrate then both grasses will be providing carbohydrate in excess of protein requirements (Table 4.1) but it has been found that excessive dietary carbohydrate can be removed via increased CO_2 output (Zanotto *et al.* 1997). These studies have been performed on grasshoppers with minimal activity and one experiment that investigated the effect of activity found that exercise increased carbohydrate requirements (Raubenheimer and Simpson 1997). In the field, it is predicted that Australian plague locusts would have variable energy requirements depending on density. High carbohydrate requirement would be required under banding and plaguing conditions. Bands of later instar nymphs have been measured walking up to 100 m h^{-1} while migratory flights can be over 500 km per night, while nymphs at

densities below 30 per m^2 move much less (Australian Plague Locust Commission 1986).

Button grass had a higher ratio of water to dry matter than Mitchell grass. The effect of differences in water quantity is equivocal (e.g. Ben Halima *et al.* 1983; Lewis 1984; Paul *et al.* 1992; Scriber 1979; Slansky Jr. and Wheeler 1991; Timmins *et al.* 1988). *Locusta migratoria* when allowed to self regulate over an entire instar, chose 1 part water to 2 parts dry matter, however the majority of water was consumed in the day preceding the moult (Lewis and Bernays 1985). Both grasses (and plants in general) have water in excess of this ratio (Table 4.1) and whether this water was assimilated is uncertain. It was not possible to collect frass as it was produced.

Dietary water may regulate nutrient intake through dilution of the haemolymph. Nutrient concentrations in the haemolymph both stimulates and inhibits feeding (Abisgold and Simpson 1987; Ben Halima *et al.* 1983; Bernays and Chapman 1974a; Raubenheimer and Gade 1994; Roessingh *et al.* 1985; Simpson and Simpson 1992; Simpson and Raubenheimer 1993a). Locusts feeding on Button grass may consume more by consuming larger meals more frequently because per meal the nutrients are more diluted than when feeding on Mitchell grass (

Table 4.2) likely to result in the locust's haemolymph having a lower osmolarity and nutrient concentration. Shorter food retention time has been associated with moister food (Goodhue 1962 as cited by Baines *et al.* 1973; Uvarov 1966) although, there appears to be other factors interacting with water determining food retention time (Barton Browne and van Gerwen 1976; Hoekstra and Beenakkers 1976). Button grass meal size might also be larger because the concentration of nutrients on the fractured plant surface will be lower providing less stimulation that results in negative feedback limiting meal duration (Barton Browne *et al.* 1975a; Barton Browne *et al.* 1975b).

Insufficient dietary water can also significantly increase metabolic costs (Paul *et al.* 1992; Scriber 1977; Slansky Jr. and Wheeler 1991; Timmins *et al.* 1988; Van'T Hof and Martin 1989) and reduce water for synthesis of new body tissue (Martin and Van't Hof 1988). However, increased efficiency of conversion of digestion to biomass associated with reduced intake was not observed. Nor did I observe the

locusts ingesting their cast exoskeletons (although potentially this was because they were sacrificed before their new exoskeleton had hardened) which is frequently observed if dietary water is excessive (Lewis 1984). Water content of the grasses could be acting as a consumption regulator (pre-ingestive factor) but did not appear to be affecting utilization efficiency.

Digestibility

When the indigestible cell wall was accounted for there was no difference in the digestibility of each grass. Many studies fail to remove the indigestible component (e.g. Yang and Joern 1994c) and conclude correctly, after diluting an artificial diet with indigestible cellulose that it is less digestible. However, this does not provide any information on the effect of the dilution on the digestible component of the diet. Where the indigestible fraction has been removed before the calculation of digestibility, there is often no difference in nutrient assimilation (e.g. Slansky Jr. and Wheeler 1991; Timmins *et al.* 1988). Recalculation of the data of Yang and Joern (1994c) found a corresponding (66%) decrease in digestibility when the 5% N diet was diluted to 3% N. However, there was a correlation with decreased food retention time and digestibility between the 1% and 3% diets.

Models predict that increased food retention time in the gut would increase digestibility (Raubenheimer and Simpson 1996) but empirical evidence to date either does not always support this (Timmins *et al.* 1988) or has been concluded when the indigestible fraction of the diet had not been removed from the analysis (Yang and Joern 1994c). Nymphs consuming Mitchell grass could have a longer food retention time as less food was consumed over a longer period of time although it is equally possible that food retention time was equal and smaller meals were ingested.

Digestibility of protein was higher than that of the non-cell wall dry matter (Fig. 4.10 vs Fig.4.11), although the trends were the same with locust age. While non-structural carbohydrate assimilation was not measured, it was predicted that this could not all have been assimilated because protein appeared to be preferentially assimilated over the remainder of the non-cell wall fraction. This strongly suggests that the major non-cell wall components (protein, carbohydrate) either have differential accessibility or digestibility in entire grass blades. Proportionally different assimilation of dry

matter components may be due to variations in the retention time of food throughout the instar as the locust 'balances' the blend of nutrients assimilated (Raubenheimer and Simpson 1996; Raubenheimer and Simpson 1998; Simpson 1982a).

Differences with age

As the locusts increased in age, growth and dry matter consumption increased per unit body weight but diet digestibility and the rate of digestion decreased, resulting in the amount assimilated remaining the same. The efficiency with which nymphs converted ingested and digested material to biomass remained the same, which was the opposite to that predicted. The adults appeared less efficient at converting intake and assimilate to body mass than the nymphs but this was most likely due to the apparent reduced growth as the adults do not moult, there is no exuviate to include in the growth. The younger nymphs were able to perform equally on each diet while the older nymphs' performance was affected more when consuming Mitchell grass than Button grass.

Pre-ingestive mechanisms appeared to be causing the performance differences observed with the older nymphs. This could be because a larger body imposes certain constraints or because different nutrients are required and the grasses differed in, for example, amino acid concentrations. Differences in dietary performance (survival, development and growth rate) on different grasses have been recorded between late instars but not when they were younger (Olfert *et al.* 1990) and between males and the same age, but larger, females (Hinks *et al.* 1990). However, evidence to date is inconclusive (e.g. Slansky and Scriber 1985). Metabolic rate usually decreases with age (Woodring *et al.* 1979) but was not observed in this study, suggesting the older instars actually had increased costs associated with feeding compared to the younger nymphs.

Reduced consumption by nymphs feeding on Mitchell grass could have resulted from either consumption of smaller meals or longer intermeal durations or a combination of both. Consumption may have been reduced on Mitchell grass because the older nymphs had less water per unit dry mass that may have led to

increased osmolarity and nutrient concentrations in the haemolymph (Abisgold and Simpson 1987; Barton Browne and van Gerwen 1976; Bernays and Chapman 1974a; Simpson and Simpson 1992; Simpson and Raubenheimer 1993a). Increasing water per unit dry matter for the locusts correlated negatively with age (Table 4.5) and this is thought to be linked to juvenile hormone concentration (Beenakkers and Van Den Broek 1974). On a wet weight basis, the protein and non-structural carbohydrate concentration in Mitchell grass was higher (Table 4.2) which may result in smaller meals being consumed and/or less often (Barton Browne *et al.* 1975a; Barton Browne *et al.* 1975b; Bernays and Chapman 1974b; Simpson and Raubenheimer 1993a). Smaller meals of Mitchell grass could also result from the differences in structure (SLA) of the two grasses as volumetric feedback from the crop terminates feeding (Bernays and Chapman 1973a; Roessingh and Simpson 1984).

Smaller meals could also have resulted from a decrease in preference or breadth of dietary choice. Reduced consumption in the first day of the instar, due to initial lack of phagostimulation, can lead to increased instar duration and reduced growth (Grabstein and Scriber 1982; Schoonhoven 1972). If for some reason Mitchell grass was less acceptable than Button grass this could have led to the patterns recorded.

Digestibility rate decreased with age and the rate for nymphs consuming Mitchell grass was lower than for those consuming Button grass. The age related decrease could be due to the predicted decreased food retention time (consumption increased relatively with age), a result of processing by mandibles of increasing size, constraints imposed by increasing gut volume to surface area ratio or a combination of all three. Increased particle size was measured with increased mandible sizes for saturniid caterpillars (Bernays and Janzen 1988) and with artificial diets where particle size is not an issue, generally AD is higher than with 'natural' foods (e.g. Slansky and Scriber 1985). The lower digestibility rate of Mitchell grass could be because Mitchell grass was unable to be digested and absorbed as quickly due to its anatomy or the differences in water, or this observation is a result of the decreased consumption of Mitchell grass.

Australian plague locusts are considered to be extreme *r* strategists (r_{max} =0.13) (Gregg 1981) and given the environment they inhabit it was predicted that developmental rate would be selected over growth rate. However, the opposite was recorded, with differences between instar duration recorded at an earlier age than differences between growth rates. Therefore, as noted by field workers (Hunter *pers. comm.*; Symmons and McCulloch 1980) the longer Button grass remains green the more likely plagues are to develop.

TABLES AND FIGURES

Table 4.1 Chemical analysis of the two treatment diets. Results averaged over the duration that locusts were fed. Number of replicates (days) = 43 for each diet except nitrogen where 10 randomly selected samples were analyzed.

	Button grass	**Mitchell grass**	
SLA ($m^2 g^{-1}$)	0.045 ± 0.001	0.025 ± 0.001	$F_{1, 84} = 513.595$ $P < 0.001$
Water (g g^{-1} dry weight)	4.864 ± 0.093	2.541 ± 0.046	$F_{1, 84} = 498.580$ $P < 0.001$
Cell wall material (% dry weight)	51.598 ± 0.566	55.304 ± 0.412	$F_{1, 84} = 28.011$ $P < 0.001$
Protein (% dry weight)	9.870 ± 0.206	10.120 ± 0.184	$F_{1, 84} = 0.816$ $P = 0.369$
Nitrogen (% dry matter)	2.364 ± 0.064	2.435 ± 0.109	$F_{1, 18} = 0.314$ $P = 0.582$
Non-structural carbohydrate (% dry weight)	19.087 ± 0.311	21.498 ± 0.476	$F_{1, 84} = 17.994$ $P < 0.001$
Ratio protein : non-struct. carbohydrate (g g^{-1})	1:1.970 ± 0.053	1:2.152 ± 0.061	$F_{1, 84} = 5.165$ $P = 0.026$
Protein (% protoplasm)	20.483 ± 0.451	22.683 ± 0.413	$F_{1, 84} = 12.931$ $P < 0.001$
Carbohydrate (% protoplasm)	39.74 ± 0.90	48.422 ± 1.304	$F_{1, 84} = 30.087$ $P < 0.001$

Table 4.2 The predicted amounts of nutrients per 100 mg intake of fresh diet.

	Button grass (mg)	**Mitchell grass (mg)**
Water	82.8	77.7
Dry matter	17.2	22.3
Within the dry matter:		
Cell wall	9.0	12.4
Protein	1.7	2.3
Carbohydrate	3.3	4.8
Unknown	3.2	2.8

Table 4.3 Mean dry weight (± se) of the newly moulted male locusts ($n = 20$ for each diet). There was no significant difference for the initial weight for nymphs feeding on either diet.

Age	Button grass diet	Mitchell grass diet	
II	2.88 ± 0.09	3.02 ± 0.19	$F_{1,38} = 0.450$, $P = 0.507$
III	5.56 ± 0.20	5.61 ± 0.18	$F_{1,38} = 0.031$, $P = 0.861$
IV	11.71 ± 0.41	12.18 ± 0.33	$F_{1,38} = 0.785$, $P = 0.381$
V	25.53 ± 0.49	25.43 ± 0.84	$F_{1,38} = 0.010$, $P = 0.922$
Adult	55.35 ± 1.22	54.21 ± 1.34	$F_{1,38} = 0.378$, $P = 0.542$

Table 4.4 Survival of nymphs feeding on Button and Mitchell grass calculated for Instars II-V and also including the first 7 days of the adult stage.

Age	Button grass %	Mitchell grass %	*P* value
Instar III	89.29	74.19	
Instar IV	93.94	74.36	
Instar V	93.55	76.19	
Total nymphal survival	89.05	73.83	0.005
Adult	82.35	90.63	
Total survival	87.71	77.19	0.049

Table 4.5 Mean water content per gram dry matter (± se) of newly moulted nymphs from the sacrificial group used to obtain the estimate for initial dry weight for the treatment groups.

Age	Water content ($g\ g^{-1}$ dry matter)
II	3.45 ± 0.11
III	3.26 ± 0.06
IV	3.06 ± 0.08
V	2.95 ± 0.10
Adult	2.71 ± 0.05

Table 4.6 Summary of results of ANOVA/ANCOVA statistical analysis. WW = wet weight, DW = dry weight. ✓ = significant difference and – no difference. The statistical values are given in Appendix III Table III.1.

Variable		Interaction diet x age	Diet	Age
Instar duration		✓	✓	✓
Final wet	**WW**	✓	✓	✓
	DW	-	✓	✓
Growth rate		✓	-	✓
Consumption	**dry matter**	-	✓	✓
	non-cell wall/protein/ non-structural carbohydrates	-	✓	✓
Consumption rate		-	✓	✓
Assimilation	**dry matter**	-	✓	✓
	protein	✓	✓	✓
	Frass	✓	✓	✓
AD	**dry matter**	✓	✓	✓
	non-cell wall material	-	-	✓
	protein	✓	✓	✓
ECI	**WW**	✓	-	✓
	DW	-	-	✓
ECD		-	✓	✓

Table 4.7 Summary of ANCOVA/ANOVA table. I.W.=initial weight (dry and wet), F.W.=final weight, I.D.=instar duration, F=frass produced, FM=fresh matter, DM=dry matter, C=consumption, Ass=assimilated, H_2O=water consumption, C.W.=cell wall consumption, nCW=non-cell wall material, Prot=protein, CHO=carbohydrate, AD=approx. digest., ECI-efficiency ingested converted to body mass, ECD=efficiency digested converted to body mass. ✓ = significant difference between Button grass and Mitchell grass and – no difference for that measured for Button and Mitchell grass.

Age	I.W.	F.W. wet	F.W. dry	I.D	C FM & DM	C H_2O	C.W	C nCW	C Prot.	C CHO.	Ass DM	Ass Prot	F	AD	AD nCW	AD Prot	ECI FM & DM	ECD
II	-	-	-	-	✓	✓	✓	✓	-/-	-/-	-	-	✓	✓	✓	-	-	-
III	-	-	-	✓	✓	✓	✓	✓	-/-	-/✓	✓	-	-	-	-	-	-	-
IV	-	✓	-	✓	✓	✓	✓	✓	-/✓	✓/✓	✓	-	-	✓	-	-	-	-
V	-	✓	✓	✓	✓	✓	✓	✓	✓/✓	✓/✓	✓	✓	✓	✓	-	-	-	-
Adult	-	-	✓	n.a	✓	✓	✓	✓	-/-	✓/✓	✓	✓	✓	✓	✓	✓	-	✓

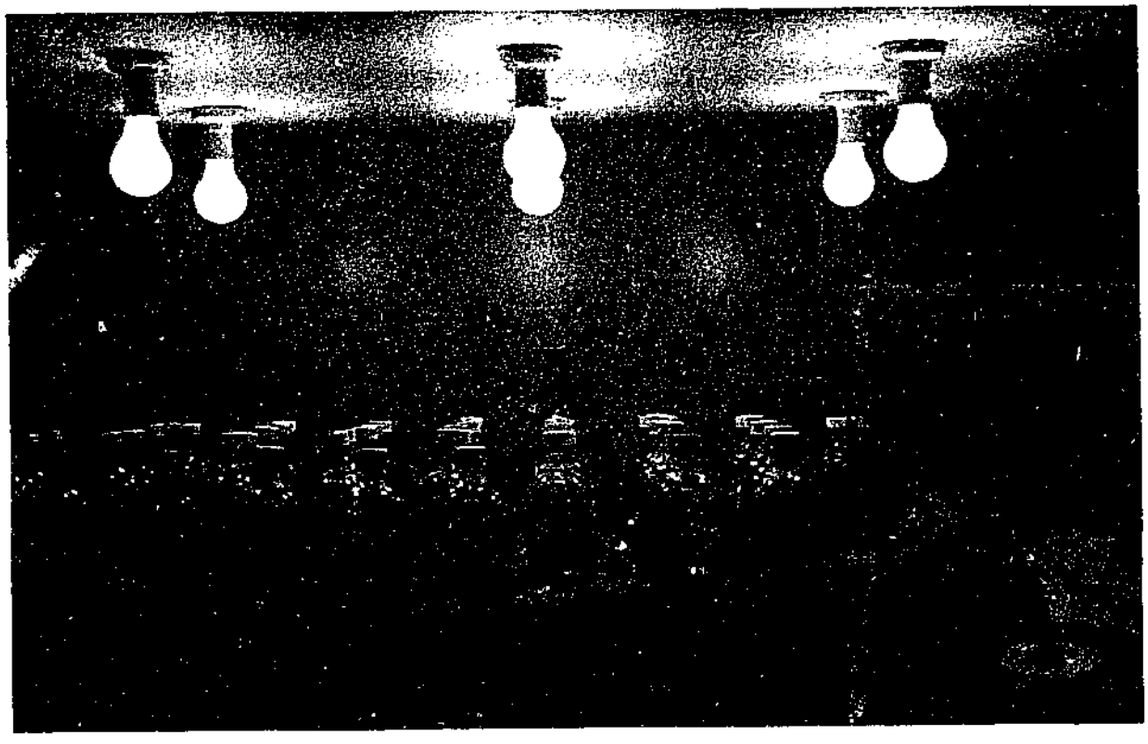


Fig. 4.1 Experimental set-up of the feeding chambers, with a close-up view of an Instar V locust feeding on Mitchell grass. Treatments were randomized over time and amongst positions.

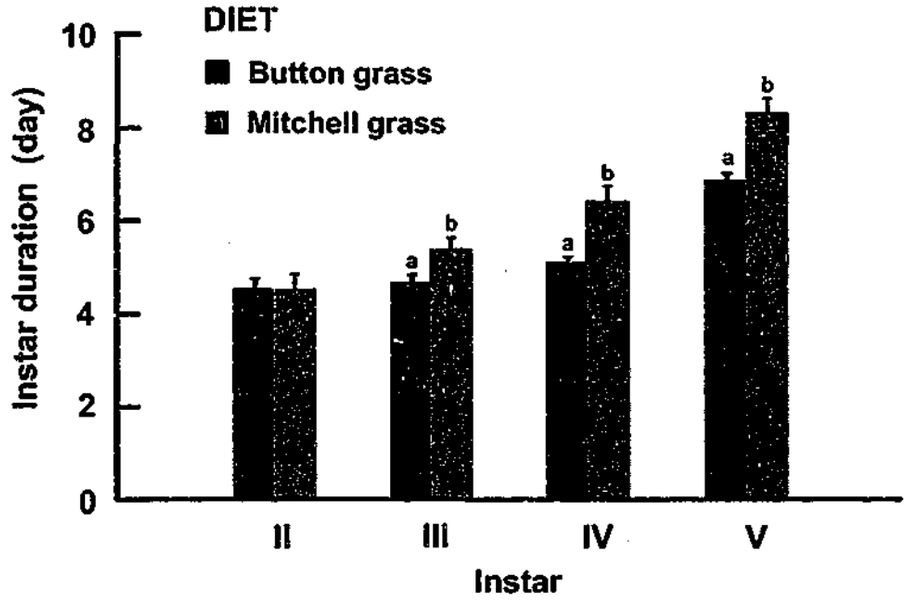


Fig. 4.2 Mean instar duration (± se) of Australian Plague Locust nymphs feeding on Button and Mitchell grasses. For each age, bars with different letters are significantly different ($P < 0.05$).

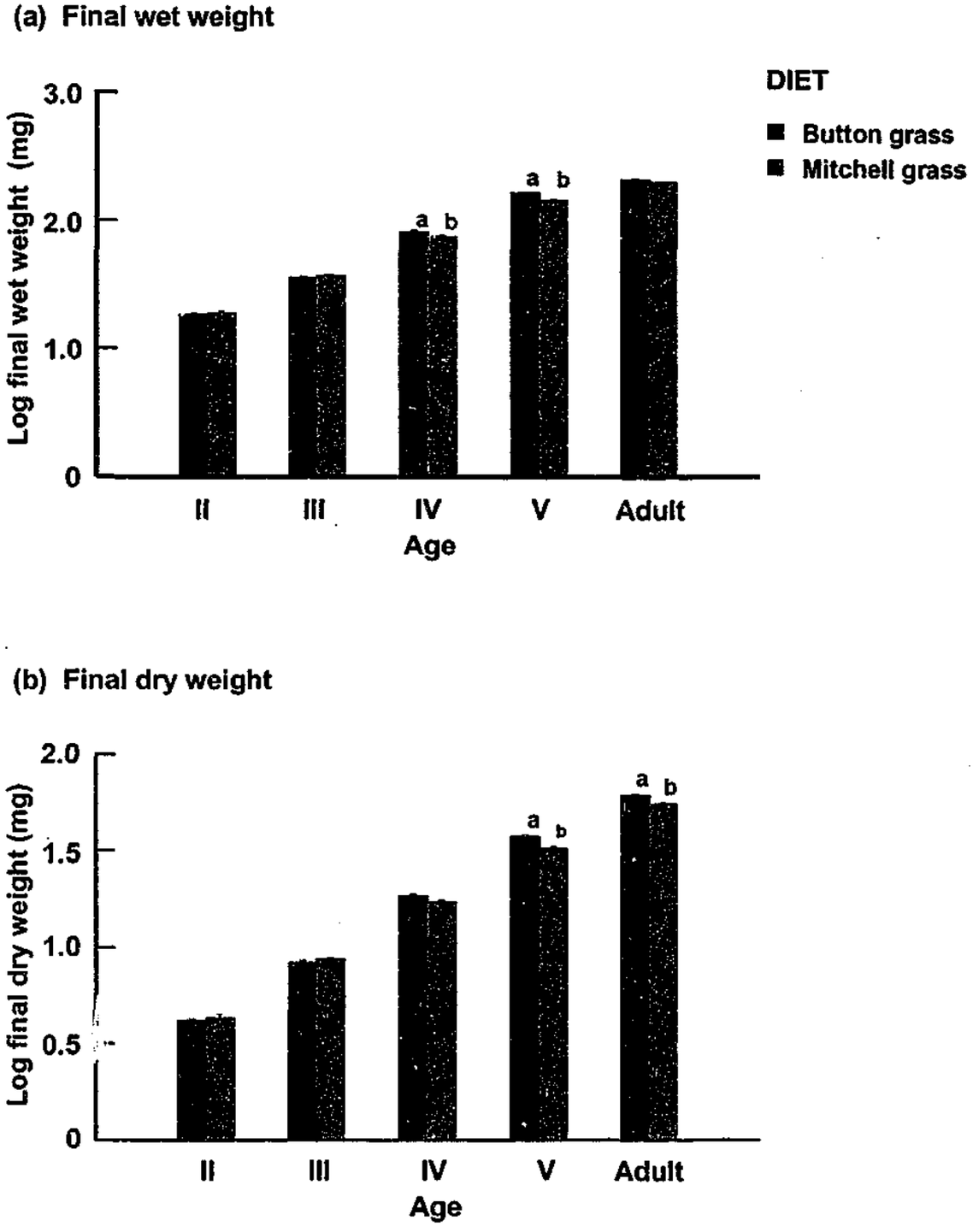


Fig. 4.3 ANCOVA-adjusted (log_{10}initial weight) of (a) log_{10}final wet weight and (b) log_{10}final dry weight, mean (± se) for locusts consuming both diets. For each age, bars with different letters are significantly different ($P < 0.05$).

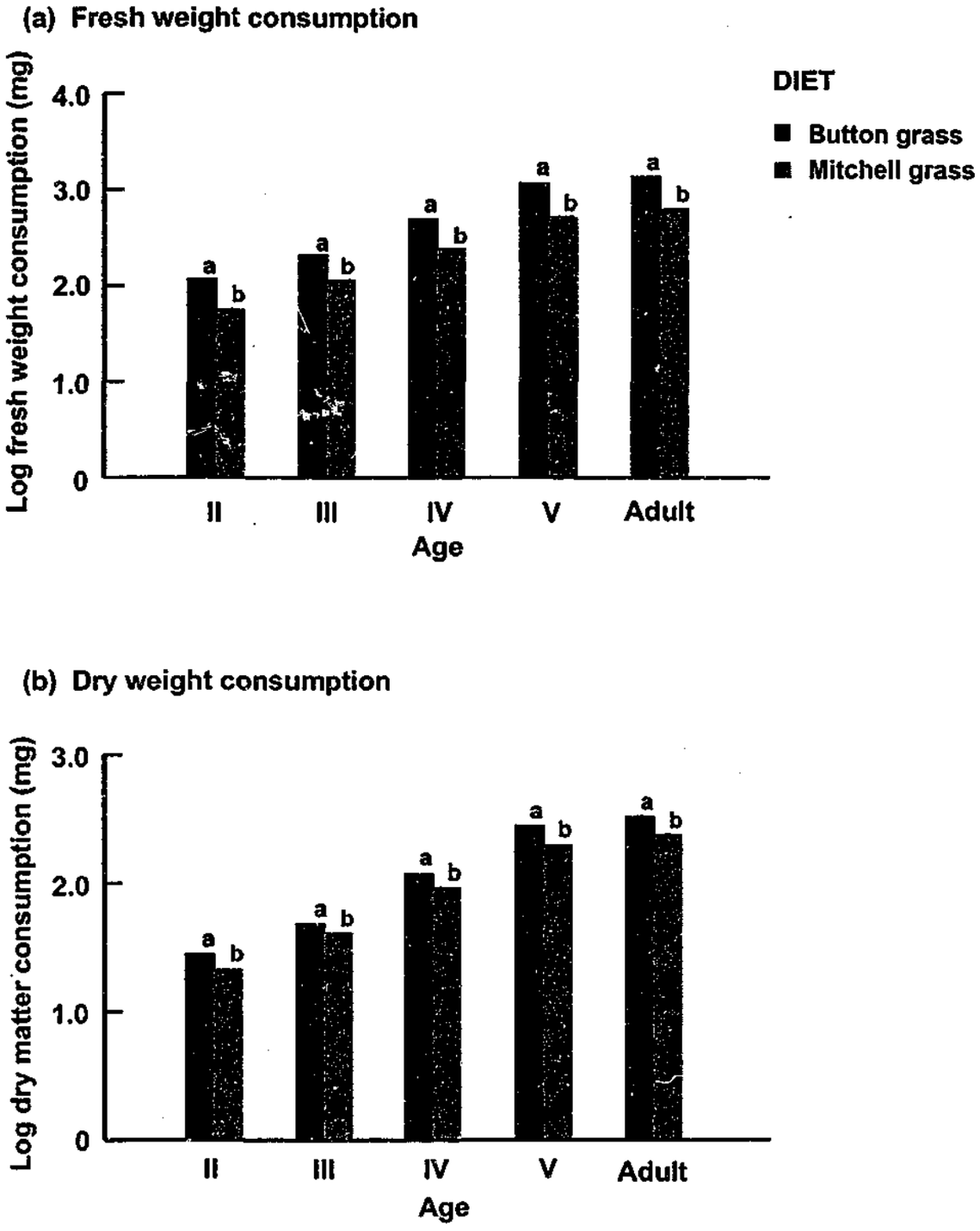


Fig. 4.4 ANCOVA-adjusted (log_{10}initial dry weight) (a) log_{10}fresh weight and (b) log_{10}dry weight consumption mean (± se) for locusts consuming both diets. For each age, bars with different letters are significantly different ($P < 0.05$).

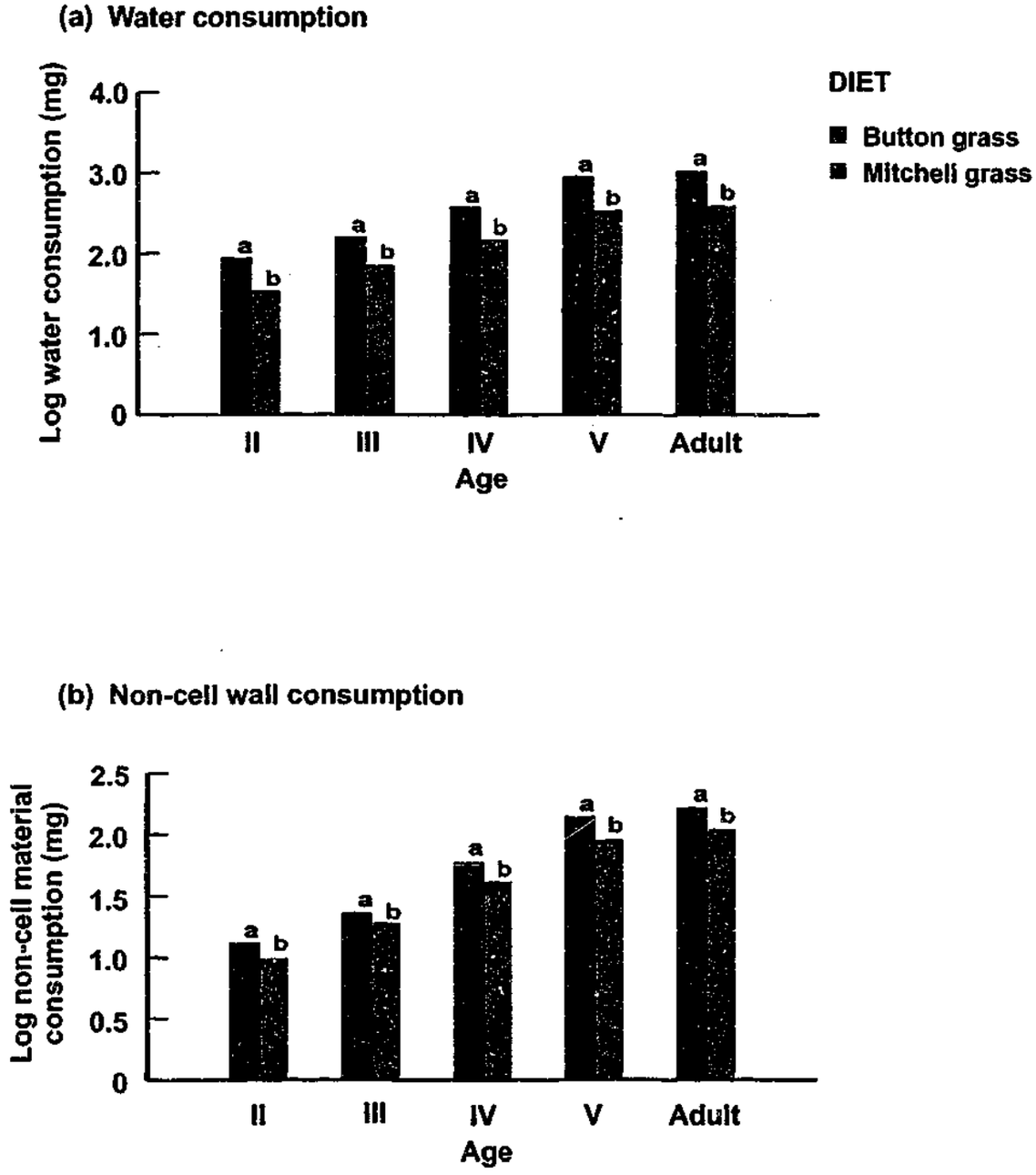


Fig. 4.5 ANCOVA-adjusted (log_{10}initial dry weight) mean (± se) (a) log_{10}total water and (b) log_{10}non-cell wall consumption for nymphs feeding on Button grass and Mitchell grass. For each age, bars with different letters are significantly different ($P < 0.05$).

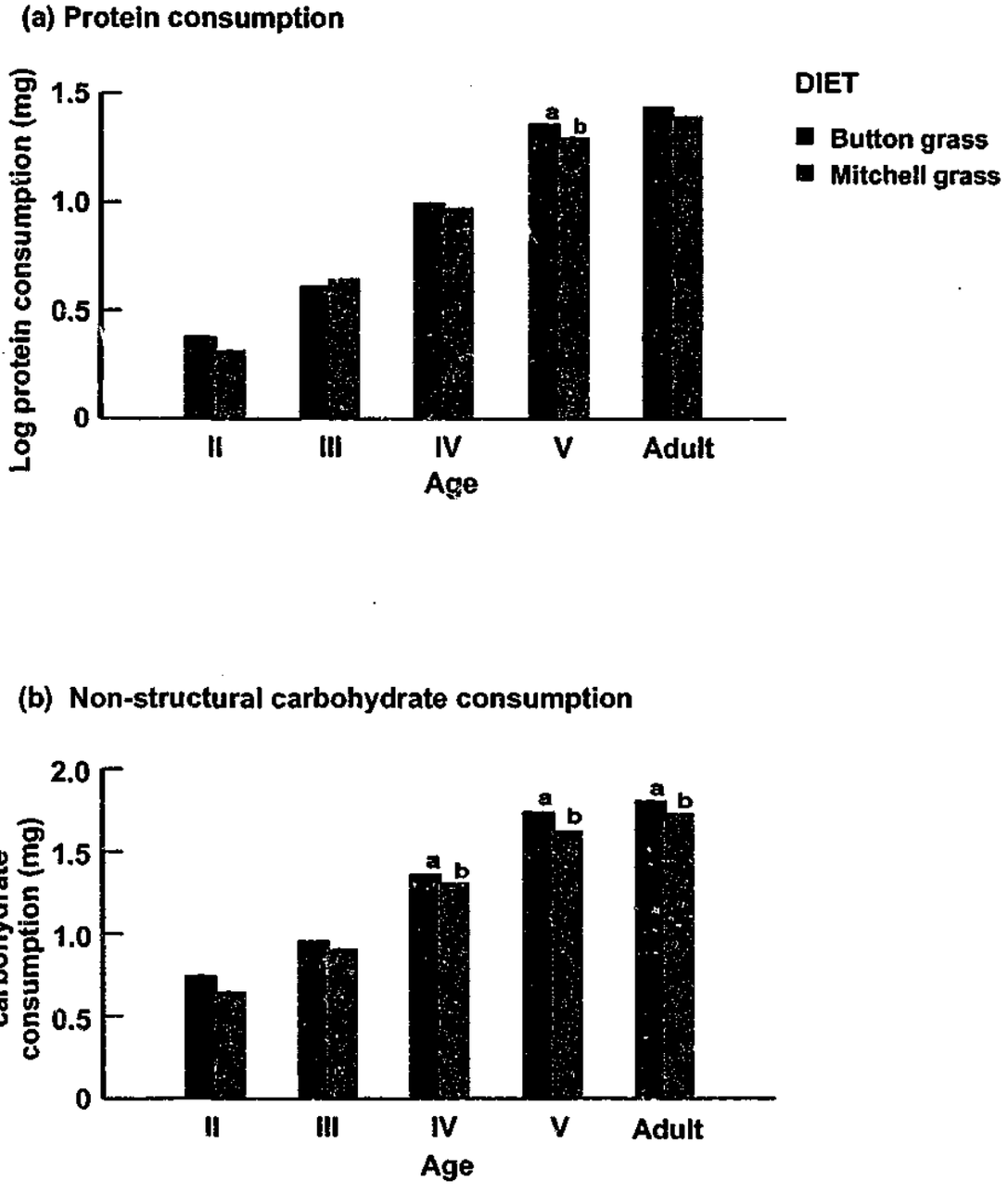


Fig. 4.6 ANCOVA-adjusted (log_{10}initial dry weight) mean (± se) (a) log_{10}total protein and (b) log_{10}non-structural carbohydrate consumption per instar for nymphs feeding on Button grass and Mitchell grass. For each age, bars with different letters are significantly different ($P < 0.05$).

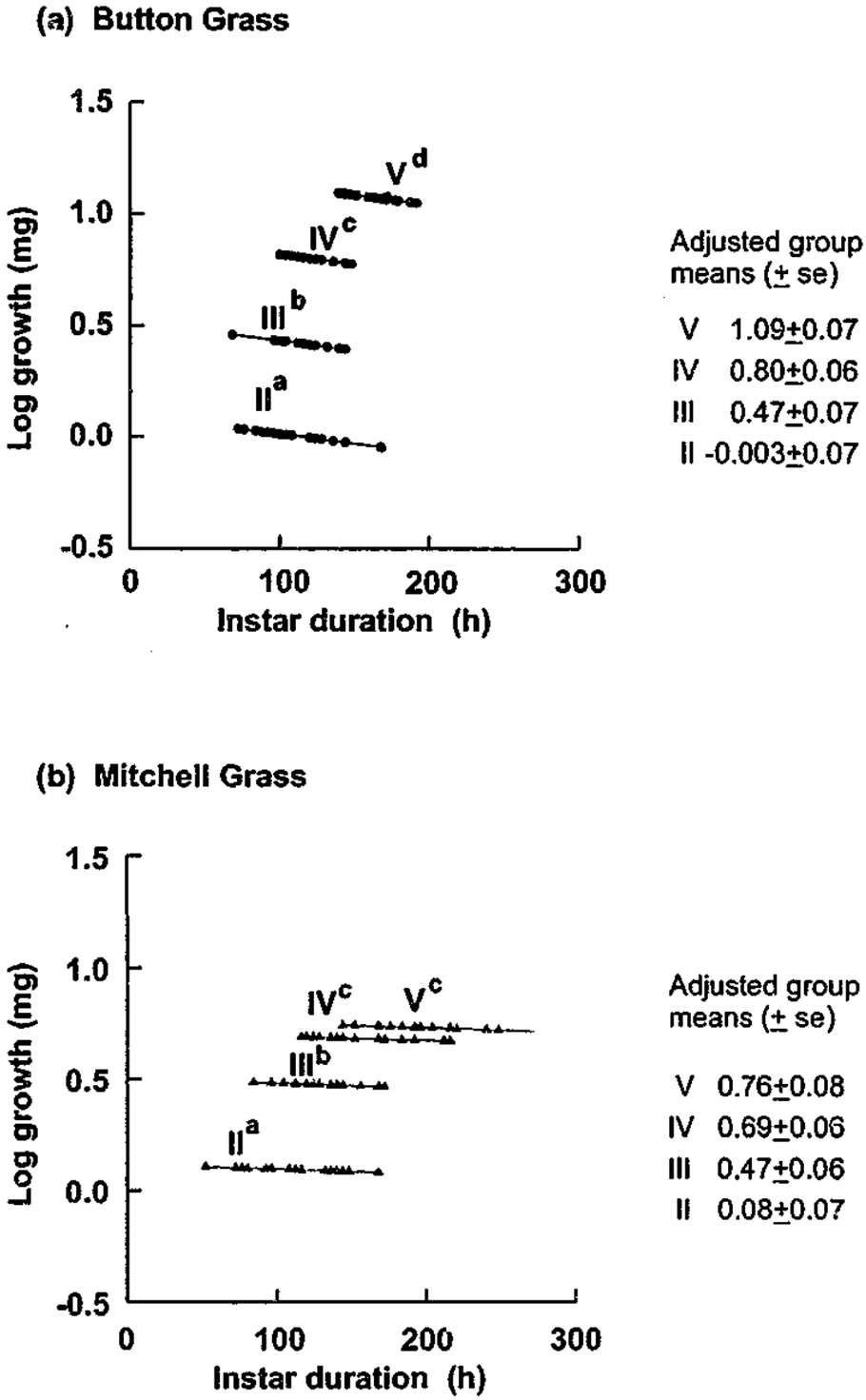


Fig. 4.7 Growth rate (ANCOVA fitted values) for nymphs feeding on (a) Button grass and (b) Mitchell grass. Lines with different letters are significantly different ($P < 0.05$).

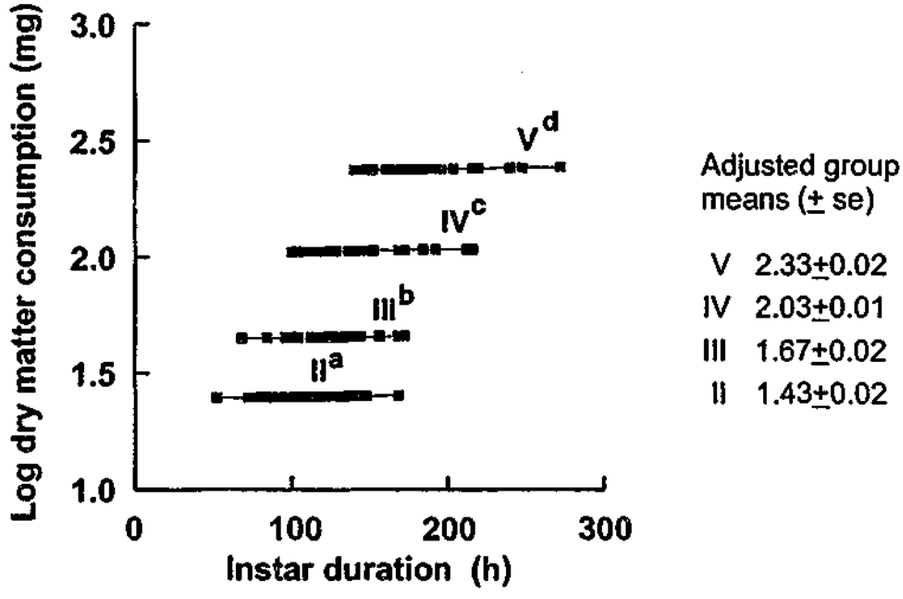


Fig. 4.8 Consumption rate for the different aged locusts. Lines with different letters are significantly different ($P < 0.05$).

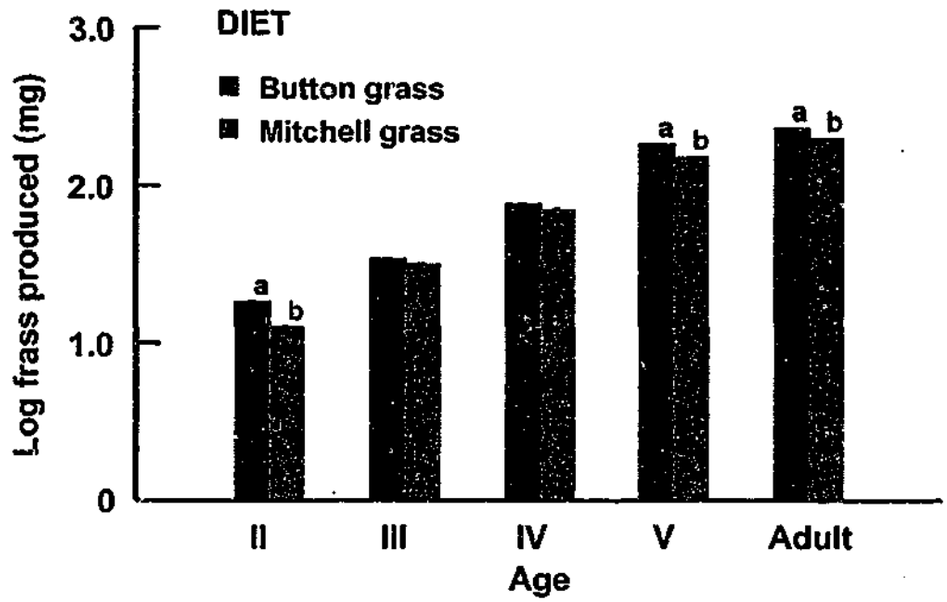


Fig. 4.9 ANCOVA-adjusted (log_{10}initial dry weight) mean (± se) log_{10}frass produced by the different aged locusts on each diet. For each age, bars with different letters are significantly different ($P < 0.05$).

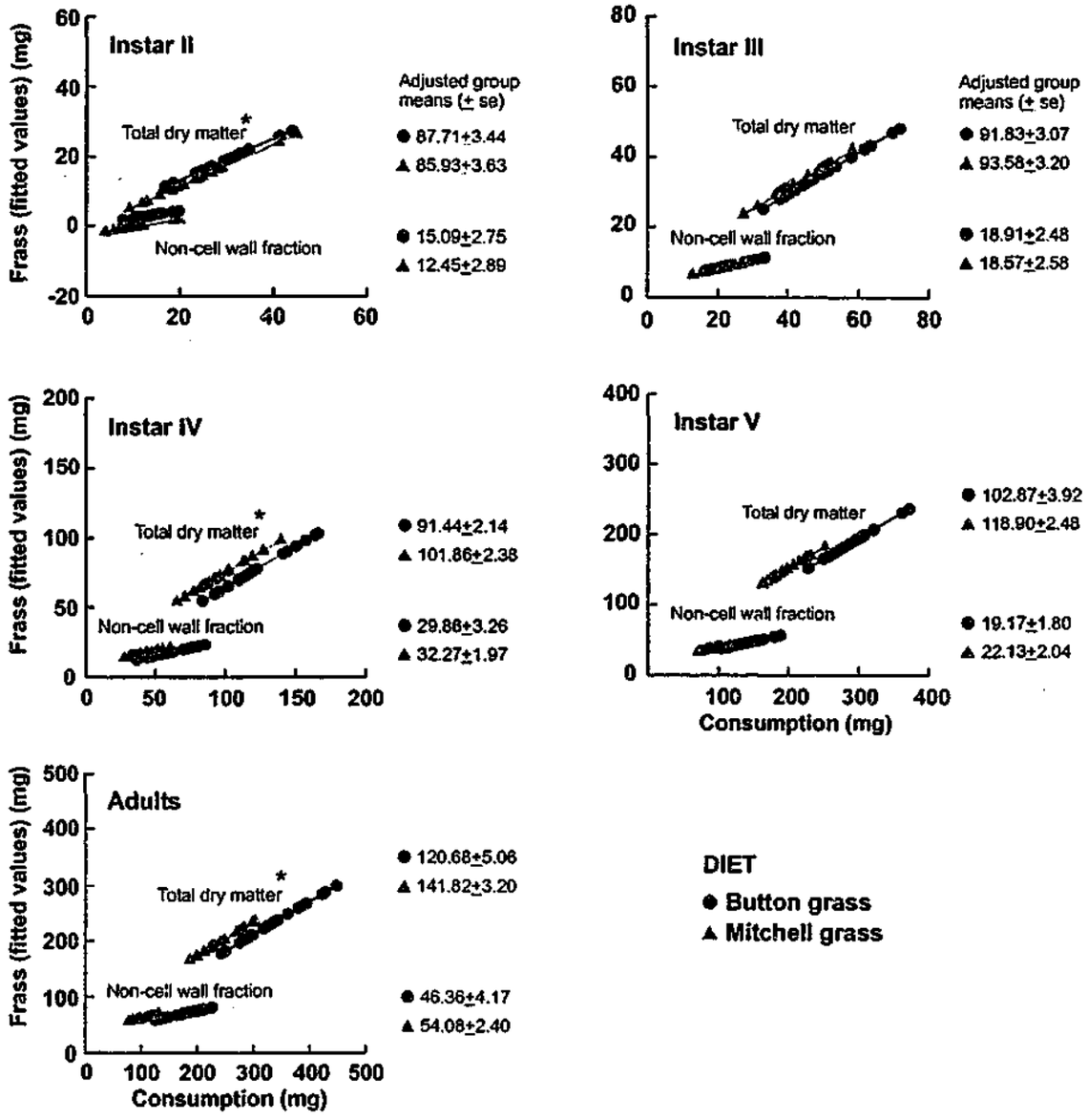


Fig. 4.10 'Utilization plots' of ANCOVAs on frass production against consumption for the total dry matter and for the non-cell wall fraction. * denotes significant ($P < 0.05$) differences between diets).

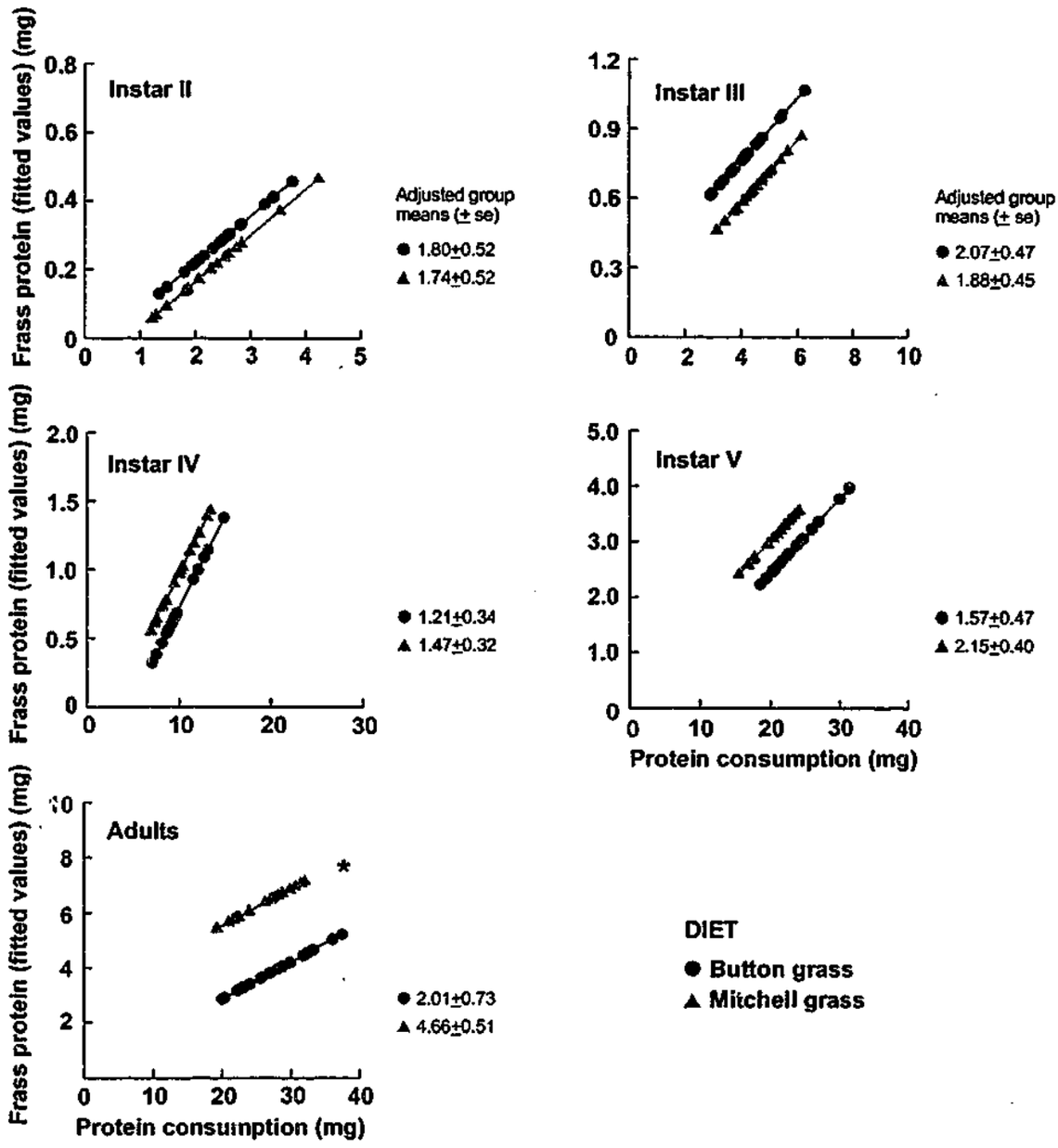


Fig. 4.11 'Utilization plots' of ANCOVAs on frass protein against protein consumption ('protein AD'). * denotes significant ($P < 0.05$) differences between diets).

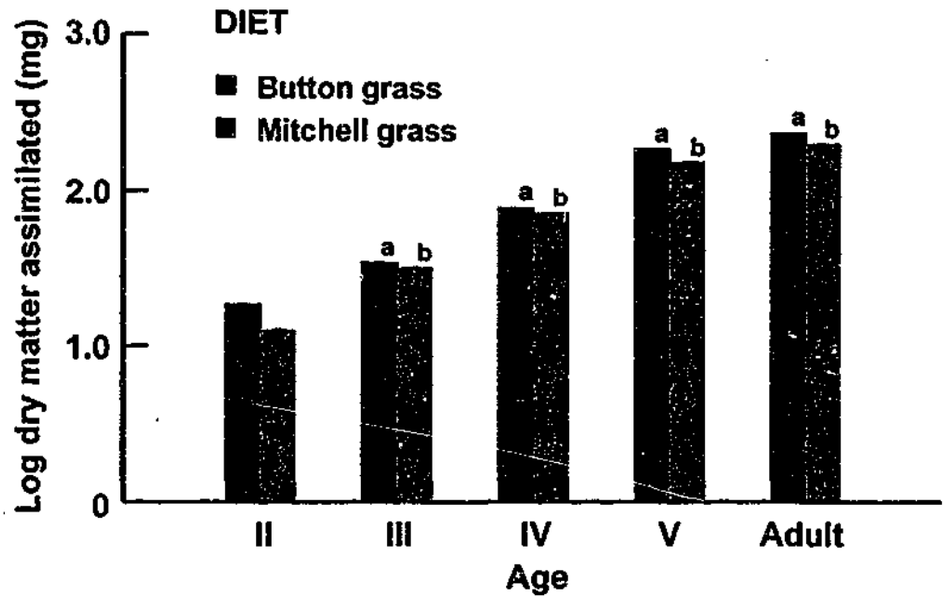


Fig. 4.12 ANCOVA adjusted (log_{10}initial dry weight) mean (± se) log_{10}assimilated Button grass and Mitchell grass by the different aged locusts. For each age, bars with different letters are significantly different ($P < 0.05$).

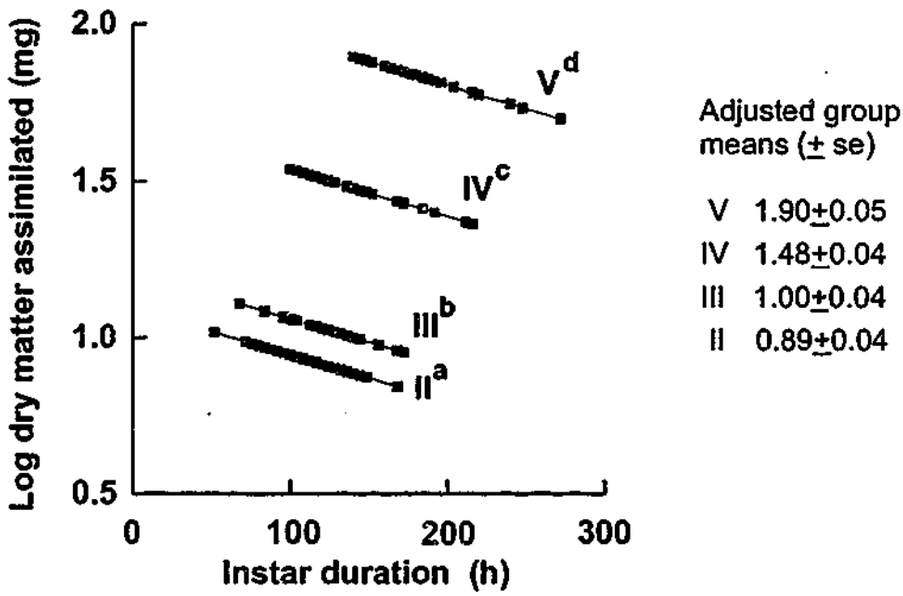


Fig. 4.13 Assimilation rate for the different aged locusts. Lines with different letters are significantly different ($P < 0.05$).

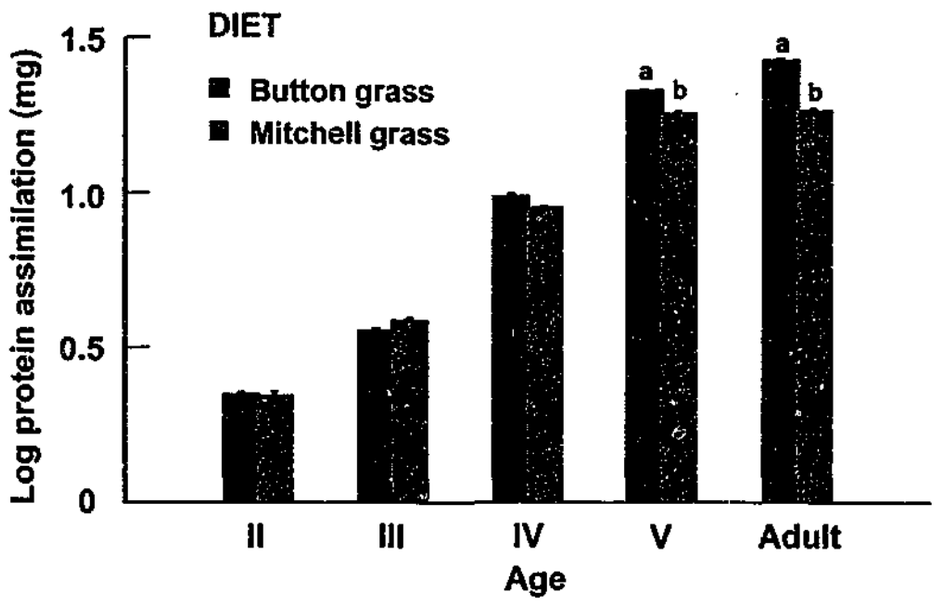


Fig. 4.14 ANCOVA adjusted (log_{10}initial dry weight) mean (± se) log_{10}assimilation of protein by the different aged locusts consuming either diet. For each age, bars with different letters are significantly different ($P < 0.05$).

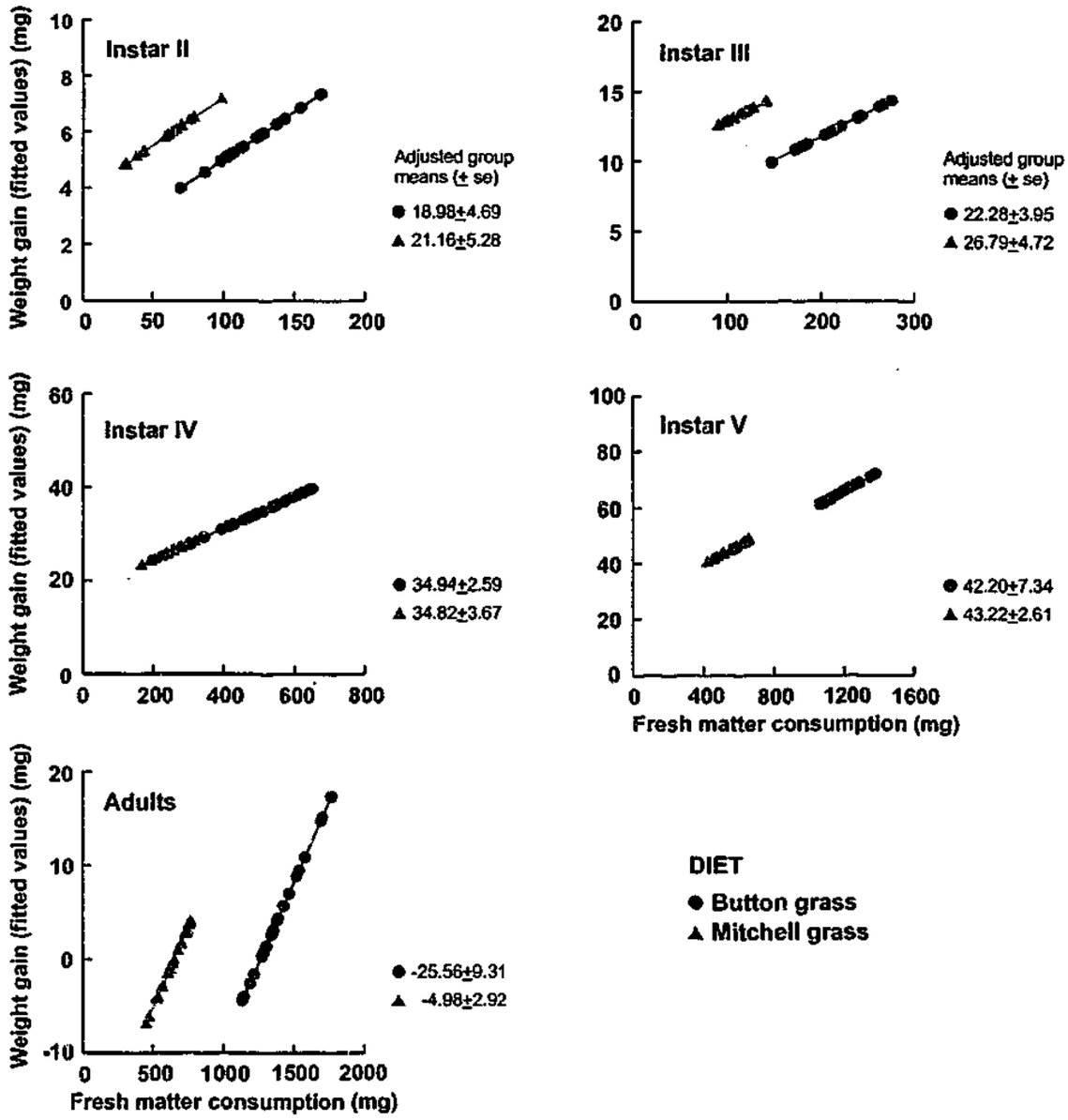


Fig. 4.15 'Utilization plots' of ANCOVAs on wet weight gain against consumption (fresh matter)('ECI').

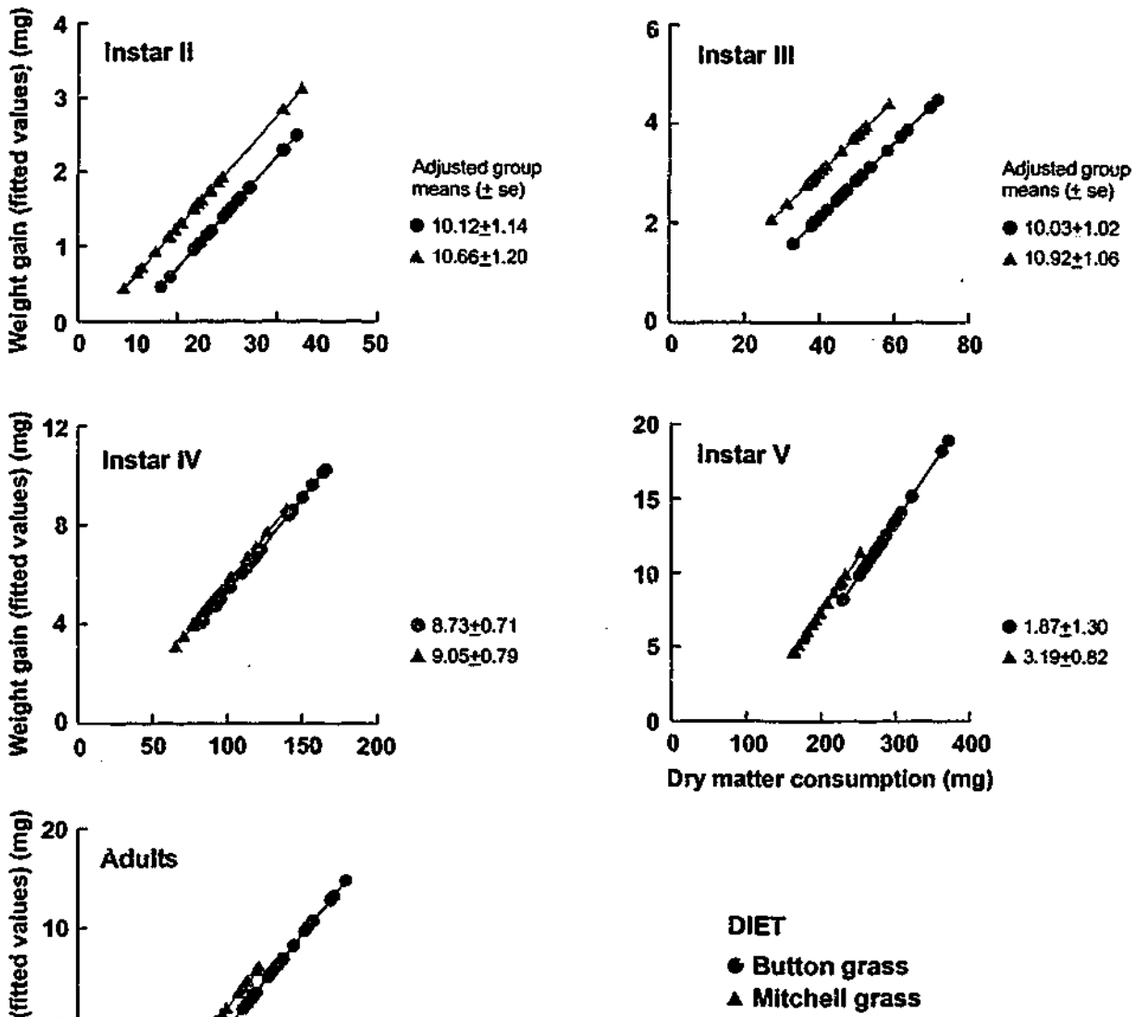


Fig. 4.16 'Utilization plots' of ANCOVAs on weight gain against consumption ('ECI').

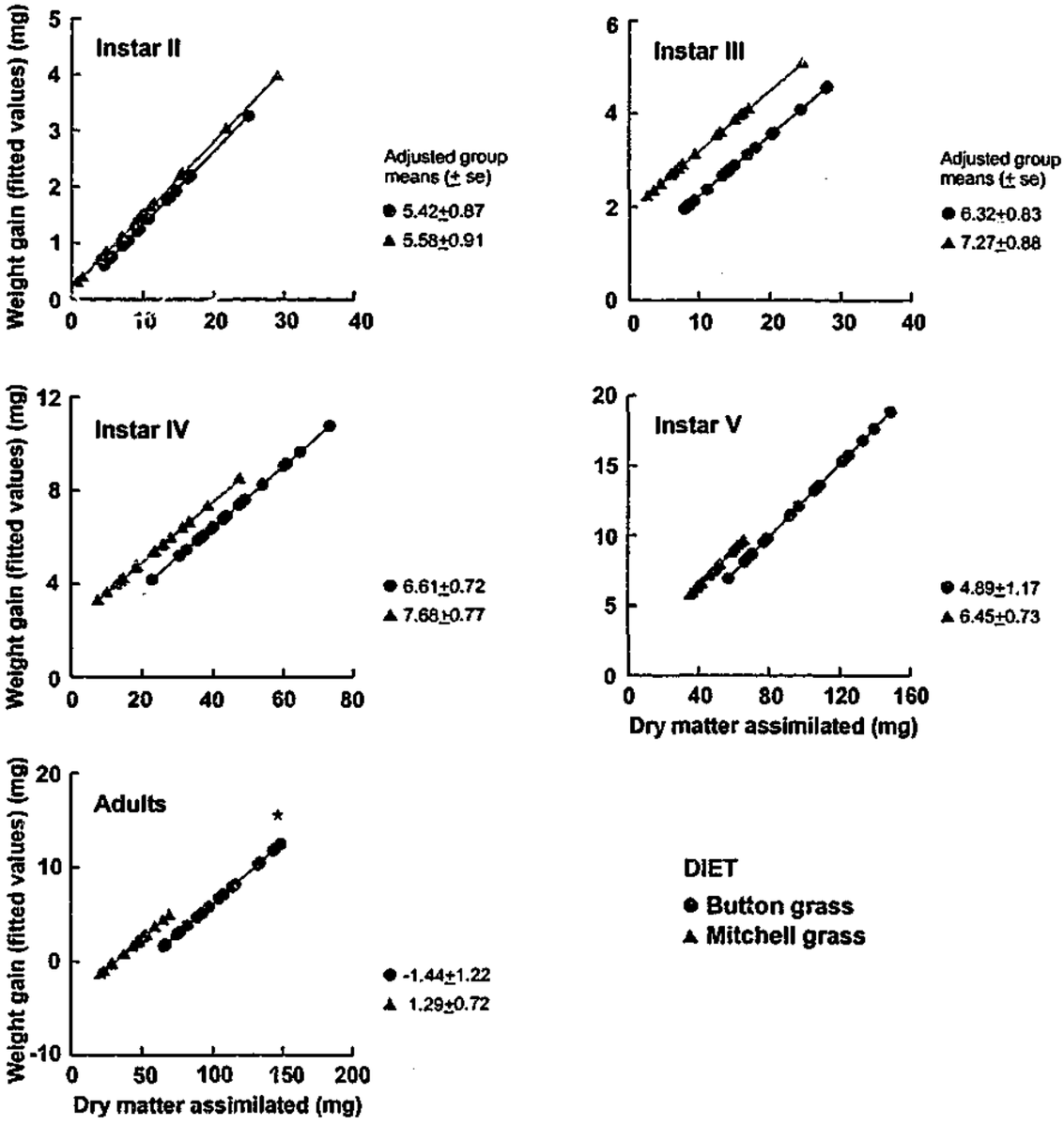


Fig. 4.17 'Utilization plots' of ANCOVAs on weight gain against assimilation ('ECD'). * denotes significant ($P < 0.05$) differences between diets).

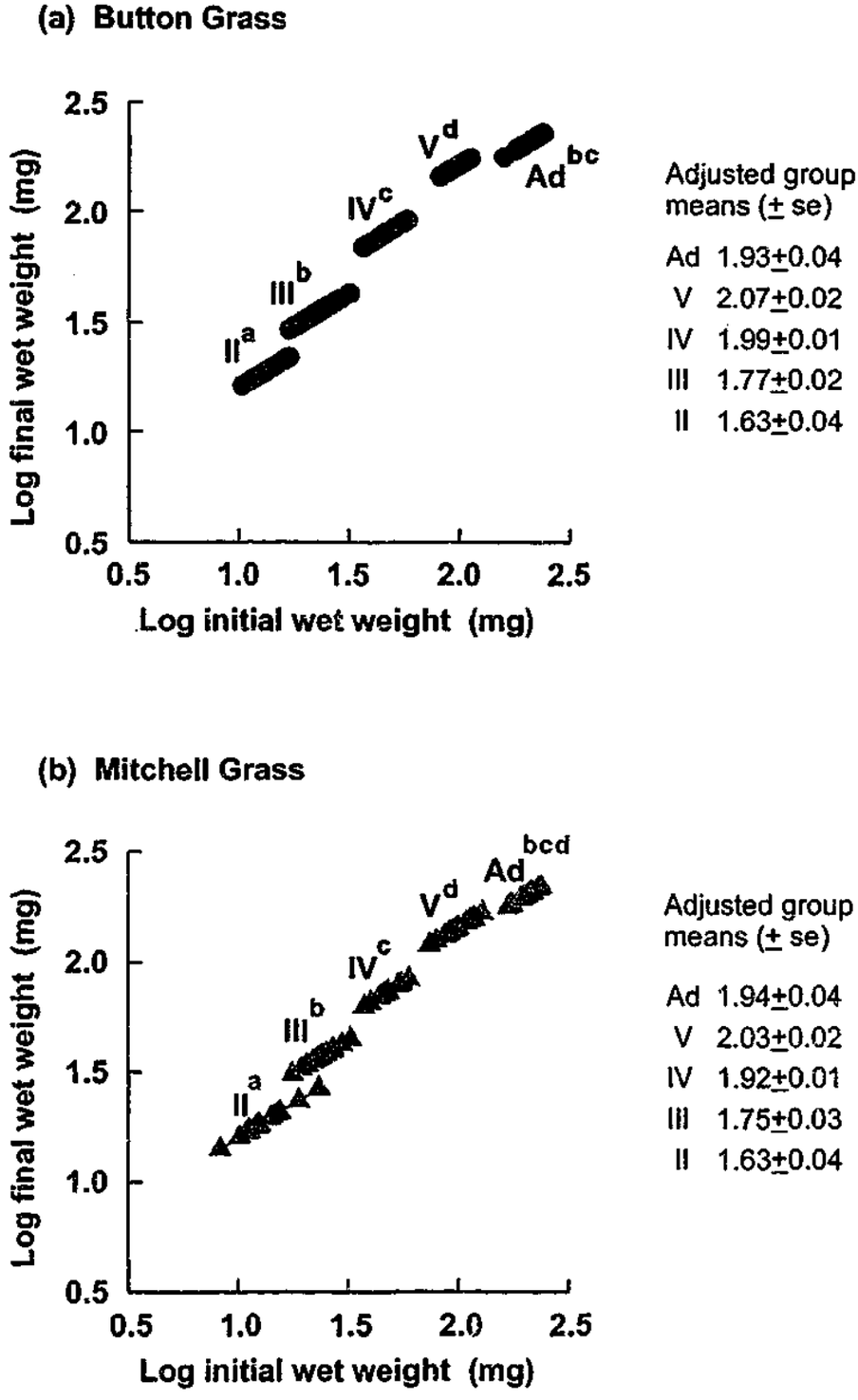


Fig. 4.18 Log_{10}final wet weight (ANCOVA fitted values) against log_{10}initial fresh weight for nymphs feeding on (a) Button grass and (b) Mitchell grass. Lines with different letters have significantly different ($P < 0.05$) means.

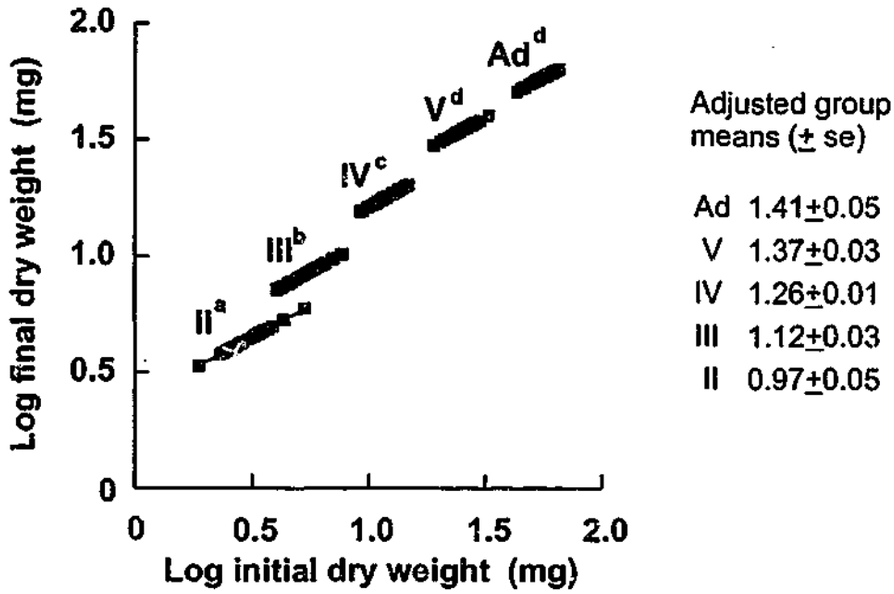


Fig. 4.19 Log_{10}final dry weight (ANCOVA fitted values) against log_{10}initial dry weight for nymphs feeding on both grass. Lines with different letters have significantly different ($P < 0.05$) means.

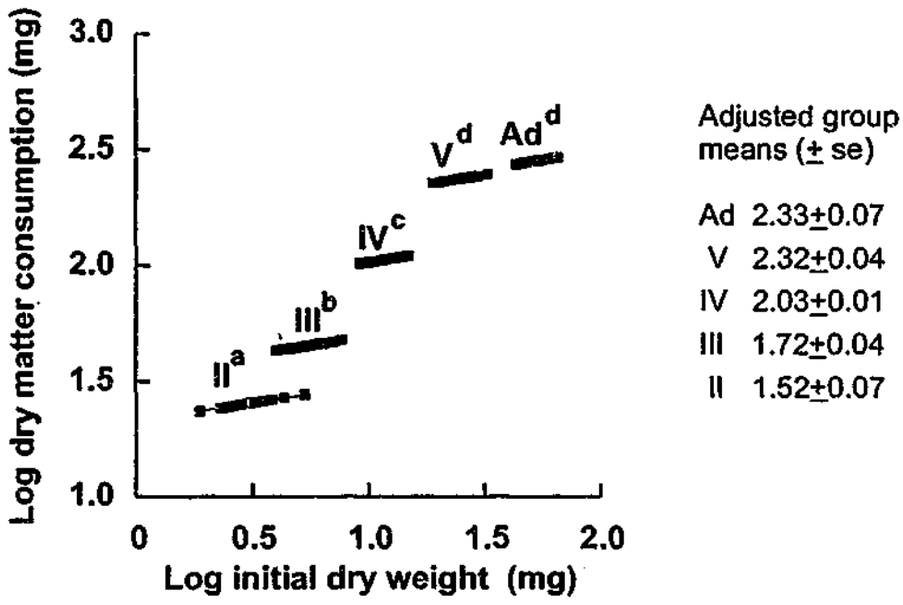
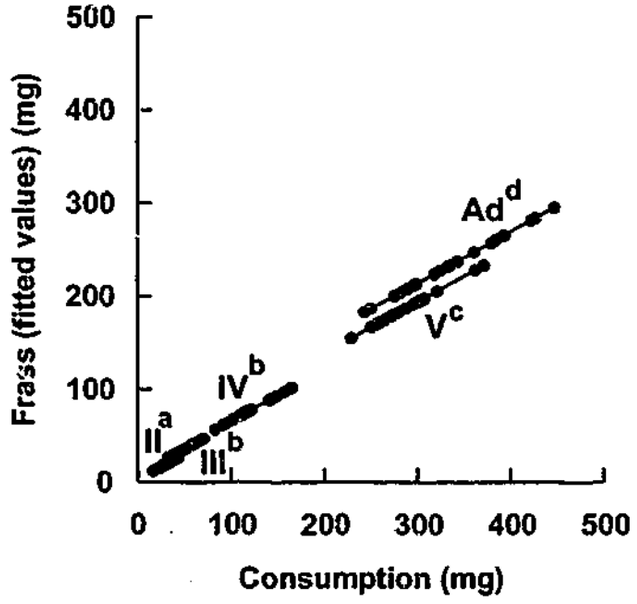


Fig. 4.20 Log_{10}total dry matter consumption (ANCOVA fitted values) against log_{10}initial dry weight for nymphs feeding on both grass. Lines with different letters have significantly different ($P < 0.05$) means.

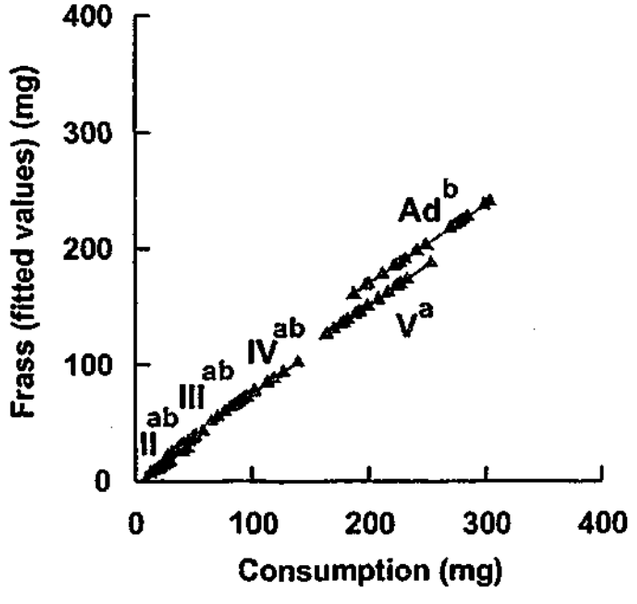
(a) Button Grass



Adjusted group means (± se)

Ad	120.68±5.06
V	102.87±3.92
IV	91.44±2.13
III	91.83±3.07
II	87.71±3.44

(b) Mitchell Grass



Adjusted group means (± se)

Ad	141.69±3.20
V	118.90±2.48
IV	101.86±2.38
III	93.58±3.20
II	85.93±3.63

Fig. 4. 21 'Utilization plots' of ANCOVAs on frass production against consumption for total dry matter fraction for nymphs feeding on (a) Button grass and (b) Mitchell grass. Lines with different letters have significantly different ($P < 0.05$) means.

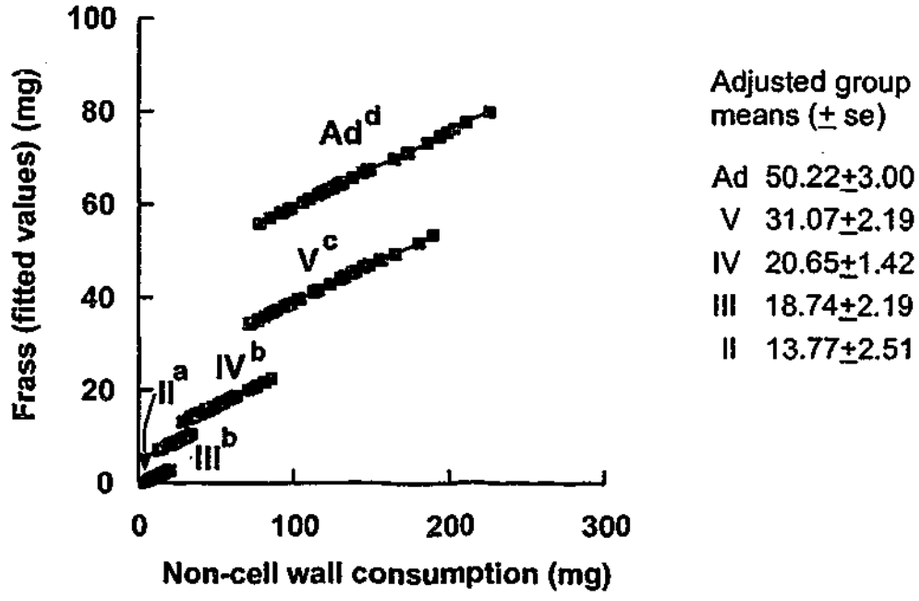


Fig. 4.22 'Utilization plots' of ANCOVAs on frass production against consumption for the non-cell wall fraction of the dry matter for nymphs feeding on both grasses. Lines with different letters have significantly different ($P < 0.05$) means.

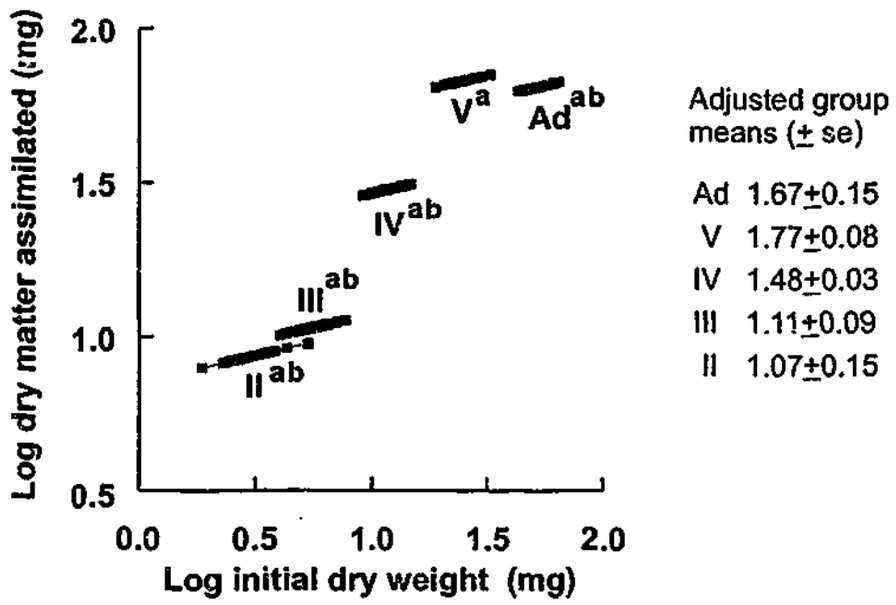


Fig. 4.23 'Utilization plots' of ANCOVAs on $\log_{10}$assimilation against $\log_{10}$initial dry weight for nymphs feeding on both grasses. Lines with different letters have significantly different ($P < 0.05$) means.

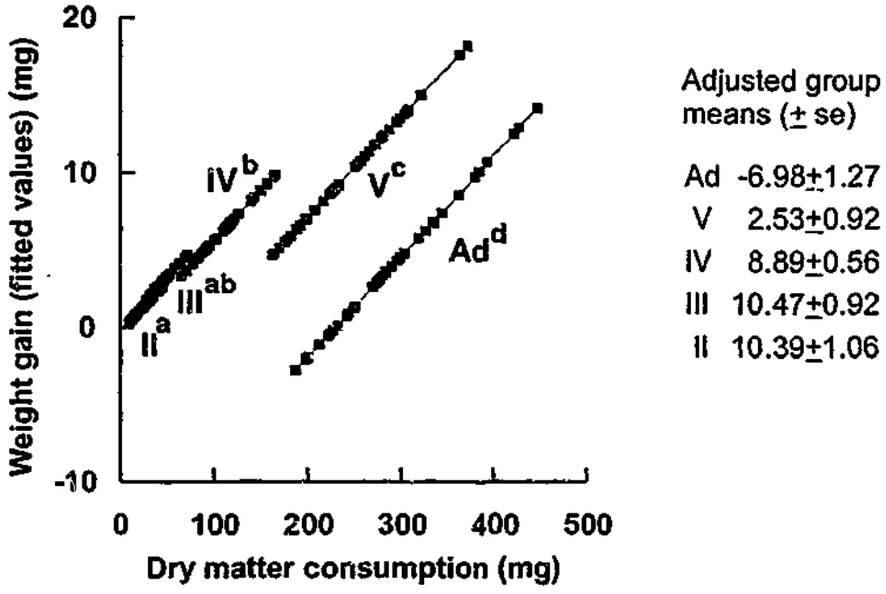


Fig. 4.24 'Utilization plots' of ANCOVAs on weight gain against total dry matter consumption ('ECI') for nymphs feeding on both grasses. Lines with different letters have significantly different ($P < 0.05$) means.

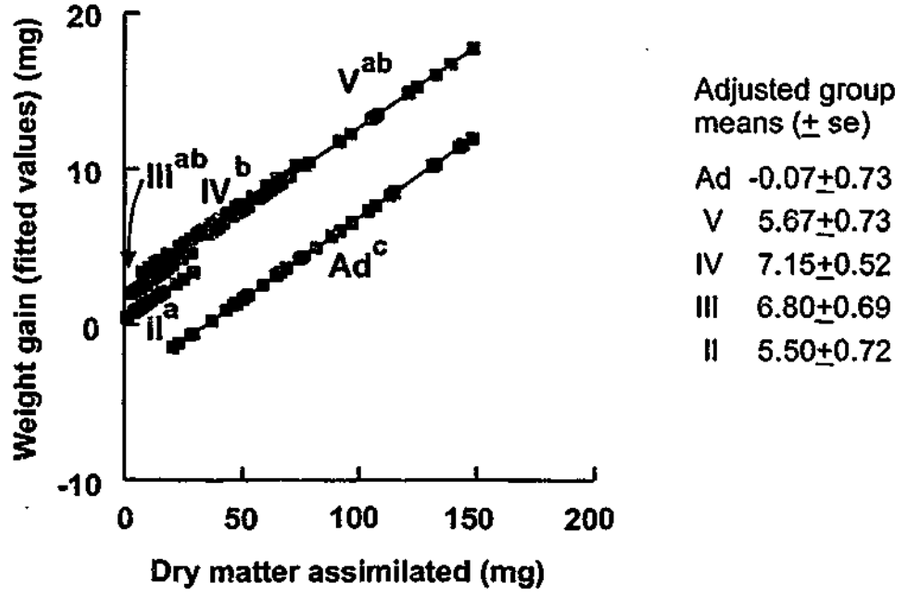


Fig. 4.25 'Utilization plots' of ANCOVAs on weight gain against dry matter assimilated ('ECD') for nymphs feeding on both grasses. Lines with different letters have significantly different ($P < 0.05$) means.

CHAPTER 5. DIGESTIVE CAPACITY AND FOOD PROCESSING

SUMMARY

1 The biomechanical properties and anatomy of the two grasses were investigated, and for Instar II to Adult locusts, the digestive capacity and how they processed their food was quantified.

2 A model describing how locusts consume a grass blade is proposed.

3 Older nymphs had a relatively smaller head and gut mass, while mandible measures increased linearly with increasing age (and thus head mass).

4 With increasing age, meal size increased but the meal duration remained the same, i.e. older nymphs consumed food more quickly. The mean food retention time was equivalent for all ages, although the intermeal duration decreased.

5 Mitchell grass was thicker, with more cells per unit thickness, and required significantly more work and specific work to fracture.

6 The older nymphs processed (number of chews and bites) Mitchell grass more per unit weight and retained it for longer than Button grass, as predicted from Chapter 4 results.

7 Therefore it appears that more time is required to digest the equivalent proportion of Mitchell grass compared to Button grass, as nymphs age. This may be because (1) anatomical differences *per se* between Mitchell grass and Button grass may cause the nutrients to be less accessible to digestive enzymes and would thus be absorbed more slowly from Mitchell grass; (2) the smaller amount of water per unit dry weight of Mitchell grass may be limiting digestion and absorption; or (3) an interaction between the above two points.

INTRODUCTION

The relationship between diet quality, intake, gut capacity, food retention time and digestibility is tightly linked in herbivores (e.g. Batzli *et al.* 1994; Yang and Joern 1994b). Growth is determined by the digestive capacity and efficiency with which nutrients are extracted and allocated to body mass. Nutrient transfer from the environment to the insect is constrained by both diet quality and digestive capacity. With respect to locusts, the majority of studies have investigated the effects of altered diet chemistry (e.g. Joern and Behmer 1997; Simpson and Raubenheimer 1993b), but it is also known that the physical properties of a diet influence consumption (e.g. Coley 1983; Nichols-Orians and Schultz 1990; Ohmart *et al.* 1987; Williams 1954). Locusts appear to release nutrients from ingested plant material by mechanically breaking the cell wall (Chapter 3). However, as diet processing is energetically expensive (Roces and Lighton 1995), to maximize growth there may be a compromise between the degree of processing, food retention time and assimilation per gram of intake. The degree to which insects are able to adjust intake and gut capacity may be limited by their small size (Weeks 1996), but there is evidence to suggest they are able to compensate to some extent for dietary nutrient dilution (e.g. McGinnis and Kasting 1967; Yang and Joern 1994b; Yang and Joern 1994c).

Physical properties of a diet influence consumption and the effort an animal must spend to gain nutrients. The tensile strength and toughness of forage affects intake by sheep and cattle (Wright and Vincent 1996). For insects, negative correlations between leaf 'toughness' and nymphal development have been recorded (Casher 1996; Coley 1983; Feeny 1970; Hewitt 1969; Stevenson *et al.* 1993). Therefore, mechanical properties are potentially an important aspect of a plant's resistance to herbivory. For insects, forage 'toughness' has been shown to influence leaf-chewing patterns more than secondary compounds (Nichols-Orians and Schultz 1990; Ohmart and Edwards 1991; Steinbauer *et al.* 1998) and grasshoppers have been observed to reject a grass after the first bite if it was 'hard' (Bernays and Chapman 1970). Both the physical dimensions of insect head capsules and mandible morphology can relate to and prevent access to leaf tissue (Boys 1981; Casher 1996).

The number of cells fractured will determine the amount of nutrients released and available for absorption. Locusts fracture grass initially with the incisor region of their mandibles before the grass is processed further in the molar region (Gangwere 1960, *pers. obs.*). Biting consists of the incisor regions of opposing mandibles acting as blades being driven through the leaf (Chapman 1964; Gangwere 1960; Hillerton 1980, *pers. obs.*). The biomechanical properties of grasses will affect the force and energy a locust requires to ingest and process them.

There are different ways to measure the biomechanical properties of materials (e.g. Lucas *et al.* 2000; Vincent 1992a; Vincent 1992b). The resistance to crack formation is most relevant when linking plant biomechanical properties and resistance to herbivory (Lucas *et al.* 2000). Fracture, or crack initiation and propagation, requires force and energy (Vincent 1992b). Fracture can be produced by tension, in-plane shear and out-of-plane shear (Vincent 1992b; Wright and Vincent 1996) and the forces and energy to fracture in these different modes can differ by factors of 5 to 10 (Vincent 1982; Vincent 1991; Wright 1992). The exact mode of action of the molar and incisor regions of the mandibles is not known. However, wear patterns on the incisors indicate they act in out-of-plane shear (e.g. like scissors). Out-of-plane shear, referred to hereafter as shearing, gives the best measure of intrinsic toughness as the fracture can be controlled and is not deflected by the veins (Lucas and Pereira 1990; Vincent 1990).

It has been argued that biologists should adopt the terminology used by engineers when trying to describe the physical properties of biological materials (e.g. Sanson *et al.* 2001; Wright 1992). Many researchers (e.g. Cherrett 1968; Steinbauer *et al.* 1998) have used a penetrometer to measure what they have termed 'toughness'. Toughness, as defined by materials engineering, is the resistance to crack propagation (Wainwright *et al.* 1976), and is measured as the energy required to cleave a unit area of material (Vincent 1992c). Penetrometer tests that measure only force and not displacement cannot measure toughness as defined by materials engineering (Lucas *et al.* 1991; Lucas and Pereira 1990; Vincent 1990). In this thesis I will use toughness in the materials engineering sense.

Generally, as insects increase in age, relative intake per gram body mass and diet digestibility is reduced but the efficiency of converting assimilate to growth is increased (reviewed by Slansky and Scriber 1985). The mechanisms behind this trend have not been elucidated and are likely to be complex (Simpson and Simpson 1990). The effects of increased body size are likely to be interrelated. Increased post-ingestive efficiency could be due to decreased mass specific metabolic rate found as insects age (Hack 1997) but could also be due to the decline in relative consumption rate (Slansky and Scriber 1985) or the increased ease of processing by larger heads (Hochuli 1994). Declines in diet digestibility could be a result of decreased surface area to gut volume and/or the relatively smaller gut in larger insects (Yang and Joern 1994b) or the processing ability of mandibles being used (Bernays and Janzen 1988).

Previous data (Chapter 4) showed that intake was proportionally higher per gram of body mass for the later instars, with nymphs consuming and assimilating more Button grass than Mitchell grass. In terms of protein, non-structural carbohydrates and the indigestible cell wall, there were negligible differences between the two grasses. However, given that the grasses appear to be structured differently. This may be a reason why the early instars were able to digest and grow on either diet equally but the later instars consuming Mitchell grass suffered reduced growth and increased instar durations.

Overall, Button grass was more digestible than Mitchell grass, which could be explained by differences in the amount of cell wall. When only the digestibility of the cell contents was examined, there was a significant decrease in digestibility for both grasses with age. As instar duration was longer for the older nymphs consuming Mitchell grass than Button grass, the digestibility rate of Mitchell grass was less.

The aim of this chapter was to attempt to integrate feeding behaviour and digestive capacity with plant structure and mandibular function for the different aged locusts on the two contrasting diets. Feeding behaviour was described in terms of meal size, processing rate and type, and food retention time.

MATERIALS AND METHODS

Grass blades were harvested as for Chapter 4. Specific leaf area (SLA) was determined daily from a subset of the leaves collected for shearing. Five leaf blades per day were haphazardly allocated for measurement of either biomechanical properties or leaf anatomy.

Biomechanical properties of the grasses

Single blades of grass were sheared using two 4 mm thick K110 steel blades, hardened and ground, mounted onto a Chatillon Universal Testing Machine. The fixed upper blade was set at an approach angle of 45° and the lower blade, which moved, was mounted horizontally, with the clearance between the blades adjusted so that minimal force was registered when the lower one was moved. The upper blade was raked at 45° and the lower blade was unraked but inclined to provide a relief angle of 5° (Sanson *et al.* 2001) (Fig. 5.1). Single grass blades were sheared at a speed of 0.191 mm s^{-1} with a 'blank' run prior to shearing each leaf blade to correct for any friction in the machine. The cross-sectional area sheared was determined by tracing the freshly sheared grass blade using a profile projector (magnification x 50). Using image analysis (Digital Data Exploration Program (Copyright © Rene Stolk)) the traced areas and shear length was determined. The average thickness of the leaf blade was estimated by dividing the area by the shear length. The work to shear (J m^{-1}) (force multiplied by displacement per unit shear length) and the specific work to shear (J m^{-2}) (work to shear divided by the average leaf blade thickness) were determined from the force displacement trace using Leaf2 v3.7 (M. Logan) (Fig. 5.2). Specific work is the work to shear per unit leaf thickness and removes the effect of differences in leaf thickness, thus giving the work required to fracture a given leaf anatomy.

Leaf anatomy

Sections of 1 μm thickness were cut from leaf blades embedded in LR White – medium grade. Leaf blade structure was examined after staining with toluidene blue pH 4.5. Sections were obtained from the middle 10% of four leaf blades from each species fixed in FAA (10% formaldehyde, 50% ethanol, 5% acetic acid).

The thickness of the entire leaf blade, the distance between the centre of the vascular bundles and the minimum distance between adjacent outer bundle sheath cells (Fig. 5.3) was recorded. All measures were recorded randomly from between the edge of the leaf blade and the mid-rib. Values were averaged from at least eight measurements per replicate. The number of cells per unit area was calculated by randomly positioning a 2.5 $x10^{-3}$ mm^2 quadrat on images of the cross-sections and counting the number of whole cells within the boundary to obtain a measure of cell packing (Fig. 5.3). Ten quadrats per image were counted and there were two images per replicate (four).

Experimental animals

Locust stocks were reared as described in Chapter 2, prior to experimentation. Locusts were reared on wheat until they moulted (timed to within 4 h), then each one was placed on either Button grass or Mitchell grass in individual digestibility chambers (as for Chapter 4). Only locusts moulting between 0600 h and 1000 h E.S.T. and whose initial weight was within one standard deviation of a previously weighed population (as for Chapter 4) were used. Fresh grass of the appropriate diet was provided every 24 h as previously outlined (Chapter 2).

I wanted to compare feeding behaviour, in terms of meal size, food retention time, and effort (number of bites and chews) to consume a meal. This type of comparison between instars is problematic since digestive physiology changes within an instar (Simpson 1982a; Simpson 1983b; Tobe and Loughton 1969), instar duration differs with respect to instar number and diet (Chapter 4), and the mandibles get 'blunter' with wear, which may affect intake and processing (Chapman 1964). It was decided to compare each instar 48 h after moulting, as this was the mid-point of the early instars and *c.* 30% into the instar for the later instars and during this period intake had stopped increasing and was steady (results tabulated from Chapter 4 daily intake values). Previous research suggested that crop emptying rate and food absorption varied the most in the first 24 h and in the last 48 h of an instar (Simpson 1983b).

Digestive capacity

Ten freshly moulted (timed to within 4h) locusts of all ages except Instar I (due to their small size) were frozen (-20°C) and while still frozen the gut and head (severed immediately behind the post-occiput) were removed. All body parts were freeze-dried to a constant weight prior to being weighed to 0.1 mg.

Feeding behaviour

Chewing behaviour

Chewing behaviour was determined by filming the locusts consuming a meal. To film the locusts, they were placed on a balsa wood stage that could rotate freely, in a thin walled rectangular glass container (52 x 26 x 76 mm, l x w x h) with fly mesh covering the open end. In the centre of the stage a hole was drilled in which the plastic vial holding the grass blade was placed. To minimize any effect this disturbance might have on feeding behaviour, i.e. an increased intermeal duration may increase the next meal size (Bernays and Chapman 1972a), the locusts were allowed to feed on a fresh blade of the appropriate diet. The diet was then replaced with a leaf blade of known area. When the locust commenced feeding from this leaf blade it was recorded using a video camera with close up lenses mounted on a tripod *c.* 50 mm from the insect. The container and stage was rotated to allow recordings of the locusts consuming the grass on an angle so that bites and chews could be determined.

A complete analysis of feeding behaviour was not undertaken to determine bout criterion, i.e. sustained feeding bouts from shorter 'sampling' events (Simpson 1990; Simpson 1995). However, when locusts were observed over a 6 h period and their behaviour recorded at 5 min intervals, the observations suggested their behaviour was similar to that reported for other locusts (Blaney *et al.* 1973). Australian plague locusts appeared to either 'nibble' for a very short period of time or consume the diet for a sustained period of time (*c.* 10 min). Observations on locusts eating for less than *c.* 30 s duration that did not feed again within the next minute were discarded and the grass blade was replaced with another of known area. A meal was considered complete if the locust ceased feeding and did not feed again within two minutes. Generally the locusts left the diet and basked in the upper section of the container.

The locusts were recorded when they fed between 1000 and 1800h. As only one locust at a time could be recorded, if another locust commenced feeding during this time it was allowed to finish that meal and then that leaf was replaced with another of known area. Any locust that did not feed between 1000 to 1800 h was returned to the colony. On completion of the meal the locust was placed in FAA, and once dead, the gut was removed and the meal contents fixed in FAA. The remaining leaf blade was kept turgid and its area determined by image analysis. Treatments (diet x age) were randomized over time.

The right mandible was extracted from each locust and a digital image made, from which the incisor length and the length and width of the molar region was measured (Fig. 5.4). The average distance between molar ridges was estimated by measuring the distances from the centre of each ridge to the next where they touched along the molar length. Care was taken to orient the mandibles so that the measure of interest was normal to the camera lens.

Meal size

Data from 10 Instar II, III, IV, V and adult locusts feeding on both diets was recorded. Instar I nymphs were too small to record and interpret their behaviour accurately. Meal size, fresh and dry weight and volume, were determined from the area eaten and the SLA and cross-sectional area from a control group of leaves harvested at the same time. Leaf area was determined by image analysis. Meal volume was estimated by multiplying the surface area eaten by the average leaf thickness. Leaf thickness was estimated by dividing the cross-sectional area of leaf blade by the length of the leaf blade. An average was taken from five leaves of each species per day.

Meal duration

Meal duration (as defined above) was determined from the video recording of the meal. A data-logging program, Win Counter, (V 2beta, program written by Peter Feil and Gordon Sanson, Monash University November, 1986) was used to analyze the number of bites and chews per meal, the video was slowed down to 63% of its original speed. Chews were defined as mandible movements occurring after a strip (mouthful) had been excised from the leaf blade before the commencement of excising the next strip.

Food retention time

Detached grass blades of both species were placed in a 1% aqueous methylene blue solution until the dye had stained the major veins. This took *c.* 2 h for Button grass blades and *c.* 3 h for Mitchell grass blades. Previous research found that meal size was not affected if the dye was not excessive (Baines *et al.* 1973). To test if the dye affected meal size (which may alter food retention time) a trial was performed using Instar V and adult locusts. Ten locusts of each age were fed either dyed or undyed Button grass blades of known area. They were allowed to feed once and then the grass blades were removed and the remaining area ascertained. No difference was found between the area of grass blade removed for dyed or undyed leaf blades ($F_{1,18} = 0.013$, $P = 0.910$).

As for the locusts recorded chewing, a fresh blade of the appropriate diet was offered to the locusts. Once this was eaten it was exchanged for a dyed leaf blade. Every five minutes the behaviour of the locust and the amount and colour of frass produced was recorded. Once the locust had fed and left the dyed grass blade it was replaced with an undyed leaf blade of the same species. When frass was produced that appeared blue in colour it was removed from the container with an aspirator. It was then determined, using a dissecting microscope, whether the frass was coloured from dyed grass or from dissociated dye passing down the gut more quickly. Food retention time was recorded for ten locusts aged from Instar II to adults. Food residence time was estimated using the medians of intake time and frass production as the median is less affected by extreme values. Any locusts only 'nibbling' at dyed leaf blades were discarded. Intermeal duration was measured as the time taken between ending consumption of the meal on the dyed grass and beginning to consume the next meal of undyed grass. Food residence time and intermeal duration was recorded for ten locusts of each age on both diets. Observations for diet and age were randomized over time.

Data analysis

The physical parameters of the two grasses were compared using ANOVA. The dry weights of the body, gut and head were $\log_{10}$-transformed to satisfy the underlying assumptions for ANCOVA. As there was no difference between days for the various parameters measured for Button grass and Mitchell grass, days were pooled and one factor ANOVA was used to compare the two grasses. Linear regressions were performed to investigate the relationship between age and the mandibular measures, as the weight of the locusts was not known. The effect of diet and age on the measures of diet processing was analyzed using two-factor ANOVA. Where there was not an interaction between factors and a significant difference only within age, a one factor ANOVA was performed combining the results from both diets with post-hoc Tukey's tests. When there was no interaction between factors and a difference within both factors, a one factor ANOVA was performed for each diet with post-hoc Tukey's tests using the $MS_{residual}$ from the original analysis. Where there was a significant interaction between factors, tests of simple main effects between levels of one factor at each level of the other factor were performed using the $MS_{residual}$ from the original analysis (Quinn and Keough 2002). Prior to analysis, box plots were used to check for normality and homogeneity of variances across the treatments. All analyses were undertaken with SYSTAT® 10 unless otherwise stated.

Due to lack of visual clarity in a few cases with the smaller instars on both diets, their chewing behaviour was excluded from the analysis and the degrees of freedom altered accordingly.

RESULTS

Digestive capacity

As body mass increased, relative gut mass decreased. There was a log-log isometric relationship between body mass and gut mass (regression equation: log gut dry weight = 0.78 $\log_{10}$ body mass dry weight – 0.71, $r^2 = 0.67$, $P < 0.001$) which did not differ among ages (Fig. 5.5). A similar relationship for head mass to body mass was found. An isometric relationship existed between log head mass against log body mass (regression equation: $\log_{10}$ head dry weight = 0.79 $\log_{10}$ body mass dry weight – 0.32, $r^2 = 0.96$, $P < 0.001$) (Fig. 5.6).

Grass physical properties

Mitchell grass blades were significantly thicker than Button grass and the SLA was significantly lower (Fig. 5.7). Button grass required both significantly less work to fracture per unit width and specific work to fracture (Fig. 5.7). The vascular bundles were significantly closer together in Button grass than Mitchell grass (Fig. 5.8a,b) and Mitchell grass had almost twice as many cells per mm^2 than Button grass (Fig. 5.8c). The vascular bundles in Mitchell grass were characterized by having both abaxial and adaxial bundle sheath extensions, while only every fourth vascular bundle for Button grass had these extensions (Fig. 5.9).

Chewing behaviour

Observations revealed that the locusts consumed the leaf blade by ingesting a mouthful and then pausing, usually to 'chew' before consuming the next mouthful. Locusts ingested the leaf blades by cutting across the outer vascular bundles, followed by bites parallel to and between the vascular bundles, with the final bites severing the outer vascular bundles to completely excise a piece of plant tissue (Fig. 5.10a, 5.11). This sequence was repeated until the meal was finished (Fig. 5.10b). With the bites parallel and between the vascular bundles the fracture path was occasionally observed running ahead of the mandibles (Fig. 5.12). Given the structure of the mandible, consuming the grass blade in this manner meant that the strip being excised by the incisors passed over the molar region with the vascular bundles running parallel to the molar ridges (Fig. 5.13). The earlier instars appeared

to find it more difficult to make the initial cut across the vascular bundles, especially of Mitchell grass. The adult locusts occasionally would sever a strip across, rather than perpendicular to, the vascular bundles.

The measures taken of the mandible increased linearly with age (Fig. 5.14). As body weight scales logarithmically with age, this means the mandible measures are relatively smaller with age. The estimated incisor length was longer than the molar width or length. For Button grass the average distance between the ridges incorporated one vascular bundle for Instar II and up to one and a half vascular bundles for the adults (Fig. 5.9). However, as the vascular bundles were closer together in Mitchell grass, at most there was one vascular bundle between the ridges.

The amount of processing (defined as bites plus chews) per unit dry weight of food consumed decreased significantly ($F_{1,84} = 40.78$, $P < 0.001$) with age but was the same for both diets (Table 5.2, Fig. 5.15a). This pattern was the same for number of bites and number of chews per mg (Fig. 5.15b & c) even though the ratio of chews per bite varied with diet and age (Fig. 5.16). The ratio of chews per bite was the same for both diets for Instars II and III but locusts consuming Mitchell grass chewed almost twice as much per bite than those consuming Button grass (Table 5.2, Fig. 5.16). There was a strong interaction between diet and age for the ratio of chews per bite (Table 5.2). There was no difference between diets for the time taken to consume a unit dry weight (Table 5.2, Fig. 5.17). The older instars consumed the grasses more quickly, but overall there was no difference in meal duration (*c.* 10.5 min) (Table 5.2). There was a significant linear negative trend between increasing age and meal duration per mg consumed ($F_{1,84} = 62.21$, $P < 0.001$).

Meal size

Locusts consumed significantly more fresh weight of Button grass than Mitchell grass (Fig. 5.18a). However, fresh weight consumption also varied with age and there was an interaction between diet and age (Table 5.1). On either a dry weight or volumetric basis meal size did not vary between diets, but did increase significantly with increasing age (Table 5.1, Fig. 5.18b, c)

Food retention time and intermeal duration

Locusts retained Mitchell grass significantly longer than Button grass (Fig. 5.19). Instar age did not affect food retention time but there was an interaction between age and diet (Table 5.3). Intermeal duration decreased significantly with age ($F_{4,82} = 5.117$, $P < 0.001$) and was significantly longer for Instar V nymphs consuming Mitchell grass (Fig. 5.20).

DISCUSSION

The results reflect the pattern of intake observed in Chapter 4. In Chapter 4 it was hypothesized that the reduced Mitchell grass intake by the later instars may be due to smaller and/or less frequent meals being consumed. The results suggest that the difference in dry weight consumption of the two diets for the later instars was due to equal size meals being consumed at different frequencies. Meal size on a dry weight basis was the same for both grass species for each locust age, although it increased with age correlating with the increase in gut weight. Meal duration was the same across diet and age since the later instars consumed the diets more quickly.

Mitchell grass compared to Button grass required significantly more work to fracture, not only because the leaf blade was thicker, but also due to the anatomy. This is reflected in the specific work to shear results, which are adjusted for leaf thickness. The effect of differing amounts and types of cell wall on mechanical properties is complex (Lucas *et al.* 2000; Wright and Vincent 1996). Mitchell grass had more cells per mm^2 and thus a potentially higher cell wall volume that would increase the force required to initiate a crack (Lucas *et al.* 1995). The amount of sclerenchyma has been shown to correlate positively with leaf strength (Vincent 1991) and Wright (1992) found the energy required to fracture increased with vascular and sclerenchyma content. The vascular bundles of Mitchell grass had sclerenchyma extensions while Button grass had more vascular bundles both in total and per mm. However, only every fourth bundle appeared to have sclerenchyma extensions (Fig. 5.9).

The methods available to measure leaf biomechanical properties have evolved from materials engineering concepts, which have been developed to measure the intrinsic properties. However, mandibles do not function exactly like shearing blades (or penetrometers) although they must provide force and energy to generate fracture as measured by blades. Mandibles are blunter than the shears used here and their exact mode of action is not known. Therefore, it is likely that blunter mandibles require more energy to fracture plant tissue. This was found by Worley and Sanson (2000) for kangaroo teeth. Force as measured using a penetrometer was correlated with leaf rejection by locusts (Bernays and Chapman 1970) indicating that biomechanical aspects relate to diet choice.

The early instars appeared to have trouble initiating fracture (across the vascular bundles), especially across the outermost edge of the leaf blade of Mitchell grass. This part of both grasses appeared to consist of densely packed sclerenchyma cells and the vascular bundles are closest together, which would increase the force required to fracture (Fig. 5.9). Locusts appear to minimize energy and force expenditure in obtaining leaf material by making the majority of the fractures required to ingest leaf material between the vascular bundles, i.e. the zone of least resistance (Fig. 5.10). An uncontrolled fracture was observed running ahead of the incisors between the vascular bundles suggesting the cellular structure did not prevent cracks forming, i.e. the locust may be providing sufficient free energy to generate fracture (Fig. 5.12). Wright (1992) found that fracture across the vascular bundles of a grass blade requires the most energy and that tearing between the vascular bundles required 69 times less energy per unit area than tearing across the vascular bundles. Only adult locusts were occasionally observed biting strips perpendicular to the vascular bundles, suggesting the younger nymphs might be unable to do this.

To fracture a material, the material being fractured must be softer than the material used to fracture it. The non-occluding surfaces of the mandible incisors consist of cuticle that is structurally different (Gardiner and Khan 1979) and is harder (Hillerton *et al.* 1982) than the surfaces that occlude. This keeps the incisors 'sharp' as the two types of cuticle wear differentially. The molar region consists of ridges of this 'harder' type of cuticle with 'normal' cuticle between the ridges (Gardiner and Khan 1979), which may be harder due to the presence of zinc (Hillerton and Vincent 1982). Locusts appear to be able to vary the force used to fracture food (Seath 1977). However, increased force would result in increased energy expenditure. There was no difference in the efficiency with which assimilate of either diet was converted to body mass although Mitchell grass required more energy to fracture. The locusts used the same number of bites to excise the same weight of leaf blade for each grass, but given that the SLA was significantly different for each grass, different bite lengths must have been used. This may have allowed the locusts to expend the same amount of energy per bite.

Animals chew their diet to reduce particle size (Lucas 1994). Particle size reduction allows transport of food from the environment into the gut, increases surface area and fractures the cell wall. The extent of fragmentation determines the accessibility of the

cell contents for digestive enzymes. Smaller mouthparts had shorter biting edges (incisors) (Fig. 5.14) that resulted in more bites required to ingest the same amount of food (Fig. 5.15b).

The molar region that characterizes the graminivorous-type mandible (Chapman 1964; Isely 1944) appears to be designed to fracture a grass blade. The parallel molar ridges, absent in forbivorous-type mandibles (Chapman 1964; Isely 1944) appear to increase the extraction of nutrients from grass plants (Boys 1981). Exactly how the molar ridges occlude was not able to be determined; it was unclear after sectioning, 2-dimensional mapping of the molar ridges from each mandible and molar wear analysis. A 3-dimensional *in situ* reconstruction is required.

While vascular bundles increase the force and energy required to fracture a leaf blade (Vincent 1982; Wright 1992) they also may 'act like sand in a pestle and mortar during chewing, providing a stiff material against which cells are pressed to cause rupture' (Wright and Vincent 1996). Boys (1981) concluded from particle size differences that the vascular bundles of a C_4 grass made it less easy to shear than a C_3 grass. However, despite the differences in particle sizes, no differences in protein or carbohydrate digestibility between the C_3 and C_4 grasses were found in that study. Despite the variations in histology, differences in digestibility of the cell contents related to locust age not grass species consumed (Chapter 4). It was expected that cell rupture related to differences in structure and the average distance between the molar ridges. The average distance between the molar ridges suggests that for Button grass the older locusts will generally encounter more vascular bundles between the ridges and thus an interaction with age and diet was expected if the vascular bundles were having a significant effect on cell rupture. Non-cell wall digestibility only differed for the adults, (Fig. 4.11) with those consuming Button grass being unable to extract the same proportion as those consuming Mitchell grass. Frass particles are the shape that would be predicted from the intake (i.e. strips), but they separate fairly easily between the vascular bundles and the cell contents generally appeared to be absent, suggesting that the cell walls had been ruptured. The decrease in digestibility of both grasses by the older nymphs may be due to larger particles, 'strips' being produced by the larger mandibles. As the size of the excised fragment increases, the ratio of cells fractured by the incisors to those remaining intact would decrease. However, the proportion of non-cell wall material extracted was very much greater

than could be explained by just leakage from the cells that would have been fractured by the incisors. So, it appears that if mechanical processing is solely responsible for the release of nutrients from within the cell walls, this is dependent on both the incisor and molar regions of the mandible and plant structure will affect the amount of nutrients accessed.

Each diet on a dry weight basis was processed (sum of chews + bites) equally, but for the older locusts consuming Mitchell grass there were more chews per bite compared to earlier instars. Given the mandible structure, it is most likely that a locust initiates a fracture with the incisor region that creates a strip, and chews simultaneously with the molar region (Fig. 5.11, 5.13). Hence, the amount of chewing recorded here could underestimate the total processing that occurred. If a locust consumes an equal surface area of both grasses, which given mandible morphology is likely to happen, they will ingest more Mitchell grass than Button grass on a dry weight basis because it is thicker and structured differently. The increased chewing observed per bite of Mitchell grass would explain this. How the locusts move the ingested grass particles around in their oral cavity was not ascertained. It would appear that as a grass strip is fed over the molar ridges it enters the start of the digestive tract and how it is manipulated for further processing is not known. Not surprisingly, given their smaller mandibles, the early instars processed their diet on a dry weight basis more slowly than the older instars. This has been previously recorded with Australian plague locusts (Bernays and Chapman 1973b) and is to be expected given that the mandibles get bigger with age and are thus able to take larger bites.

With increasing age, meal size increase reflected gut capacity. Gut capacity increased linearly with age, but as body size increased exponentially, the later instars had a relatively smaller gut. Meal size was obviously not limited by total intake as more water was ingested when feeding on Button grass (Fig. 5.18a). Baines *et al.* (1973) and Simpson (1983b) recorded that fluid liberated during mastication passed more quickly down the gut than the solids.

Lowered digestibility of the diets by the older nymphs could be because they have a decreased ability to release nutrients and/or a decreased ability to digest and absorb them. With increasing age, meal size increased, the mandibles were proportionally larger, and food retention time was the same in a relatively smaller gut.

Gut size decreased relative to body size with increasing age. There was a log-linear relationship between gut dry weight and body dry weight for the Australian plague locust, contrary to the relationship measured by Yang and Joern (1994b) for grasshoppers in Nebraska, USA, although this could have been because Australian plague locusts were at the lower end of their recorded weight range, i.e. where the relationship is linear. Previous researchers (Yang and Joern 1994b) have used gut dry weight as a measure of gut volume. However the exact relationship is unknown and gut volume is extremely difficult to obtain (Young Owl 1994). Gut weight changes with diet quality upon moulting (Yang and Joern 1994b) and during an instar (Simpson 1982a) mostly due to changes in the weight of the anterior caeca (Chapman 1988b). It is not known if the gut is able to alter its size during an instar in response to diet. If gut length increases linearly (evidence suggests that body length does (Chapman 1982; Luong-Skovmand and Balanca 1999)) but weight increases logarithmically, then it must be assumed that the ratio of surface area to volume is being conserved.

The later instars consumed equivalent dry weight amounts of either grass, but retained Mitchell grass for longer. The intermeal duration measured was longer only for Instar V nymphs. However, given the total intake, instar duration, meal size and food retention time for all ages, it was predicted for Instar IV nymphs and older, consuming Mitchell grass that the intermeal duration would be longer than for nymphs consuming Button grass. For all ages, generally Mitchell grass intake was lower, meal size and food retention time was slightly higher and instar duration longer. As the diets were very similar on a dry weight basis, and equivalent dry weight amounts were consumed for either grass, the amount of feedback in terms of protein, should be approximately the same. For Instars II – IV protein assimilation was equal for both diets, just the rate varied. Therefore, the hypothesis that smaller Mitchell grass meals may have been consumed due to negative feedback occurring at a faster rate than when consuming Button grass is not supported. The results suggest that Mitchell grass consumption compared to that of Button grass is lower for the later instars because of an increased intermeal duration, which is most likely linked to increased retention time of Mitchell grass.

Several factors could be interacting to limit digestibility. A correlation between increasing particle size and increasing mandible incisor length has been observed

(Bernays and Janzen 1988) that would most likely result in decreasing the ratio of cells with fractured cell walls to intact cells. If fewer cells were fractured due to the increased size of the mandibles, less nutrients would be digested. Moore (1993) found a positive correlation between leakage of nitrogen from cell contents with the number of cut ends (fractured cells) and no significant difference between incubation times of 2 h and 4 h. Observation of the particle sizes produced by the different aged locusts suggested that with increasing age, the particle sizes also increased. Also, with increasing age, relatively more grass was processed in a relatively smaller gut per unit time. The increased ratio of food to gut surface area (assuming mass and surface area is proportional) could be limiting both nutrient digestion and absorption through such factors as relatively reduced enzyme production, or sites available for absorption.

Decreased digestibility of grasses has been previously observed with increasing locust age (Bailey and Mukerji 1976; Beenakkers *et al.* 1971; Hoekstra and Beenakkers 1976; Mehrotra *et al.* 1972; Smith 1959). However, in the only study investigating an age effect on digestibility, using ground artificial diets, adults were able to digest more of the diet than Instar VI (Yang and Joern 1994c). If this is a 'real' trend, then it suggests that digestibility is not limited by gut constraints but by the locusts' processing ability, i.e. fracture of cells. However, this result should be regarded with caution as food retention time also increased with age (Yang and Joern 1994c). The digestibility measures were determined over an entire instar while the data reported in this chapter were for one meal consumed when instar intake was at its highest. However, during this time digestibility is also highest (Simpson 1982a; Simpson 1983b).

Median food retention time for both diets was very short (88 vs 106 mins). This result was predictable given the locusts were offered continuous access to food, at the time within the instar when intake was highest and at temperatures where growth is maximized. Temperature, meal size, feeding regime and diet quality affect gut emptying rate (Baines *et al.* 1973; Yang and Joern 1994c). Although longer food retention times have been previously reported for other locusts (Baines *et al.* 1973; Yang and Joern 1994c) and Australian plague locusts (Bernays and Chapman 1973b; Hochuli 1987, Cooper *pers. comm.*), frass of a marker meal of *Bromus* was recorded within 90 min of consumption for *Locusta* with continuous access to food (Baines *et*

al. 1973). Uvarov (1966) also reported food retention times within the range I recorded.

Mitchell grass was retained *c.* 22% longer than Button grass. A longer retention was expected given that meal sizes were the same for each diet and significantly more Button grass was consumed within an instar (Chapter 4). Thus the later instars consuming Mitchell grass, which consumed significantly less over a longer time, retained their meal for longer. Therefore, the intermeal duration would be longer for the later instar nymphs consuming Mitchell grass. Baines *et al.* (1973) showed that grasses with higher water content are retained for less time although water was probably not the only factor that varied between the grasses. Unexpectedly, food retention time did not differ with age although there was an interaction with diet. For the earlier instars with a smaller gut, smaller meals were consumed but held for the same length of time.

An apparent conundrum was that food retention time was equivalent across ages but the intermeal duration decreased with age. This suggests a meal was being egested at different rates with age, i.e. the younger nymphs egested a meal over a greater time range than the older nymphs. The results suggested that Instar II compared to instar V nymphs produced on average 2.5 more pieces of frass and took *c.* 35 min longer to egest the remains of the meal. This same pattern was recorded by (Abisgold and Simpson (1987) for *Locusta migratoria* feeding on diets of differing ratios of protein.

Models predict that increased food retention time will increase the quantity of nutrients absorbed (Raubenheimer and Simpson 1996). However, data to support this are equivocal. Many studies fail to account for the indigestible component (e.g. Yang and Joern 1994c) and conclude correctly that, following dilution of an artificial diet with indigestible cellulose, it is less digestible. However, where the indigestible fraction has been removed before the calculation of digestibility, there was often no difference in nutrient assimilation (e.g. Slansky Jr. and Wheeler 1991; Timmins *et al.* 1988). Yang and Joern (1994c) reported a corresponding (66%) decrease in digestibility when the diet was diluted 66%. Hence it appeared that the locusts were able to digest the same proportion of nutrients present in the diet. Also, digestibility was found to decrease with increased food retention time associated with the dark phase (night) and smaller meals taken after the mid-instar (Simpson 1982a; Simpson

1983b). Further research is required to determine the effect of food retention time on digestibility. The study by Yang and Joern (1994c) suggests there may be a threshold level below which digestibility is affected. Although there was a significant difference in the retention time, the same proportion of the non-cell wall component of Button and Mitchell grasses were digested by Instars IV and V.

As the early Instars appeared to be able to egest a meal over a longer period of time, they may have the capacity to differentially excrete particles. If the cell wall is a barrier to nutrient release and some cell types are more resilient to fracture than others (Caswell and Reed 1975), it would be advantageous to excrete uncrushed cells faster than crushed ones. Potentially the early instars could preferentially egest uncrushed particles at a faster rate than crushed particles. A mechanism for this is difficult to envisage, as the foregut is relatively small and it is thought very little mixing of food particles occurs here (Baines *et al.* 1973). However, to date the role the sclerotized spines play in food processing has not been elucidated (Hochuli *et al.* 1992) but it has been proposed that their most likely role is in that of regulating food through the foregut (Hochuli *et al.* 1994).

It appears that food retention time is driven primarily by intake, as food movement through the gut appears to be driven primarily by the intake of the next meal pushing that already present through the gut (Chapman 1985b). However, it is not this simple, as the best predictor of intake is defecation (Simpson and Ludlow 1986). Although, there are muscles associated with all parts of the gut, their role in moving food through the gut is not clear. It is thought that the midgut muscles may be important in fluid circulation but are too feeble to move solids (Baines 1979). The muscles of the foregut and hindgut appear to be more developed (Chapman 1985a). The foregut of most locusts are characterized by a lining of sclerotized spines (Hochuli *et al.* 1992; Hochuli *et al.* 1994; Uvarov 1966; Williams 1954) that are thought to be involved in both the peristaltic movement of food through the gut and holding the food particles against the influx of digestive enzymes from the midgut (Hochuli *et al.* 1994).

As meal sizes (dry weight) were equal, the decrease in consumption of Mitchell grass was not due to inhibition or lack of excitation during a meal (Bernays and Chapman 1972a; Bernays and Simpson 1982; Simpson 1990), rather factors controlling intermeal duration. Intermeal duration appears to be controlled by the volume and

pressure of the haemolymph itself, as well as the osmolarity and nutrient concentration of the haemolymph (Abisgold and Simpson 1987; Abisgold and Simpson 1988; Barton Browne *et al.* 1976; Bernays and Chapman 1974a; Simpson and Simpson 1992). Simpson and Ludlow (1986) recorded a correlation between increasing meal size and the decreased probability of feeding. The ratio of water per unit dry matter in locusts decreased with increasing age and was lower for locusts consuming Mitchell grass. In addition locusts feeding on Mitchell grass ingested less water per unit dry matter intake than those feeding on Button grass. For nymphs feeding on Mitchell grass this may lead to relatively higher nutrient concentrations in the haemolymph following a meal than for nymphs feeding on Button grass. Nymphs consuming Mitchell grass will ingest significantly more non-structural carbohydrates per meal (Fig. 4.1), which may also act to increase intermeal duration. Absorption of digested material is more rapid than simple diffusion would explain and there is evidence to suggest that there is probably considerable interaction between amino acids and sugars affecting absorption (Turunen 1985). The increased intermeal duration for the older locusts feeding on Mitchell grass could be due to (1) increased concentration of nutrients *per se*, (2) a specific nutrient preventing the haemolymph returning to a pre-meal state as quickly as occurred with nymphs consuming Button grass, or (3) nutrients being released more slowly, which would also prolong the post-feeding state of the haemolymph. That this did not occur with the younger locusts could be because the earlier instars have different nutrient requirements (Simpson and Simpson 1990) or the increased ratio of water to dry matter in the nymphs buffered the effect. Fewer nutrients were consumed by the early instars feeding on Mitchell grass compared to Button grass but this did not affect development rate. So whether the increase in retention time of Mitchell grass allowed for increased nutrient digestion and absorption or the increase in food retention time was due to factors preventing the intake of a meal needs to be ascertained.

For Australian plague locusts inhabiting an environment in which nutrients decrease rapidly as they develop, it appears to be essential that they consume plant tissue with a particular ratio of water to other nutrients to maintain growth rates. As the plants dry out there is likely to be an interaction between water and nutrient concentration of the leaf blades. Mitchell grass withdraws nutrients, including water, from the leaf blades as the soil dries out (Phelps and Gregg 1991). If decreasing digestibility is a

result of increase in size, then females that are bigger and develop more slowly are more likely to be affected by reductions in diet quality.

TABLES AND FIGURES

Table 5.1 Results of ANOVA of meal size, dry weight, fresh weight and volume for locusts feeding on Button grass and Mitchell grass.

Type of analysis	Source of variation	df	MS	F	P
Fresh weight	DIET	1	1.20×10^6	32.84	< 0.001
	AGE	4	5.16×10^5	14.13	< 0.001
	AGE x DIET	4	2.34×10^5	6.41	< 0.001
	Residual	88	3.66×10^4		
Simple Main Effects:					
Age = 2	DIET	1	1.03×10^4	0.28	0.897
Age = 3	DIET	1	3.00×10^4	0.82	0.367
Age = 4	DIET	1	4.11×10^4	1.012	0.292
Age = 5	DIET	1	5.65×10^5	15.45	< 0.001
Age = 6	DIET	1	1.49×10^6	40.85	< 0.001
Diet=Bg	AGE	4	7.18×10^5	19.69	< 0.001
Diet=Mg	AGE	4	4.30×10^4	0.83	0.512
Dry weight	DIET	1	0.04	0.30	0.870
	AGE	4	28.79	19.36	< 0.001
	AGE x DIET	4	1.88	1.26	0.291
	Residual	88	1.49		
Meal volume	DIET	1	1066	2.71	0.103
	AGE	4	79.40	20.18	< 0.001
	AGE x DIET	4	7.47	1.90	0.118
	Residual	88	3.94		

Table 5.2 Results of ANOVA of diet processing measures of the different aged locusts feeding on Button grass and Mitchell grass.

Type of analysis	Source of variation	df	MS	F	P
Bites + chews per mg dry weight diet consumed (processing/mg)	DIET	1	381067.43	0.325	0.57
	AGE	4	1.22×10^7	10.43	< 0.001
	AGE x DIET	4	110413.78	0.094	0.984
	residual		1.17×10^6		
Bites per mg	DIET	1	4.18×10^5	0.59	0.444
	AGE	4	8.22×10^6	11.65	< 0.001
	AGE x DIET	4	41219.64	0.06	0.994
	residual		7.05×10^5		
Chews per mg	DIET	1	838.15	0.01	0.915
	AGE	4	5.01×10^5	6.89	< 0.001
	AGE x DIET	4	2.10×10^4	0.29	0.884
	residual		7.26×10^4		
Ratio of chews per bite	DIET	1			
	AGE	4	0.66	39.09	< 0.001
	AGE x DIET	4	0.22	16.52	< 0.001
	residual		0.04	5.47	0.006
Simple Main Effects:					
Age = 2	Diet	1	< 0.001	0.001	0.994
Age = 3	Diet	1	0.04	0.99	0.322
Age = 4	Diet	1	0.81	20.23	< 0.001
Age = 5	Diet	1	0.41	10.11	0.002
Age = 6	Diet	1	11.35	33.54	< 0.001
Meal duration per mg consumed (min mg^{-1})	DIET	1	35.13	0.17	0.68
	AGE	4	3364.94	16.43	< 0.001
	AGE x DIET	4	11.46	0.06	0.99
	Residual	79	204.85		
Meal duration	DIET	1	29.54	0.94	0.335
	AGE	4	8.90	0.28	0.887
	AGE x DIET	4	59.02	1.88	0.122
	residual	79	31.35		

Table 5.3 ANOVA results for food retention time by different aged locusts consuming Button grass of Mitchell grass.

Type of analysis	Source of variation	df	MS	F	P
Food retention time (min)	DIET	1	8290.76	13.85	< 0.001
	AGE	4	574.84	0.96	0.434
	AGE x DIET	4	1911.74	3.19	0.017
	Residual	79	598.68		
Simple Main Effects:					
Age = 2	Diet	1	213.70	0.36	0.552
Age = 3	Diet	1	990.13	1.65	0.202
Age = 4	Diet	1	4025.88	6.72	0.011
Age = 5	Diet	1	10348.00	17.28	< 0.001
Age = 6	Diet	1	505.85	0.84	0.361

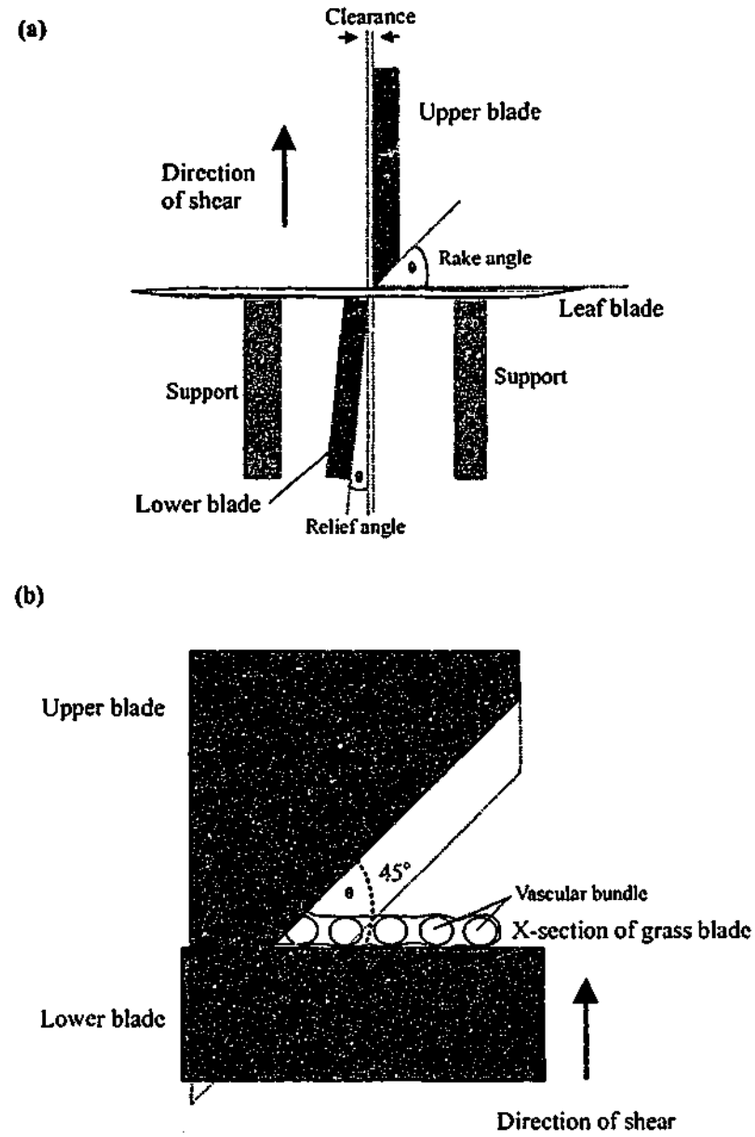
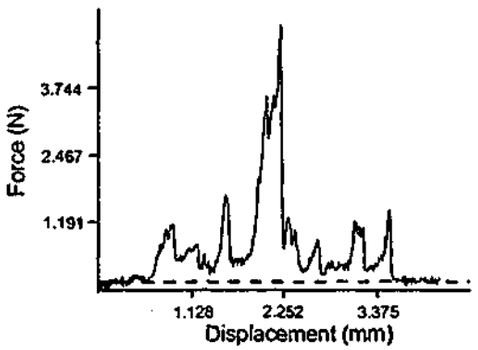


Fig. 5.1 Diagram illustrating the set-up that was used to shear the grass blade leaves. (a) Side view illustrating the arrangement of the blades, giving the rake and relief angles and the clearance between the upper and lower blades. The grass blade is laid across the supports and lower blade which moves upward at 0.191 mm s^{-1}, while the upper blade, which is attached to the force transducer, is fixed, thus severing the leaf blade. (b) Front view illustrating the position of the upper blade relative to the moving lower blade at different parts of the shear cycle. The 45° approach angle of the upper blade means that at any point during the shear cycle different tissue types are being sheared (following Sanson *et al.* 2001).

(a) Button grass



(b) Mitchell grass

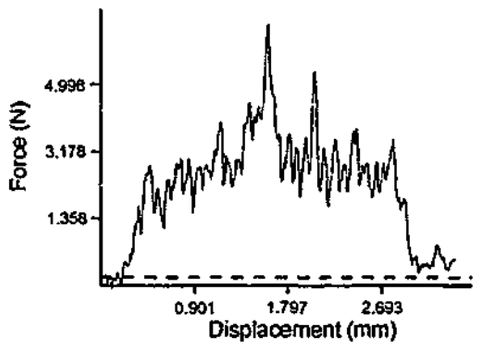
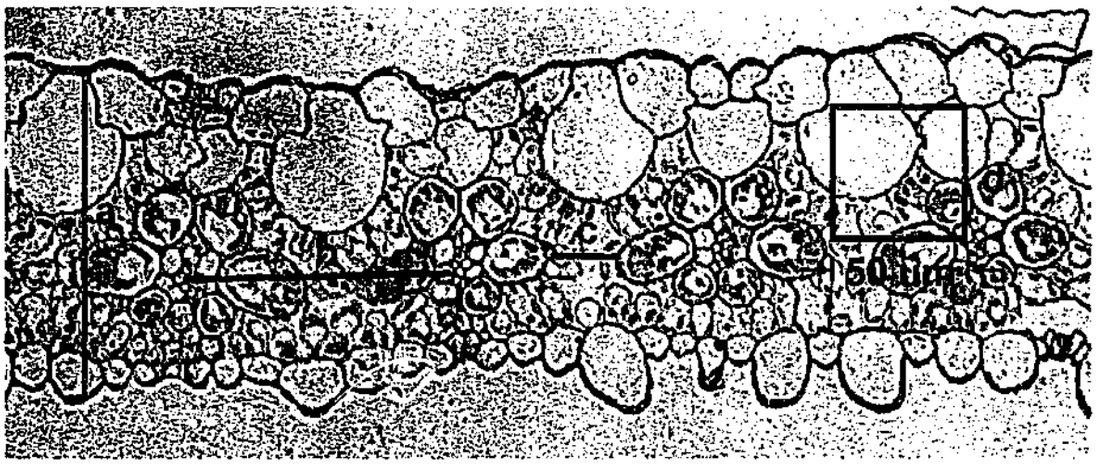


Fig. 5.2 Typical force-displacement traces for (a) Button grass and (b) Mitchell grass generated when shearing a grass blade. The peaks and troughs do not necessarily represent the minimum and maximum forces, since the blade may be cutting through multiple tissue types (i.e. vascular bundles and non-vascular tissue) at any one point (depending on the width of the tissue and distance between tissue types).

(a) Button grass



(b) Mitchell grass

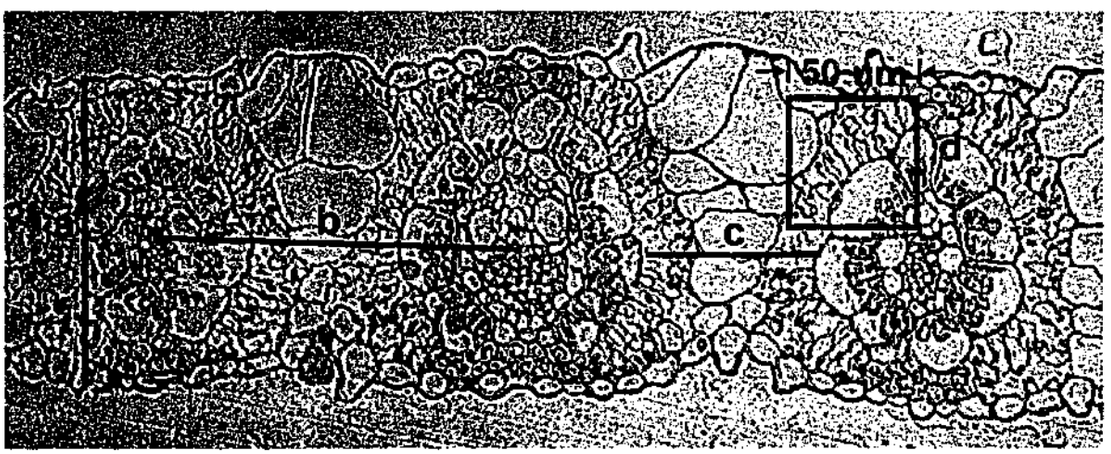


Fig. 5.3 The measures made from the cross-sections of (a) Button grass and (b) Mitchell grass; a = leaf thickness; b = distance from the centre of adjacent vascular bundles; c = distance between adjacent bundle sheath cells; d = quadrat used to count cell numbers.

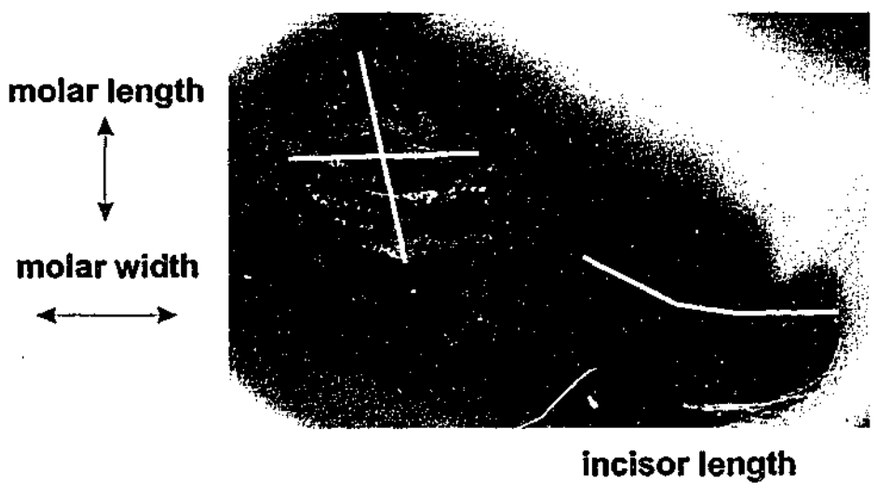


Fig. 5.4 Image of the right mandible showing where the measurements of incisor length, molar width and length were made. This image was taken on a slight angle to illustrate the position of the measurements. For the analysis, separate images were taken of the incisor and molar regions that were normal to the camera lens.

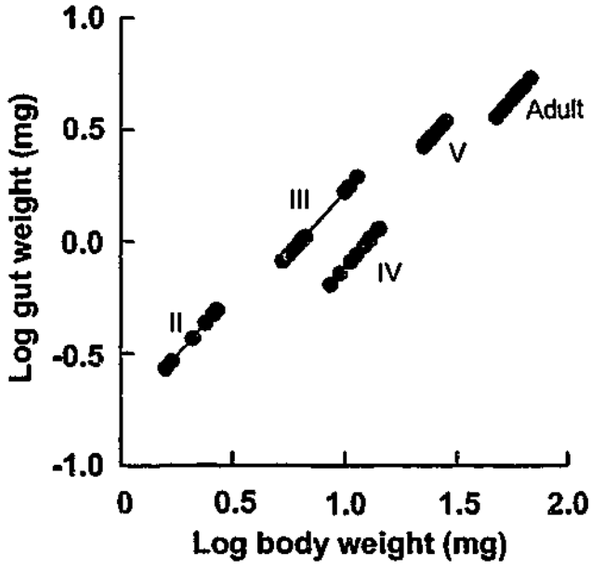


Fig. 5.5 ANCOVA-derived relationship between $\log_{10}$ gut and $\log_{10}$ weight of the remainder of the body for Instar II-V and adult locusts.

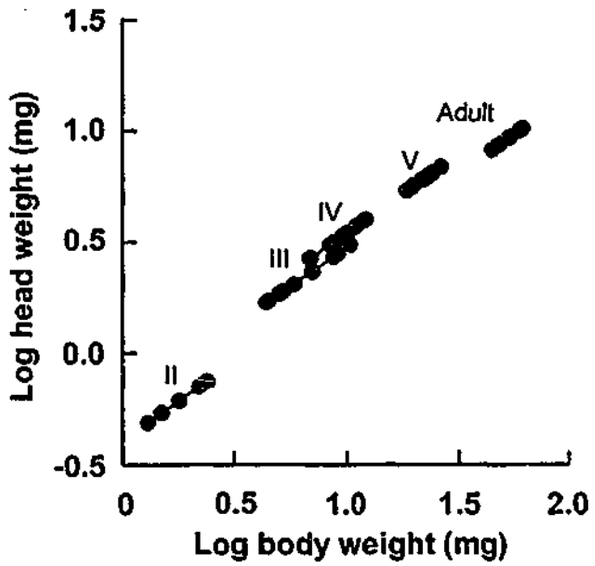


Fig. 5.6 ANCOVA-derived relationship between $\log_{10}$ head and $\log_{10}$ weight of the remainder of the body for Instar II--V and adult locusts.

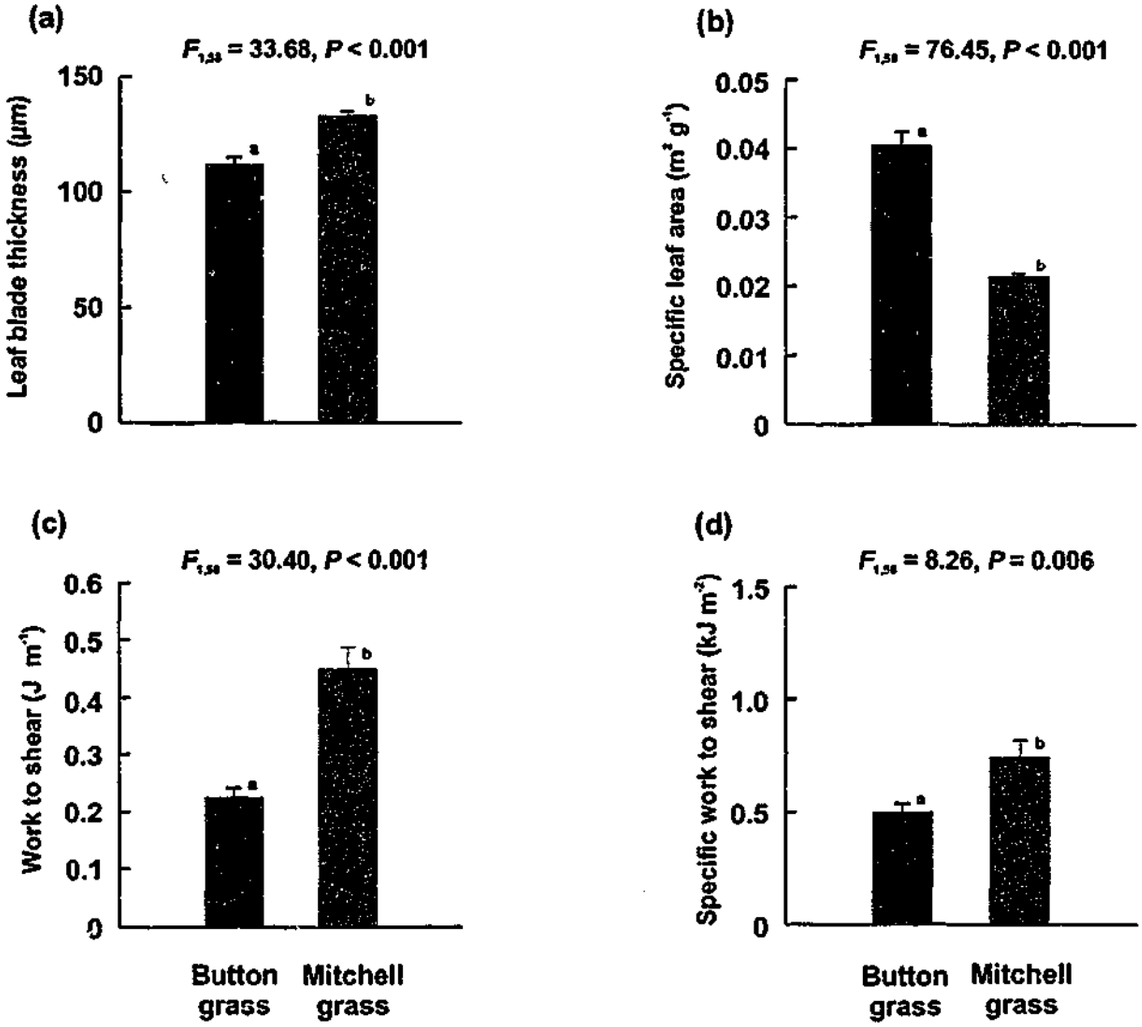


Fig. 5.7 Physical and biomechanical properties of both grasses; (a) leaf blades thickness, (b) specific leaf area (m^2 g^{-1}), (c) work to shear (J m^{-1}), and (d) specific work to shear (kJ m^{-2}). The F and P values given are for ANOVA, $n = 30$.

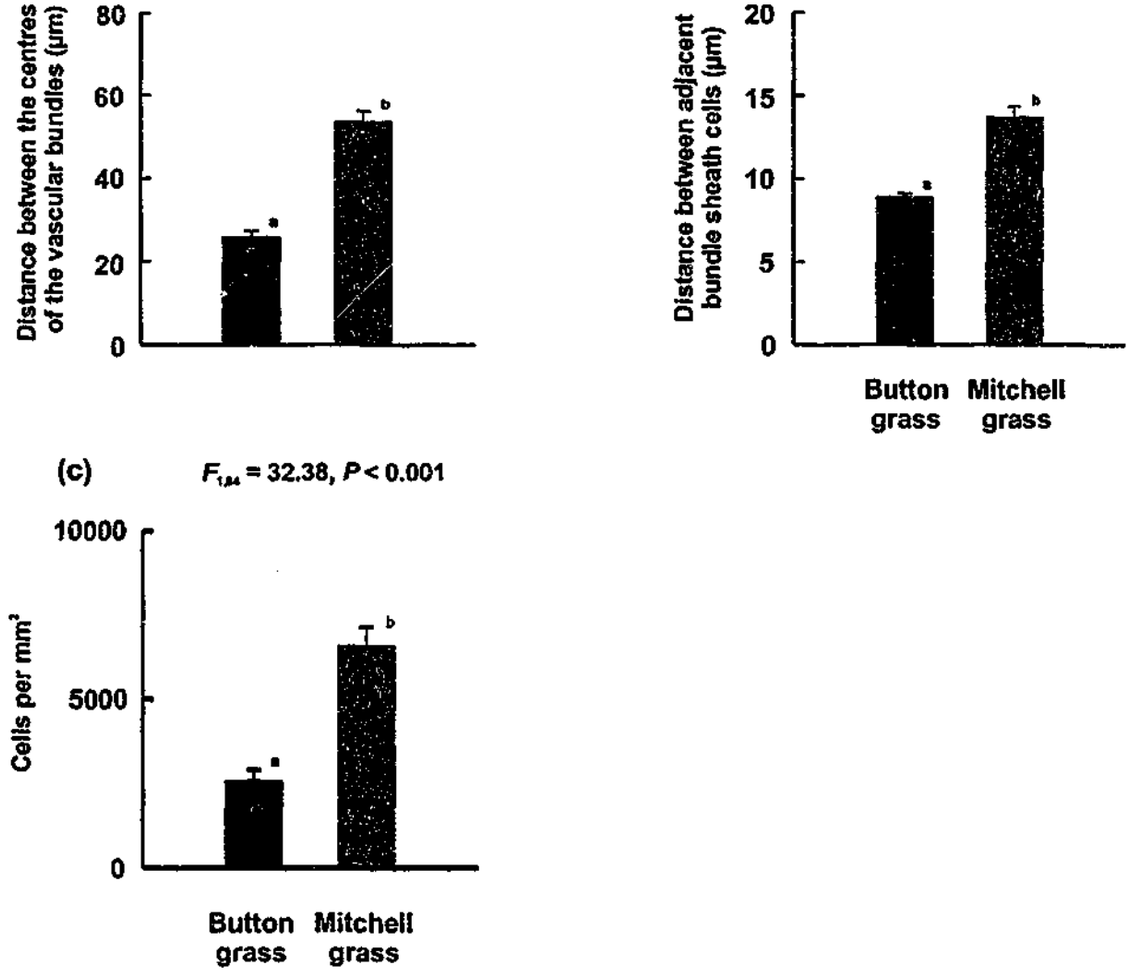


Fig. 5.8 Leaf parameters measured from the thin sections of Button grass and Mitchell grass. (a) average distance between the centre of the vascular bundles, (b) average distance between adjacent bundle sheath cells, and (c) average number of cells per mm^2. The F and P values given are for ANOVA, $n = 35$.

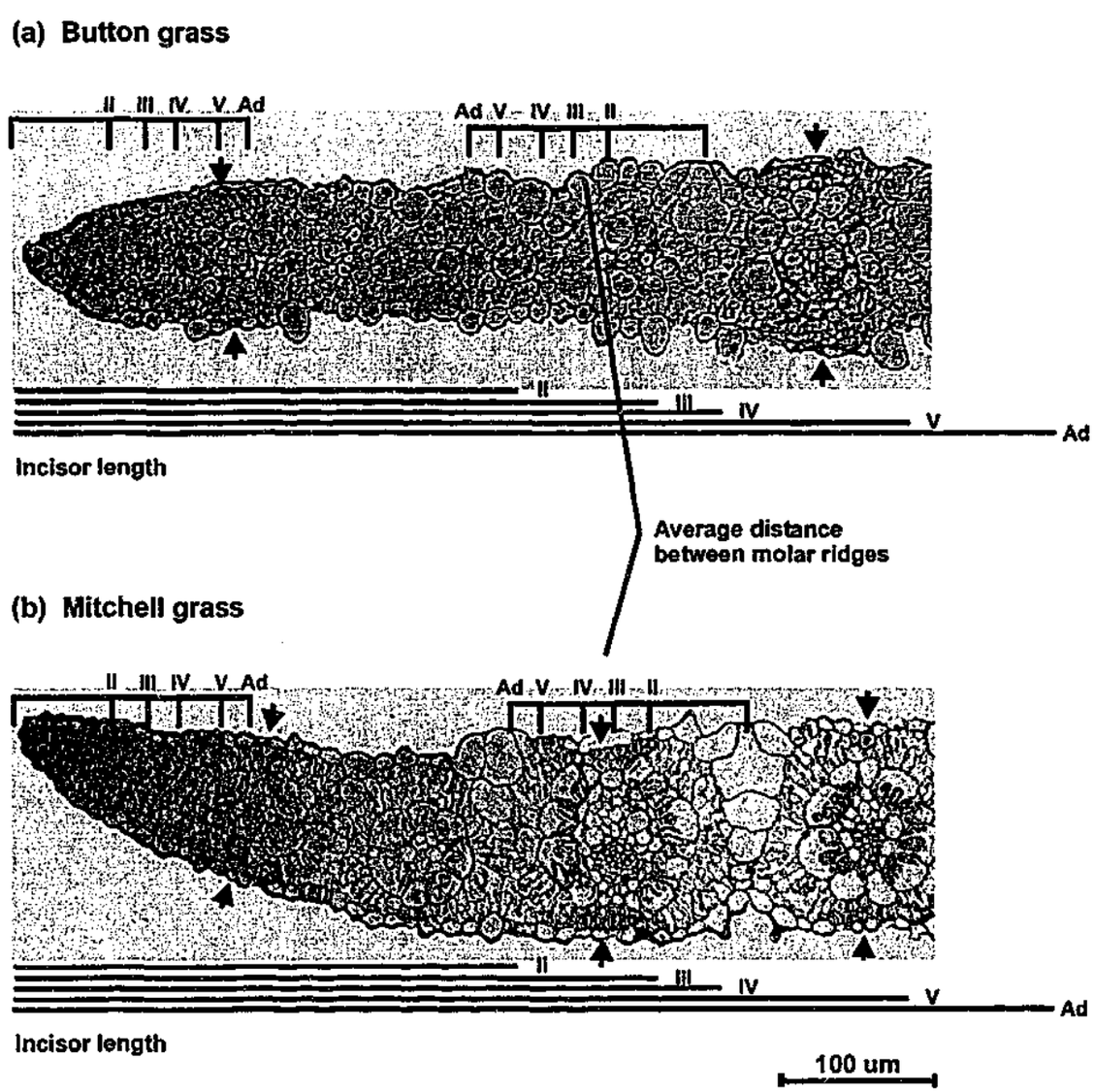


Fig. 5.9 Diagram illustrating the interaction of the mandibles with the cellular structure of both grasses. (a) Button grass; and (b) Mitchell grass. For the different aged locusts the incisor length and the average distance between the molar ridges has been shown, drawn to scale. Thus the larger the insect the larger the number of vascular bundles trapped between the five molar ridges. When opposing molar ridges crush the plant the ratio of cells crushed to those not crushed will be higher for the smaller nymphs. The arrows identify the abaxial and adaxial sclerenchyma bundle sheath extensions, ('girders').

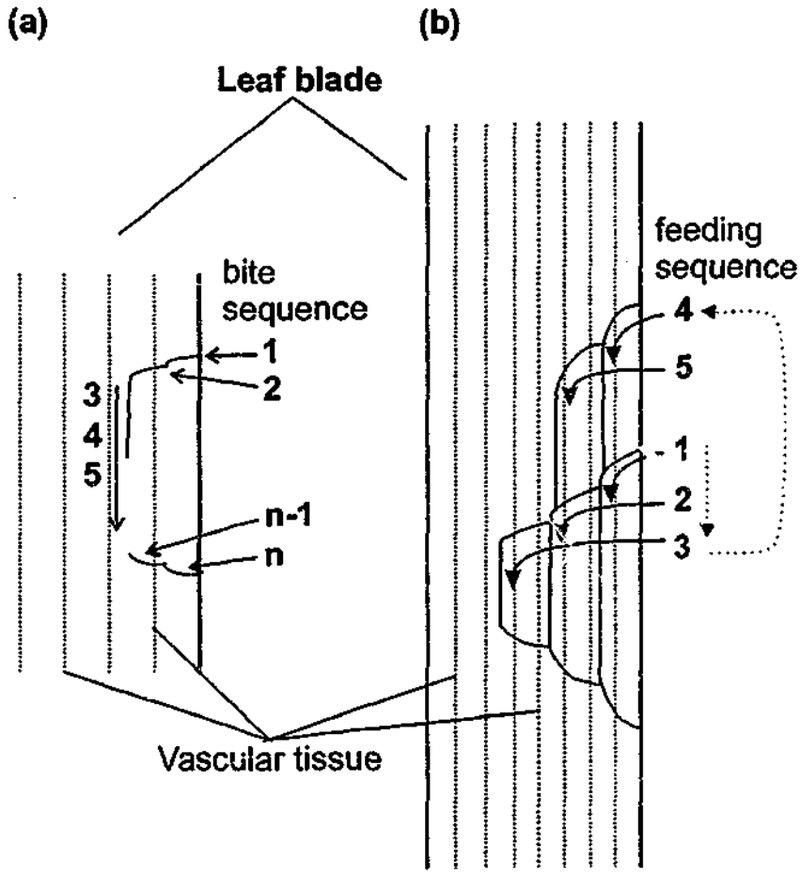


Fig. 5.10 Diagram illustrating (a) the typical bite sequence and (b) the feeding sequence of locusts consuming either Button or Mitchell grass. (a) Locusts initially sever the outer vascular bundles (bites 1 and 2) and then proceed to fracture between the vascular bundles (bites 3, 4, 5, etc.) and finish by severing the outer vascular bundles (bites n-1, n) to form feeding sequence 1. (b) This sequence of bites is repeated following the feeding sequence outlined until the meal is completed. The solid arrows indicate the direction of bites, and the dotted arrows indicate the direction of feeding sequences. The number of vascular bundles per strip is dependent on the age (size) of the locust consuming the grass blade.

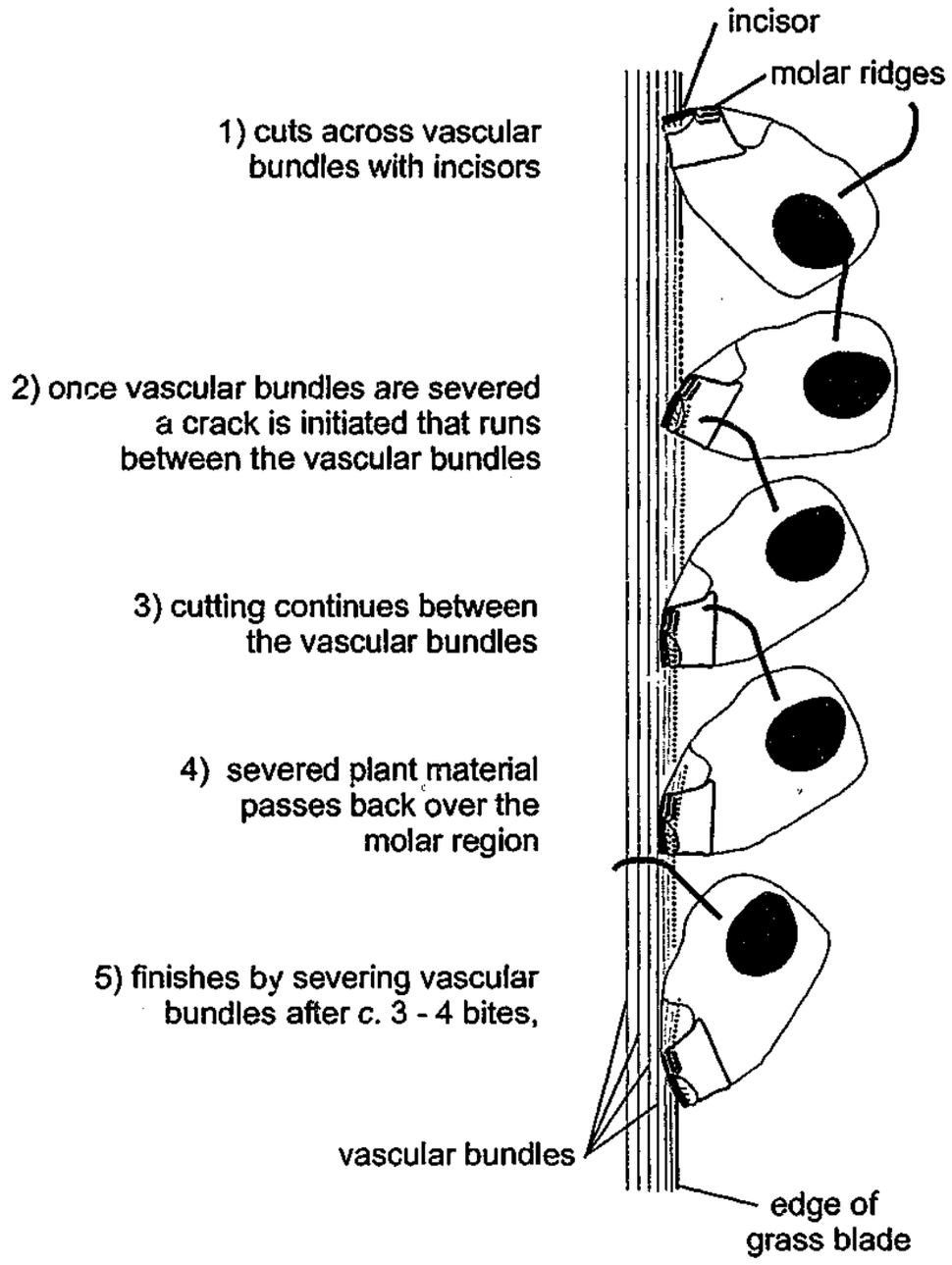


Fig. 5.11 Reconstruction of the locust consuming a blade of grass.

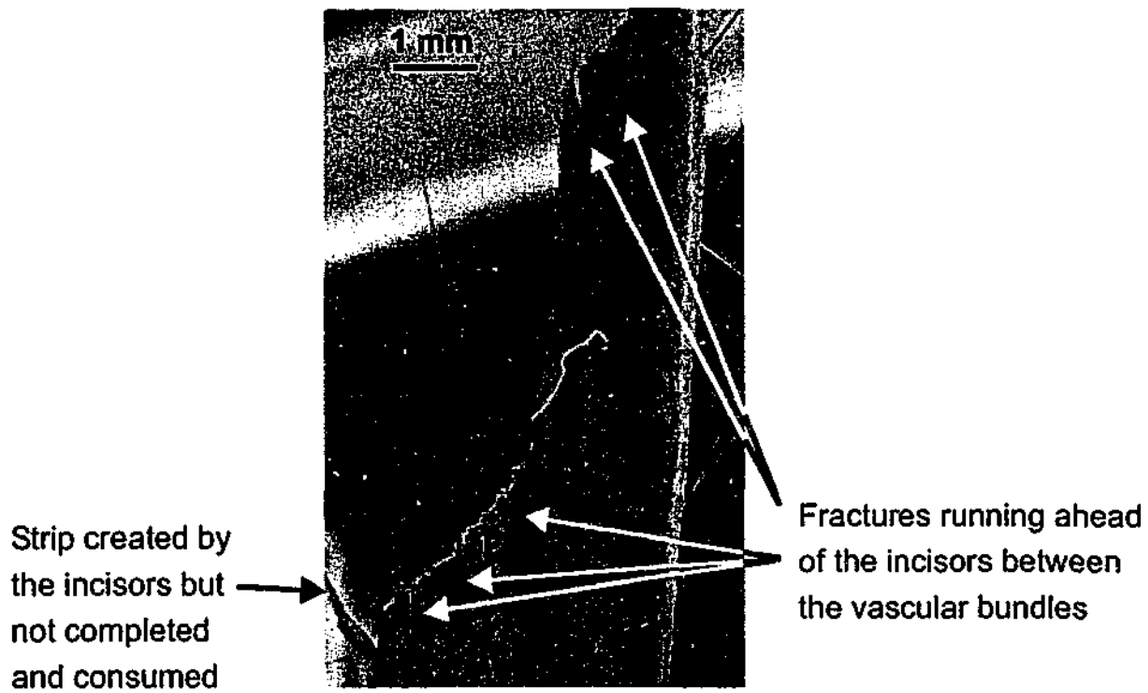


Fig. 5.12 Image showing (i) fractures running ahead of the path of the incisors between the vascular bundles and (ii) a strip created on the edge of the grass blade by the incisors but not completed and consumed. These fractures were created by an adult male Australian plague locust feeding on Mitchell grass.

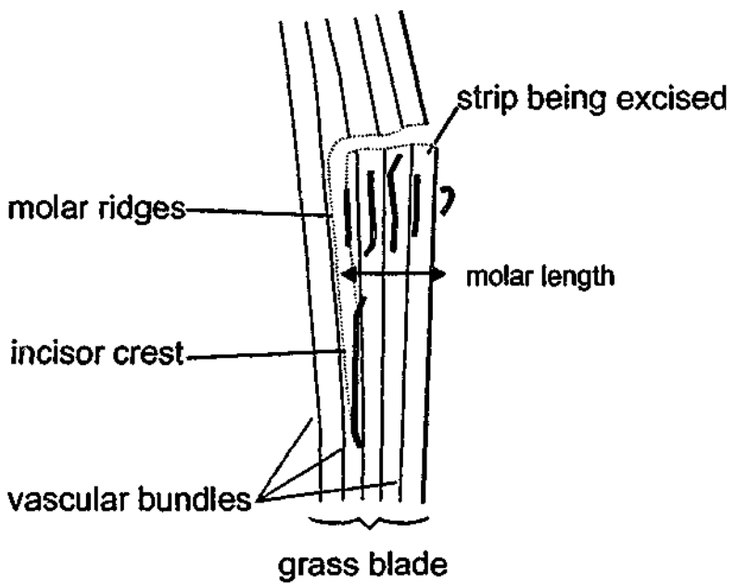


Fig. 5.13 Alignment of the incisor and molar ridges from the right mandible superimposed on a grass blade. The left mandible closes over the right. The molar ridges and incisor crest are drawn to scale, the plant is not. Mandible size increases linearly with age, i.e. an Instar II mandible is a proportionally smaller Instar V mandible. The number of vascular bundles trapped between the molar ridges depends on the age (size) of the locust. There is no advantage in the locust incising deeper than the molar length as it would not be fully processed.

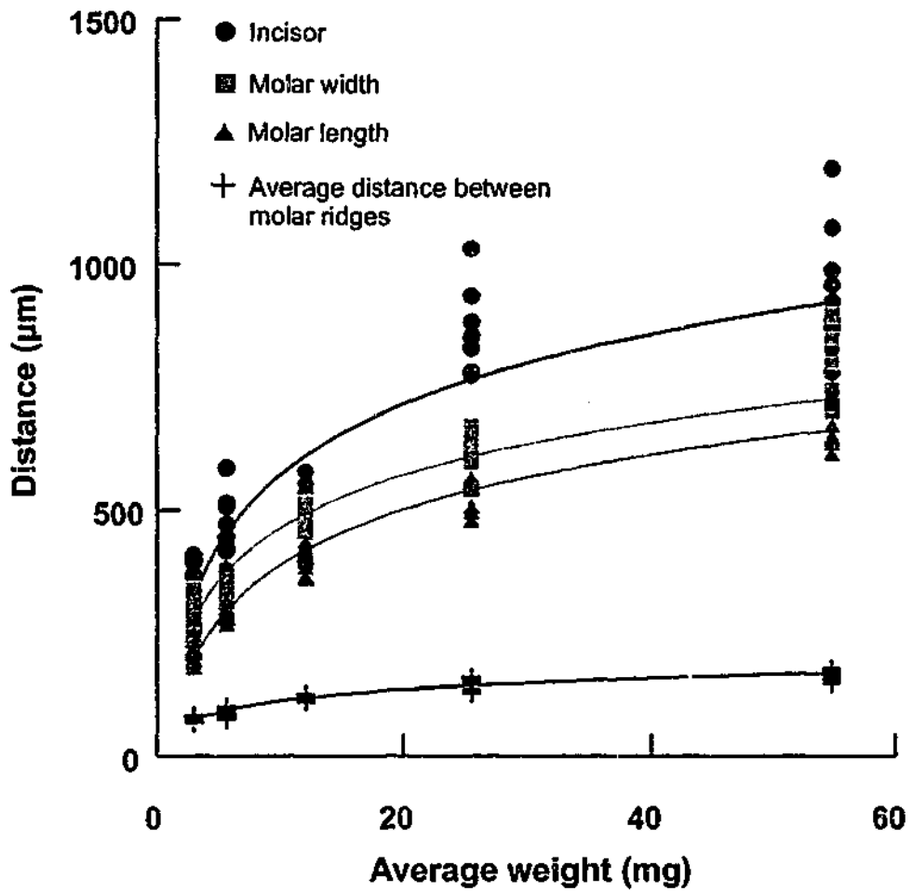


Fig. 5.14 Relationship between the length of incisor, molar width, molar length, and average distance between molar ridges for the average weight of Instars II to V and adults. Fitted line is log smoothed.

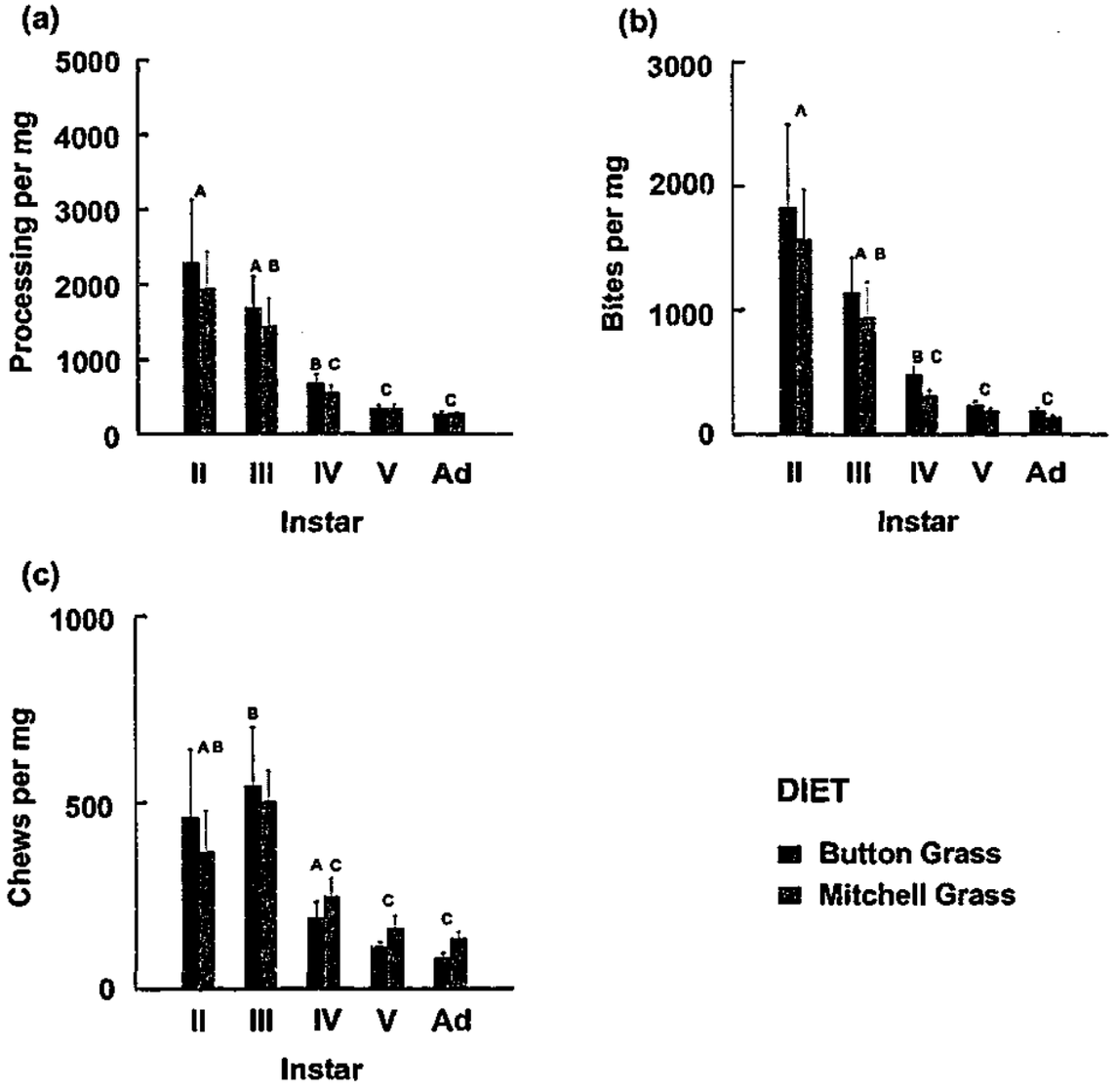


Fig. 5.15 Measures of food processing ability for each age on both diets (a) total processing efforts of locust (total number of bites + chews) per mg consumed; (b) number of bites per mg dry weight consumed; (c) number of chews per mg consumed. Different letters above the bars represent differences ($P < 0.05$). Capital letters represent differences between ages and small letters between diets at each age.

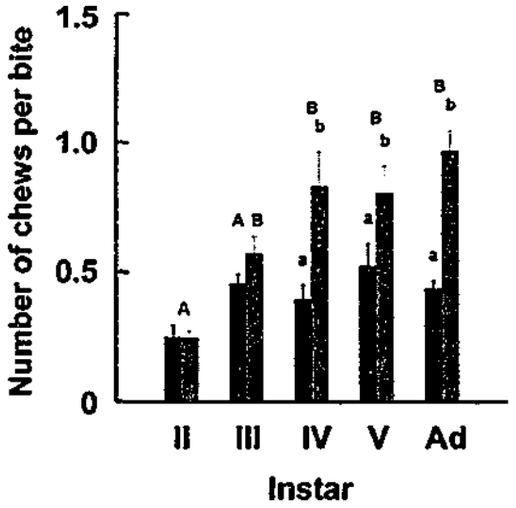


Fig. 5.16 Ratio of chews per bite for each aged locust consuming each diet. Different letters above the bars represent differences ($P < 0.05$). Capital letters represent differences between ages and small letters between diets at each age.

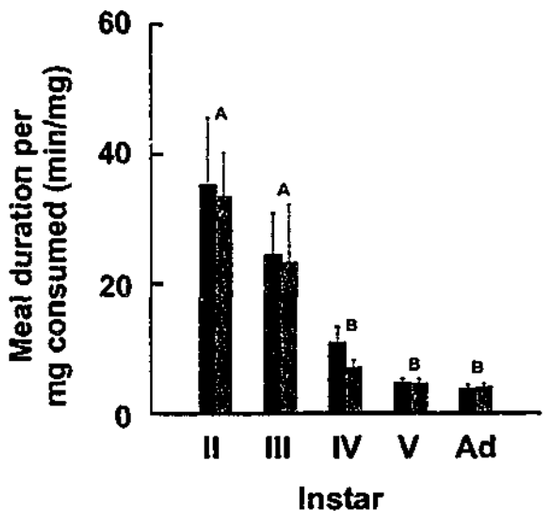


Fig. 5.17 Time taken to consume 1 mg of each diet by each locust age. Capital letters represent differences ($P < 0.05$) between ages when diets are combined.

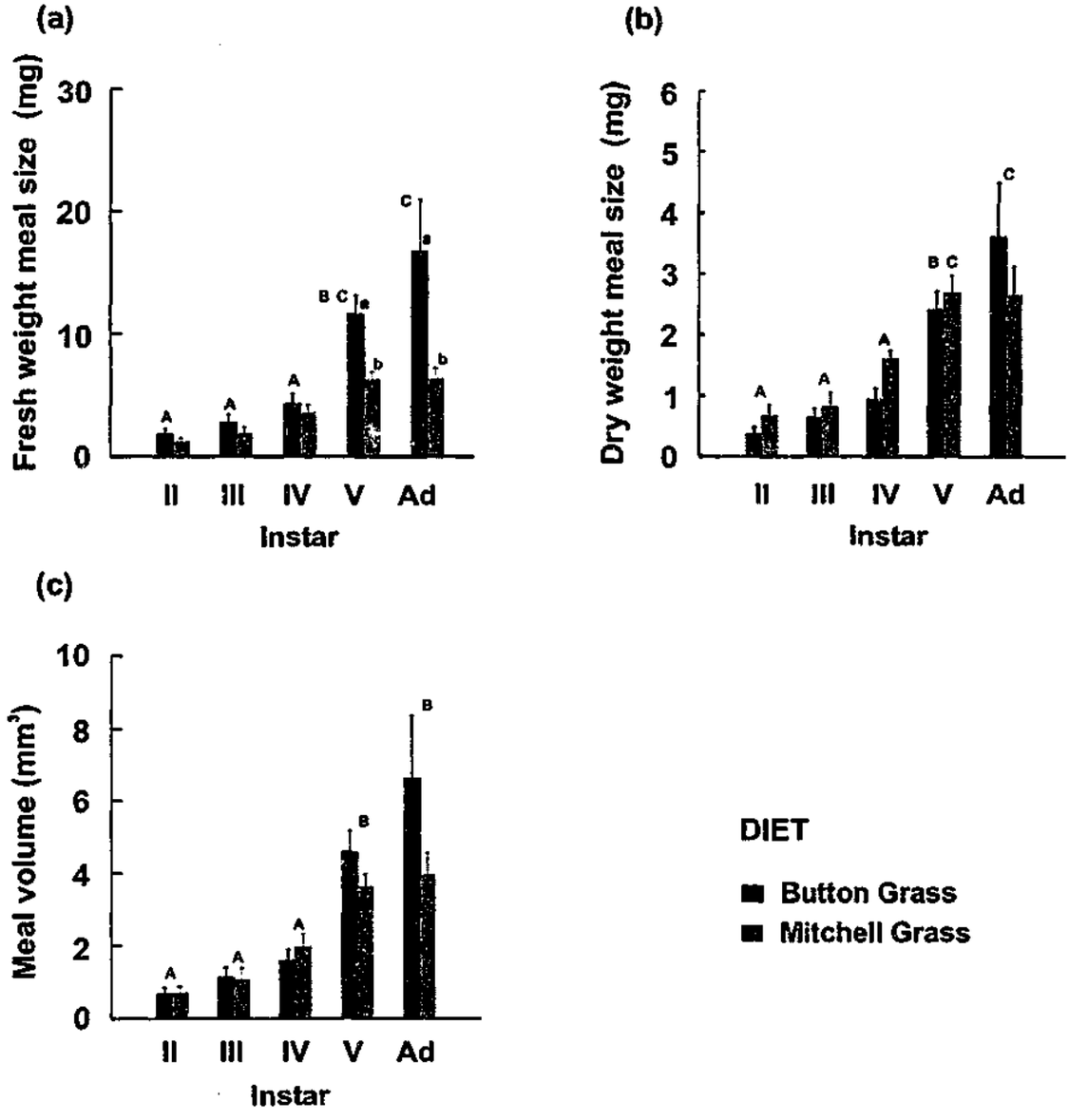


Fig. 5.18 Meal size for locusts feeding on Button grass and Mitchell grass on Day 3 of each instar; (a) fresh weight, (b) dry weight, (c) volume. Different letters represent significant differences ($P < 0.05$). Capital letters represent differences between ages and small letters between diets at each age.

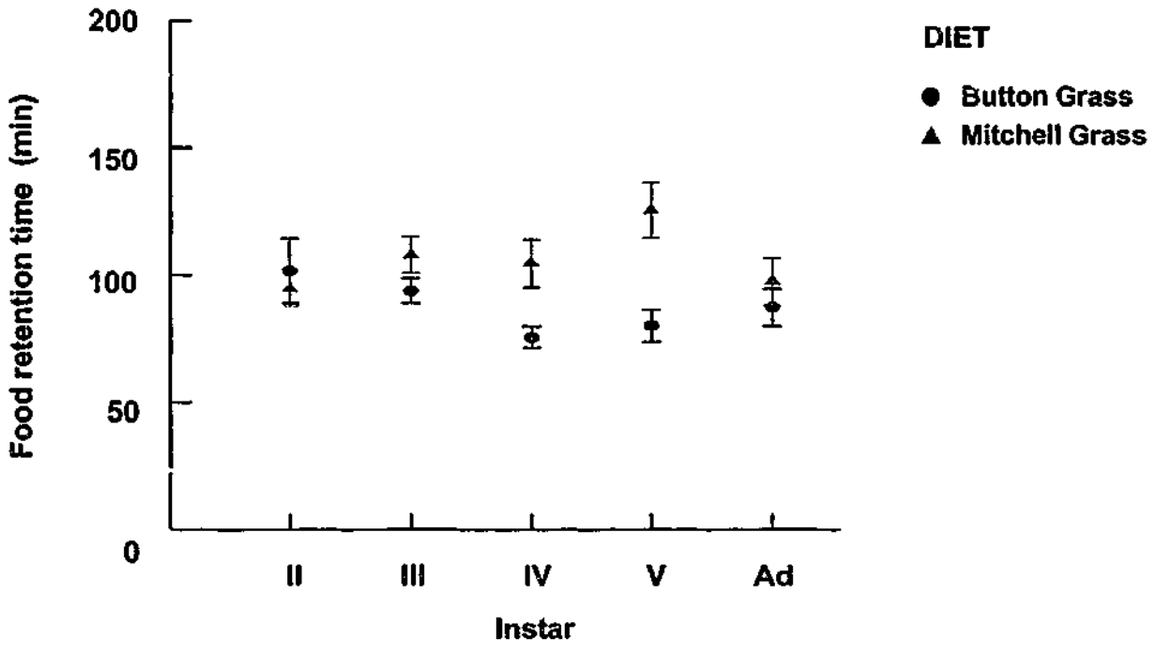


Fig. 5.19 Food retention time for a meal of either Button grass or Mitchell grass on Day 3 of each instar.

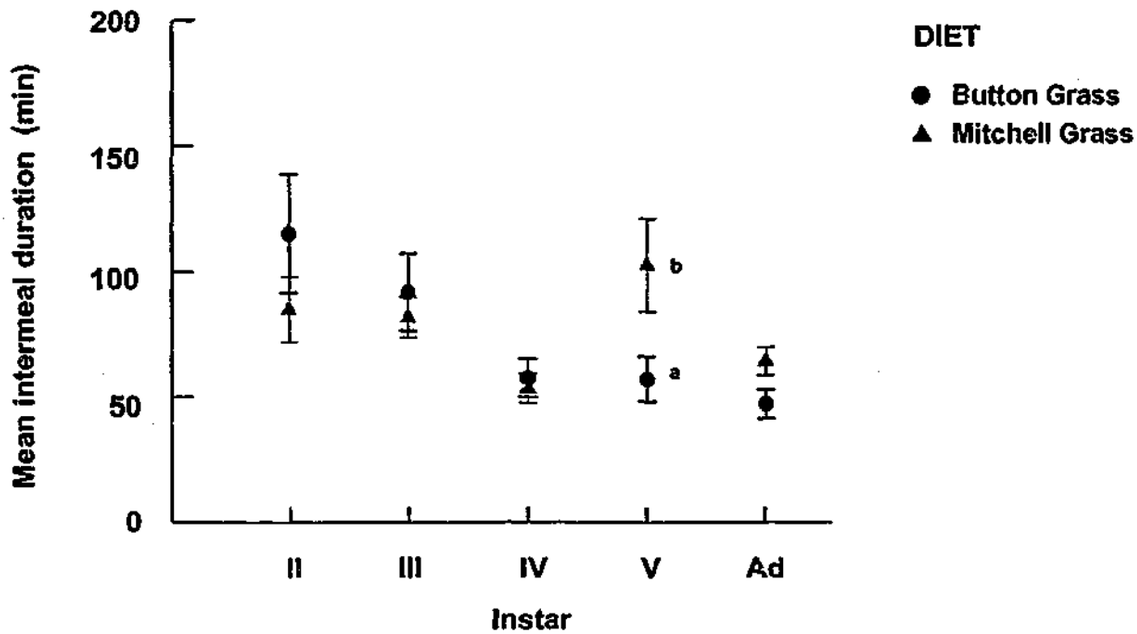


Fig. 5.20 Mean intermeal duration when feeding on both grasses on day 3 of each instar.

CHAPTER 6. THE LONG-TERM EFFECTS OF FEEDING ON EITHER BUTTON GRASS OR MITCHELL GRASS

SUMMARY

1. Nymphs were (1) reared on either grass from hatching, or (2) reared on wheat and then switched to either grass at the commencement of Instar V and dietary utilization was compared over Instar V.

2. The nymphs reared on wheat were larger than those reared on the treatment diets. However, the treatment diets themselves had more of an effect on dietary utilization than differences in body size.

3. Nymphs reared on either treatment diet appeared to compromise body size rather than increase instar duration in response to inadequate dietary nutrients. However, nymphs reared on Mitchell grass had a relatively bigger head. For these nymphs, consumption was increased, but the costs associated with converting ingested material to biomass were also higher.

4. The larger wheat-reared nymphs consumed significantly less than the smaller treatment reared nymphs contrary to that expected from differences in body size.

5. Body size affected dietary utilization. Large Instar V nymphs were able to consume and assimilate nutrients at a significantly faster rate, but the efficiency with which they converted ingested material to biomass was significantly lower than for the small nymphs resulting in no differences in growth rate.

6. Digestibility of the diet was not affected by body size or rearing treatment.

INTRODUCTION

Phenotypic plasticity may be an important component of adaptation to variable environments as it may increase the niche breadth that an organism can occupy (Bradshaw 1965). With respect to nutrition, if an organism is restricted to a sub-optimal diet for a long duration, in order to maintain growth it may be necessary tomake adjustments that affect intake and/or assimilation.

Theoretically there are various ways in which the digestive system might be modified so as to increase nutrient assimilation, for example, alteration of food retention time, gut volume, and particle size processing. Phenotypic plasticity of body parts has been measured in mammals, birds and insects. In response to reduced nutrient availability, in both mammals and insects, the digestive tract enlarges, increasing digestive efficiency (Bezzobs 1995; Green and Millar 1987; Gross *et al.* 1985; Yang and Joern 1994b). Large heads relative to body size are associated with and can be induced in insects eating a 'tough' diet (Bernays 1986; Bernays and Hamai 1987; Thompson 1992). Grasshoppers appear to have the capacity to alter gut size within an instar (Chapman 1988b; Simpson 1982a) in response to the diet being consumed.

However, evidence that this phenotypic plasticity provides advantages to digestion is equivocal, particularly in insects. Thompson (1992) measured an increase in consumption rate in grasshoppers correlated with a dietary induced increase in muscle mass and cross-sectional area and mandible size, which occurred in relatively larger heads. In contrast, while Hochuli (1994) recorded a significant positive correlation between head size and larval mass (corrected for initial mass) in caterpillars, neither consumption nor assimilation were affected by larger heads. In response to reduced diet quality, a grasshopper, *Melanoplus differentialis*, reduced the food residence time but the ratio of gut to body remained unaltered (Yang and Joern 1994c). However, in a second experiment a significant linear increase in gut to body ratio was correlated with decreased dietary nutrients (Yang and Joern 1994b).

The experiments performed in Chapters 4 and 5 examined the short-term (one instar) effect of Button grass versus Mitchell grass diet on digestive processing and life history characteristics of the locusts. Since grasshoppers can show phenotypic adjustment to their diet, the same may be true of the Australian plague locust.

Therefore, the major aim of this experiment was to determine long-term effects of each diet. I examined whether survival, development time and growth rate of Australian plague locusts on the two contrasting grasses were affected by altered digestive capacity, and whether this adjustment was sufficient to allow compensation for the differing nutritional characteristics of the diets. The results of Chapter 4 indicated that Mitchell grass was an increasingly 'poorer' resource for the locusts the older the instar. Nymphs reared from hatching on Mitchell grass were predicted to show greater plasticity than those reared on Button grass. Two comparisons were made (1) long-term Mitchell grass with Button grass diet and (2) short- versus long-term Button grass or Mitchell grass diets.

The differences observed between locusts consuming Button grass and Mitchell grass in Chapters 4 and 5 were hypothesized to be due to either the constraints imposed by the increase in body size associated with increasing age rather than ontogeny itself. With increasing age, body size increased and the larger nymphs were able to grow faster as they consumed and assimilated dry matter at a faster rate although they were less efficient at digesting the non-cell wall component. Therefore, the effect of body size within an instar, i.e. by the removal of ontogenetic effects, was investigated in a second experiment.

MATERIALS AND METHODS

Experimental animals

Locust stocks were reared as described in Chapter 2, prior to experimentation. 90% of the colony was the second generation of field-caught grasshoppers (Griffith District, NSW). The other 10% were from the long-term colony maintained at Monash University.

Experimental design

Long-term effects of diet

Nymphs were randomly allocated to each of the following treatments.

BBG: reared continuously on Button Grass from hatching to Adult.

MMG: reared continuously on Mitchell Grass from hatching to Adult.

WBG reared on wheat from hatching to Instar IV and then reared on Button grass from the start of Instar V.

WMG reared on wheat from hatching to Instar IV and then reared on Mitchell grass from the start of Instar V.

The emergence of Instar I nymphs was timed to within eight hours, i.e. every eight hours during daylight new emergents were removed from the sand containers they had been incubated in and placed in cloth cages with diets (wheat, Button grass or Mitchell grass) allocated randomly to each 8-hour cohort. Locusts that emerged during the night were returned to the colony and not utilized in the experiment. Each cohort generally consisted of between five and 10 egg pods. They were placed in cloth cages and reared communally until they reached Instar III. At some time within the third instar all the females from each diet were removed and returned to the colony. The remaining males were each placed in an individual digestibility chamber. They were then reared on the same diet until Instar V when those reared on wheat were randomly allocated to Button grass or Mitchell grass. The diets were presented as previously described (Chapter 2).

A digestibility trial was performed on the entire Instar V for each locust. Moulting was timed to within 4 h during the lights-on phase. At the completion of the instar, within 4 h of moulting to the adult stage, the locusts were frozen at -20°C. They were later dissected while still frozen into guts, head (severed immediately behind the post-occiput) and remaining body and then freeze-dried. Any grass remaining in the gut was removed prior to freeze-drying. After drying, each portion was weighed separately for each grasshopper to 0.1 mg.

Ten freshly moulted locusts from each rearing treatment were harvested throughout the experiment to determine mass allocation to body parts and water content so that the initial dry weight of the locusts could be estimated.

It was impossible to determine the instar stage of each cohort in the cloth cages due to sexual dimorphism of this species in terms of size, i.e. Instar II females are the same size as Instar III males. Therefore, as this species is difficult to sex visually from outside the cages earlier than the Instar IV, visual inspection was leading to under-estimation of development rate. However, it was thought the stress of handling each locust daily would be detrimental to subsequent performance so locusts were allowed to develop until they reached Instar III and were then removed and placed in a digestibility chamber as previously described.

Effect of Body Size of Instar V Nymphs on Digestive Performance

To investigate if digestive performance was affected by body size within an instar, a digestibility trial on Mitchell grass was performed on small (less than 90 mg) and large (greater than 125 mg) Instar V nymphs reared on wheat. From the experiment on the long-term effects of diets it was found that the population had a mean fresh weight of 110.0 ± 2.6 mg. Only nine nymphs were available in the designated size ranges. Dry weight was estimated from previously sacrificed insects.

Plant analysis

The control portion of the diets collected daily for the duration of the experiment was analyzed for cell wall material, protein and non-structural carbohydrates as previously described in Chapter 4. Protein digestibility was not ascertained as the results from Chapter 4 showed that protein assimilation was correlated with protein intake ($F_{1, 95}$.= 4769.389, $P < 0.001$).

Data analysis

The plant chemical constituents were compared over the duration of the experiment (21 days) using scatterplots and one-way ANOVA. One-way ANOVA was used to check if, by chance, the chemistry of the grasses offered to the different treatments varied as expected from measurements of samples collected daily (as per Chapter 4).

Intake of total dry matter, water and protein was corrected as outlined in Chapter 2 for the 'new' constant temperature room. Insect performance across the four treatments was compared using one-way ANOVA and ANCOVA as for Chapter 4. Box plots were used to check for normality and homogeneity of variances across the treatments. The BBG treatment ended up having only nine nymphs due to higher mortality in the initial instars and Instar V. This resulted in a smaller variance than for the other three treatments for some measured parameters. The data were not transformed as the variance for all treatments was normally distributed, the variances of the other three treatments were homogeneous and the plots of residuals were not skewed.

When significant differences were found, planned post-hoc testing of adjusted means was performed. The planned post-hoc comparisons were (1) the comparison of nymphs consuming either Button grass or Mitchell grass reared either on the wheat and the treatment grass, (2) nymphs reared on wheat versus nymphs reared on the treatment grasses, and (3) nymphs consuming Button grass versus those consuming Mitchell grass. As initial body mass was not equal for either experiment, the ANCOVA-fitted slopes were plotted for each factor to show the relationship among treatments.

RESULTS

Experiment 1

Plant chemistry

Button grass when compared to Mitchell grass had significantly higher SLA and water, significantly less cell wall and non-structural carbohydrates, a lower ratio of protein to non-structural carbohydrates and the same amount of protein (Table 6.1). The chemistry averaged for the duration of the experiment (21 days) was not different from that offered to the appropriate treatment.

Locust performance

Survival of Instar V nymphs was higher for nymphs feeding on Mitchell grass than those consuming Button grass (Table 6.2). There was no difference in instar duration for locusts consuming Button or Mitchell grass nor was it influenced by nymphal rearing diet (Table 6.3).

Locusts reared on Mitchell grass or Button grass did not differ in their initial fresh weight, dry weight, and thus ratio of water to dry matter (Table 6.4). Similarly, the locusts reared on wheat and allocated to either diet had the same initial weights and ratio of water to dry matter (Table 6.4). However, the nymphs reared entirely on Mitchell grass or Button grass had significantly lower dry and fresh weights than those raised on wheat. The nymphs raised on Button grass had a significantly higher ratio of water to dry matter than those reared on wheat (Table 6.4). There was no difference in the mass of the gut relative to the rest of the body for freshly moulted Instar V nymphs reared on any of the three diets. The mass of the head relative to the rest of the body was significantly heavier for nymphs reared on Mitchell grass than for those reared on wheat (Fig. 6.1). However, there was no difference in actual head size of locusts reared on wheat and Mitchell grass ($P = 0.753$), but the heads of nymphs reared on Button grass were significantly lighter ($P = 0.025$). Regardless of nymphal rearing diet, locusts consuming Mitchell grass gained significantly more biomass than those consuming Button grass and nymphal rearing diet did not influence biomass gained (Table 6.5, Fig. 6.2a). On a dry weight basis, the pattern was the same (Table 6.5, Fig. 6.2b). There was no difference detected between either

the head or gut and the remainder of the body for either treatment at the completion of Instar V (Table 6.5, Fig. 6.3).

Digestive Capacity

More fresh Button grass was consumed than fresh Mitchell grass and this was not influenced by the nymphal rearing diet (Table 6.5, Fig. 6.4a). The nymphs reared on wheat, that were significantly heavier initially, consumed significantly less dry weight of either diet than those reared and fed on the treatment diets ($P = 0.005$) (Table 6.5, Fig. 6.4b). For nymphs feeding on Mitchell grass, those reared on Mitchell grass consumed significantly more than those reared on wheat ($P = 0.002$). Ignoring rearing treatment, there was no difference in the dry weight of Button grass or Mitchell grass consumed ($P = 0.391$). However, due to the respective properties of the two diets, nymphs consuming Button grass ingested significantly more water ($P < 0.001$) and non-cell wall material ($P < 0.001$) and less non-structural carbohydrates ($P < 0.001$) than those feeding on Mitchell grass (Table 6.5, Fig. 6.5a,b,d). For nymphs reared on wheat, those consuming Mitchell grass ingested significantly less protein than those feeding on Button grass (Fig. 6.5c). Nymphs reared on wheat ingested significantly less non-structural carbohydrates than those reared on either of the treatment diets ($P = 0.002$). Nymphs consuming Button grass assimilated significantly more dry matter ($P < 0.001$) (Fig. 6.6). Button grass was significantly more digestible than Mitchell grass ($P < 0.001$) (Table 6.5, Fig. 6.7a), due to the larger amount of cell wall material in the dry matter (Table 6.5, Fig. 6.7b).

Nymphs consuming Mitchell grass were more efficient at converting fresh matter ingested ($P = 0.008$), and ingested and assimilated dry matter to biomass ($P < 0.001$), than those consuming Button grass (Table 6.5, Fig. 6.8, 6.9). For nymphs feeding on Button grass, those reared on Button grass gained significantly more weight per unit intake than those reared on wheat ($P = 0.040$). Nymphs reared on the treatment diets were able to convert significantly more dry matter consumed and assimilated to biomass compared to their counterparts reared on wheat ($P < 0.001$, $P = 0.003$ respectively).

Nymphs reared on wheat gained biomass at a faster rate than those reared on the treatment diets ($P = 0.008$), although rearing diet did not affect consumption or assimilation rate (Fig. 6.10, Table 6.5). Nymphs consuming Mitchell grass gained biomass and assimilated nutrients at a significantly higher rate ($P < 0.001$ for both) although both diets were consumed at the same rate.

Experiment 2

Plant chemistry and locust performance

There was no difference between the Mitchell grass diet offered to each treatment (Table 6.6). The initial fresh and dry weight of the large Instar V nymphs was approximately 60% higher than that of the smaller nymphs. The large nymphs had significantly more water per gram dry matter (Table 6.7) and a significantly heavier gut than the small nymphs (Fig. 6.11a). However, there was no difference in the weight of the heads relative to the remainder of the body (Fig. 6.11a).

The final weight of the large nymphs was the same as the small nymphs given the initial weight (Table 6.8, 6.9), but there was no difference in the proportions of the two different sized nymphs (Fig. 6.11b). The instar duration of the large nymphs was 6.35 ± 0.22 days, the same as that for the small nymphs, 6.87 ± 0.30 days ($P = 0.185$). More of the large nymphs survived (75 %) compared to the small nymphs (53 %).

The large nymphs were able to consume and assimilate nutrients at a significantly faster rate than the small nymphs (Table 6.8, 6.9). However, the small nymphs were significantly more efficient at converting ingested material to biomass (Table 6.8, 6.9). This resulted in there being no significant difference for growth, intake, assimilation and digestibility when body weight was accounted for (Table 6.8, 6.9). There was no difference in the digestibility rate ($F_{1, 16} = 12.560$, $P = 0.129$). Because of the small sample size there was a risk of a Type II error for the final dry weight, consumption, assimilation and efficiency of converting intake and assimilates to biomass values obtained for the two different body sizes (Table 6.10).

DISCUSSION

No differences in digestive capacity were recorded between nymphs reared on Button grass or Mitchell grass. However, the nymphs reared on Mitchell grass had a significantly larger head than those reared on wheat. Relative head size correlated positively with the energy required to fracture the three grasses (*unpub. data*; B Davies *pers. comm.*). Nymphs reared on Button grass and Mitchell grass had a smaller body due to nutrient limitations in the diets but nymphs consuming Mitchell grass maintained a larger head. These nymphs with relatively bigger heads were able to consume more Mitchell grass relative to their body size and at a faster rate, resulting in significantly more protein being ingested than for nymphs with a smaller head. However, this did not result in more nutrients being assimilated and thus there was no difference in growth. Relative increases in head size may be a neutral consequence of diet acting on the jaw muscles but it is unlikely that increases in musculature does not have a positive effect on food processing and therefore it appears to be an adaptive process of allocation.

Large heads, relative to body mass, are associated with feeding on 'tougher' diets (Bernays 1986; Bernays and Hamai 1987; Thompson 1992) which is correlated with an increase in mandible size (Thompson 1992) and cross-sectional area of mandibular muscle mass (Bernays and Hamai 1987). This increase in head size was rapid, with nymphs adjusting their head size after one instar. Previous research has been unable to determine if the increase in relative head size was due to either increased resistance encountered when excising leaf fragments and/or higher processing rates (Bernays 1986; Thompson 1992). In this study less Mitchell grass was consumed by the nymphs reared on wheat compared to those reared on Mitchell grass. Results from Chapter 5 suggests that, as there was no difference in actual head size of nymphs reared on wheat and Mitchell grass that the same amount of processing occurs per unit weight consumption. Upon moulting to the Adult stage, the head of nymphs reared on wheat and then fed Mitchell grass had increased to the same relative size suggesting that plant leaf toughness maybe driving the increase in head size. However, there is evidence to suggest that larger heads may increase intake rate (results this chapter and Chapter 5). While larger head sizes may not increase the amount of nutrients consumed, as this is limited by gut capacity, it may decrease the

time spent leaf cutting which can decrease the risk of predation (Mueller and Dearing 1994). Also, larger heads may increase feeding niche breadth, by enabling the excising of leaf pieces of 'tougher' leaf species (Braby 1994; Nakasuji 1987). As noted in Chapter 5, Instar II nymphs appeared to have the most trouble initiating fracture across the vascular bundles of Mitchell grass. For a lot of insects, where plant toughness increases with time they may need to divert resources from body growth to head growth, which may impact on insect fitness.

The results obtained for Australian plague locusts reared on wheat and then fed either treatment diet differed from those in Chapter 4. Previously, Instar V nymphs feeding on Mitchell grass, had a longer instar duration with lower survival, consumed and assimilated significantly less resulting in lower growth than for nymphs consuming Button grass, although the efficiency with which both diets were converted to biomass was the same. In this experiment, the duration of Instar V was the same regardless of rearing or treatment diet with higher survival of nymphs feeding on Mitchell grass. Although significantly more Button grass was assimilated, the superior growth rate of nymphs fed Mitchell grass was due to increased efficiency of converting digested material to body mass.

Nutrient intake may be increased when there is an imbalance of one nutrient with respect to another (Raubenheimer and Simpson 1996). However, as Button grass appeared to have a more favourable (*sensu* Simpson and Raubenheimer 1993b) ratio of protein to carbohydrate than Mitchell grass, it was predicted more Mitchell grass would have been assimilated to gain similar amounts of protein from both diets.

The pattern of grass chemistry was the same as previously recorded for both grasses in Chapter 4. However, for both grasses, the cell contents contained significantly less protein and more carbohydrates making the ratio of protein to soluble carbohydrates higher, and for Button grass the amount of water per unit dry weight was lower than previously measured. Previously it was hypothesized that the poorer performance by nymphs feeding on Mitchell grass was due to factors limiting intake. One of these factors was the lower ratio of water to dry matter found in Mitchell grass compared to Button grass. It was hypothesized that if the ratio of water to dry matter increased and/or the proportion of nutrients within the dry matter, particularly protein, decreased, Mitchell grass intake may increase due to a reduction in factors controlling

intermeal duration. The amount of water and protein was lower in the experiment reported here. However, as meal size appeared to be determined by dry weight intake (Chapter 5), to consume the same amount of protein, locusts would have consumed more water than would have been consumed by locusts feeding in the previous experiment. This suggests that water may have been a factor prolonging intermeal duration in nymphs feeding on Mitchell grass in the previous experiment.

As previously observed, there was no difference in the amount of non-cell wall material digested from either grass. However, significantly more Button grass was assimilated while no difference in consumption of the two grasses was recorded. The increase in Button grass assimilated appears to be because it was slightly more digestible and the non-significant result appears to be due to the small sample size (9) of the Button grass reared nymphs.

Nymphs consuming Mitchell grass were more efficient at converting assimilated dry matter to biomass than those consuming Button grass. However, the reasons for this are unclear. Increased efficiency is due to either reduced processing costs or reduced costs with general living not associated with feeding. As the amount of dry matter consumed was the same for both grasses, previous data would suggest that the same amount of processing would occur. Therefore, it is unlikely that the lower costs are due to reduced biting and chewing. While nymphs consuming Mitchell grass had a slightly shorter instar duration it would be difficult to attribute this to the increased efficiency.

Body size affected the rate of nutrient consumption and assimilation, and the efficiency with which nutrients were converted to biomass. Wheat-reared nymphs were almost 40% heavier than those reared on the treatment diets, but they did not differ in the pattern of allocation to the factors that influence their digestive capacity, except for the heads of Mitchell grass-reared nymphs, as already discussed. Large Instar V nymphs feeding on Mitchell grass consumed and assimilated relatively more nutrients and although they were less efficient at converting them to biomass, they were able to do this at a faster rate resulting in more growth compared to the small Instar V nymphs. However, the larger wheat-reared nymphs consumed relatively less than their counterparts reared on the treatment diets. As relative gut sizes of nymphs did not differ with treatment, data from Chapter 5 suggests that all nymphs would

have consumed the same dry matter sized meals. However, the larger wheat-reared nymphs consumed significantly less. This suggests that the intermeal duration of these larger nymphs would have been longer. Corresponding with the pattern observed between the large and small Instar V nymphs, the larger wheat reared nymphs converted nutrients to biomass less efficiently than the smaller nymphs reared on the treatment diets.

Larger nymphs had significantly lower water per unit body weight regardless of rearing treatment which may have led to increased haemolymph nutrient concentration, or alternatively, there maybe some initial reluctance to consume a novel diet which could also explain this pattern (Grabstein and Scriber 1982). It was predicted that compared to their larger counterparts the smaller nymphs would have a relatively higher intake rate. This was observed with the different sized wheat-reared nymphs switched to Mitchell grass but not when comparing nymphs reared on the treatment diet and wheat.

The treatment diets had a far greater effect than body size on dietary utilization. Body size may indirectly affect digestion. All organisms have enzymes that function optimally within a narrow temperature range. Australian plague locust growth is maximized and instar duration is minimized when body temperature is between 35-40°C (Gregg 1983, Hunter 1983). Models predict that the larger an insect is, the greater the temperature homeostasis (May 1985), which field data supports for the Australian plague locust (Gregg 1981). Laboratory studies have found that growth rate was a function of the thermal conditions experienced by the caterpillar (Stamp 1990). Maximal growth rates of insects reared at their optimal temperature appears to be mostly due to optimal enzyme function not temperature effects on food processing or metabolic rate (Lindroth *et al.* 1997; Reynolds and Nottingham 1985; Stamp 1990).

Insects exploiting environments that only provide conditions suitable for growth for a short period of time need to be able to maximize growth rate. Studies have shown that thermoregulation allows *Taeniopoda eques* to successfully develop in southern Arizona deserts. By increasing its body temperature an average of 5°C per day, *T. eques* is able to make up the 158 degree-day deficit between the 850 degree-days it requires to complete its lifecycle and the average 692 degree-days provided by the

environment between rain-induced hatching and death from frost (Whitman 1986; Whitman 1988). Australian plague locusts also need to maximize development to ensure they are able to reach adulthood and gain sufficient body mass to migrate before their food supply dries up, so increased body size may allow increased consumption and nutrient assimilation which results in greater growth although it would be expected that processing costs would be higher.

It is hypothesized that final body size is important to Australian plague locusts as it may be an important factor influencing migratory and reproductive capacities but the locust is also under very strong selection pressure for shorter development times (Gregg 1981). The effects of temperature and diet quality have been found to have both a direct and interactive effect on insect performance (Lindroth *et al.* 1997), therefore, very small changes in either could strongly affect fitness measures. It appears a lack of physiological adaptation to a new diet could result in reduced feeding and final growth. If an insect is forced to 'switch' diets mid-development this could also result in reduced fitness.

TABLES AND FIGURES

Table 6.1 Chemical analysis of the two treatment diets. Results (mean ± se) are averaged over the duration the locusts were fed. Number of replicates (days) = 21 for each diet.

	Button grass	**Mitchell grass**	
SLA ($m^2\ g^{-1}$)	0.042 ± 0.001	0.021 ± 0.001	$F_{1,40} = 185.237$, $P < 0.001$
Water ($g\ g^{-1}$)	3.97 ± 0.09	2.46 ± 0.31	$F_{1,40} = 22.509$, $P < 0.001$
Cell wall material (% dry matter)	48.15 ± 0.65	58.01 ± 0.89	$F_{1,40} = 80.716$, $P < 0.001$
Protein (% dry matter)	8.55 ± 0.29	8.57 ± 0.35	$F_{1,40} = 0.004$, $P = 0.952$
Non-structural carbohydrate (% dry matter)	22.74 ± 0.61	27.11 ± 0.58	$F_{1,40} = 27.225$, $P < 0.001$
Ratio protein : non-structural carbohydrates ($g\ g^{-1}$)	1 : 2.74 ± 0.13	1 : 3.32 ± 0.20	$F_{1,40} = 5.755$, $P = 0.021$

Table 6.2 Percentage survival of Instar V Australian plague locust nymphs feeding on either Button grass or Mitchell grass after being reared on either the treatment diet or wheat.

Treatment	Survival (%)
Wheat: Button grass	84.0
Wheat: Mitchell grass	80.8
Button: Button grass	76.9
Mitchell: Mitchell grass	90.9

Table 6.3 Mean instar duration (± se) of Instar V Australian plague locust nymphs feeding on either Button grass or Mitchell grass after being raised on either the treatment diet or wheat. There was no significant difference between treatments ($F_{3,65} = 0.779, P = 0.532$).

Treatment	Duration (days)
Wheat: Button grass	6.4 ± 0.2
Wheat: Mitchell grass	6.1 ± 0.2
Button: Button grass	6.4 ± 0.2
Mitchell: Mitchell grass	6.1 ± 0.1

Table 6.4 Initial fresh and dry weight of Instar V nymphs after being raised on either of the treatment diets or wheat (mean ± se). Column values with different letters are significantly different ($P < 0.05$).

Treatment	Fresh weight (mg)	Dry weight (mg)	Ratio water:dry matter ($g\ g^{-1}$)
Wheat: Button grass	110.8 ± 3.6^{a}	26.9 ± 0.9^{a}	$3.1 \pm 0.1^{a,b}$
Wheat: Mitchell grass	115.2 ± 3.8^{a}	27.9 ± 0.9^{a}	$3.1 \pm 0.1^{a,b}$
Button: Button grass	86.2 ± 4.0^{b}	18.2 ± 0.8^{b}	3.8 ± 0.1^{c}
Mitchell: Mitchell grass	86.8 ± 2.9^{b}	19.9 ± 0.7^{b}	$3.4 \pm 0.1^{b,c}$
	$F_{3,65} = 17.793$, $P < 0.001$	$F_{3,65} = 29.816$, $P < 0.001$	$F_{2,27} = 8.562$, $P = 0.002$

Table 6.5 Results of ANCOVA of performance measures of the locusts feeding on Button grass and Mitchell grass reared on either the treatment diet or wheat. *Significant interaction of the treatment x covariate term, with results given for Dunnett's test ($P < 0.05$) according to the Johnson-Neyman Technique. *Post hoc* testing of adjusted means was performed using pairwise comparisons with *P*-values corrected for multiple testing with the sequential Holm method.

Type of analysis	Source of variation	df	MS	F	P
Initial wt log gut	Treatment	2	0.045	3.521	0.044
	Log remainder of body	1	0.338	26.354	< 0.001
Interaction P =0.520	Residual	26	0.013		
Initial wt log head	Treatment	2	0.009	3.769	0.037
Interaction	Log remainder of body	1	0.059	23.401	< 0.001
P = 0.898	Residual	26	0.003		
Final wet weight	Treatment	3	1096.104	3.756	0.015
Interaction	Log init wet wt.	1	11858.9	40.633	< 0.001
P = 0.937	Residual	64	291.857		
Final dry weight	Treatment	3	166.650	8.265	< 0.001
Interaction	Log init dry wt.	1	720.674	35.741	< 0.001
P = 0.157	Residual	64	20.164		
Final wt. log gut	Treatment	3	0.011	0.791	0.503
Interaction	Log remainder of body.	1	0.066	4.646	0.035
P = 0.539	Residual	64	0.014		
Final wt. log head	Treatment	3	0.002	2.009	0.122
Interaction	Log remainder of body	1	0.136	109.840	<0.001
P = 0.072	Residual	64	0.001		

Type of analysis	Source of variation	df	MS	F	P
Consumption: total fresh matter					
Interaction	Treatment	3	1.230×10^6	23.056	< 0.001
P =0.546	Initial dry weight	1	6.009×10^5	11.261	0.001
	Residual	64	5.336×10^4		
Consumption: total dry matter					
Interaction	Treatment	3	7404.675	3.654	0.017
P =0.060	Initial dry weight	1	22236.4	10.973	0.002
	Residual	64	2026.487		
Consumption: water					
Interaction	Treatment	3	1.178×10^6	28.918	< 0.001
P =0.756	Initial dry weight	1	3.919×10^5	9.618	0.003
	Residual	64	4.075×10^4		
Consumption: non-cell wall material					
Interaction	Treatment	3	6033.766	14.012	< 0.001
P =0.251	Initial dry weight	1	2573.800	5.977	0.017
	Residual	64	430.619		
Consumption: protein					
Interaction	Treatment	3	52.702	2.991	0.037
P =0.250	Initial dry weight	1	45.600	2.588	0.113
	Residual	64	17.620		
Consumption: non-structural carbohydrates					
*Interaction	Results of Wilcox procedure				
P =0.035	bbg vs mmg	Lines not significantly different over data range			
	bbg vs wbg	Lines significantly different			
	bbg vs wmg	Lines not significantly different over data range			
	mmg vs wbg	Lines not significantly different over data range			
	mmg vs wmg	Lines not significantly different over data range			
	wbg vs wmg	Lines not significantly different over data range			

Type of analysis	Source of variation	df	MS	F	P
Assimilation					
Interaction	Treatment	3	2342.901	5.706	0.002
P =0.616	Initial dry weight	1	301.050	0.733	0.395
	Residual	64	410.632		
Frass (AD)					
Interaction	Treatment	3	1904.523	7.616	< 0.001
P =0.208	Consumption	1	8.233×10^4	329.247	< 0.001
	Residual	64	250.066		
Non-cell wall frass (AD)					
Interaction	Treatment	3	468.404	1.110	0.351
P =0.291	Non-C.W. consumption	1	3273.866	7.761	0.007
	Residual	64	421.811		
Fresh weight growth (ECI)					
Interaction	Treatment	3	816.949	2.796	0.047
P =0.331	Wet weight consumption	1	110.662	0.379	0.540
	Residual	64	292.191		
Dry weight growth (ECI)					
Interaction	Treatment	3	221.402	12.387	< 0.001
P =0.320	Consumption	1	149.561	8.370	0.005
	Residual	64	17.873		
Dry weight growth (ECD)					
Interaction	Treatment	3	218.013	10.861	< 0.001
P =0.686	Assimilation	1	8.782	0.437	0.511
	Residual	64	20.073		

Type of analysis	Source of variation	df	MS	F	P
Growth rate					
Interaction	Treatment	3	214.509	7.214	< 0.001
P =0.677	Duration	1	98.055	3.298	0.074
	Residual	64	29.733		
Consumption rate					
Interaction	Treatment	3	2003.083	4.883	0.004
P =0.191	Duration	1	327.182	0.798	0.375
	Residual	64	410.224		
Assimilation rate					
Interaction	Treatment	3	1458.536	0.649	0.586
P =0.924	Duration	1	8109.851	3.609	0.062
	Residual	64	2247.214		

Table 6.6 Chemical analysis of the Mitchell grass diet (mean ± se) offered to the two different sized Instar V nymphs ($n = 9$).

	Large	Small	
Water (g g^{-1} dry weight)	2.35 ± 0.02	2.34 ± 0.02	$F_{1,16} = 0.008$, $P = 0.929$
Cell wall material (% dry matter)	55.6 ± 0.6	55.7 ± 0.6	$F_{1,16} = 0.006$, $P = 0.939$
Protein (% dry matter)	9.6 ± 0.2	9.8 ± 0.2	$F_{1,16} = 0.528$, $P = 0.478$
Non-structural carbohydrates (% dry matter)	23.6 ± 0.6	23.7 ± 0.7	$F_{1,16} = 0.002$, $P = 0.966$

Table 6.7 Initial fresh and dry weight of Instar V nymphs.

Treatment	Fresh weight (mg)	Dry weight (mg)	Ratio water:dry matter ($g\ g^{-1}$)
Large	128.49 ± 1.68	30.53 ± 0.40	5.44 ± 0.24
Small	81.38 ± 2.10	19.33 ± 0.50	6.32 ± 0.19
	$F_{1,13}$ = 227.650 P < 0.001	$F_{1,13}$ = 227.650 P < 0.001	$F_{1,13}$ = 7.953 P 0.012

Table 6.8 ANCOVA-adjusted means for the locust performance parameters for large and small Instar V nymphs consuming Mitchell grass. * significantly different ($P < 0.05$) (statistics given in Table 6.9).

	Large	Small	
Wet weight final	201.16 ± 16.39	136.01 ± 16.39	
Dry weight final	49.06 ± 4.79	29.31 ± 4.79	
Consumption: wet weight	875.56 ± 141.42	589.99 ± 141.42	
Consumption: dry weight	278.63 ± 46.62	179.97 ± 46.62	
Assimilation	42.99 ± 7.58	27.42 ±7.58	
Frass (AD)	184.42 ± 4.09	183.18 ± 4.09	
Non-cell wall frass (AD)	56.52 ± 4.13	53.71 ± 4.13	
Growth (ECI)	11.66 ± 126	16.85 ± 1.26	*
Growth (ECD)	11.85 ± 1.61	16.63 ± 1.61	
Growth rate	14.65 ± 1.64	13.85 ± 1.64	
Intake rate	261.21 ± 14.26	197.38 ± 14.26	*
Assimilation rate	40.22 ± 2.12	30.18 ± 2.12	*

Table 6.9 Results of ANCOVA of performance measures of the large and small Instar V locusts feeding on Mitchell grass. *Significant interaction of the treatment x covariate term, with results given for Dunnett's test ($P < 0.05$) according to the Johnson-Neyman Technique.

Type of analysis	Source of variation	df	MS	F	P
Initial wt gut	Treatment	1	0.589	0.415	0.529
Interaction	Remainder of body	1	2.344	1.650	0.217
P =0.482	Residual	16	1.420		
Initial wt head	Treatment	1	0.014	0.064	0.803
Interaction	Remainder of body	1	1.754	7.871	0.013
P = 0.919	Residual	16	0.223		
Final wet weight	Treatment	1	947.576	4.148	0.060
Interaction	Initial wet wt.	1	23.828	0.104	0.751
P = 0.079	Residual	15	228.435		
Final dry weight	Treatment	1	81.009	4.445	0.052
Interaction	Initial dry wt.	1	15.473	0.849	0.371
P = 0.110	Residual	15	18.226		
Final wt. gut	Treatment	1	3.790	3.094	0.099
Interaction	Remainder of body.	1	0.273	0.223	0.644
P = 0.089	Residual	15	1.225		
Final wt. head	Treatment	1	0.162	1.280	0.276
Interaction	Remainder of body	1	4.172	32.983	< 0.001
P = 0.262	Residual	15	0.126		

Type of analysis	Source of variation	df	MS	F	P
Consumption: total fresh matter					
Interaction	Treatment	1	1.692×10^4	1.066	0.318
$P = 0.120$	Initial dry weight	1	2308.204	0.145	0.708
	Residual	15	1.587×10^4		
Consumption: total dry matter					
Interaction	Treatment	1	2020.575	1.171	0.296
$P = 0.113$	Initial dry weight	1	306.573	0.178	0.679
	Residual	15	1725.076		
Assimilation					
Interaction	Treatment	1	50.338	1.103	0.310
$P = 0.137$	Initial dry weight	1	3.098	0.068	0.798
	Residual	15	45.646		
Frass (AD)					
Interaction	Treatment	1	4.250	0.037	0.849
$P = 0.702$	Consumption	1	1.642×10^4	144.288	< 0.001
	Residual	15	113.766		
Non-cell wall frass (AD)					
Interaction	Treatment	1	20.022	0.181	0.677
$P = 0.844$	Non-C.W. consumption	1	983.075	8.888	0.009
	Residual	15	110.610		
Dry weight growth (ECI)					
*Interaction	Results of Wilcox procedure: Lines significantly different between -0.487				
$P = 0.030$	and 22.94, i.e. the lines are significantly different for the data range				
Dry weight growth (ECD)					
Interaction	Treatment	1	54.173	3.376	0.086
$P = 0.077$	Assimilated	1	119.700	7.460	0.015
	Residual	15	16.046		

Type of analysis	Source of variation	df	MS	F	P
Growth rate					
Interaction	Treatment	1	2.587	0.1131	0.741
P =0.780	Duration	1	17.126	0.749	0.400
	Residual	15	22.883		
Consumption rate					
Interaction	Treatment	1	1.637×10^4	9.475	0.008
P =0.480	Duration	1	273.520	0.158	0.696
	Residual	15	1727.279		
Assimilation rate					
Interaction	Treatment	1	404.758	10.621	0.005
P =0.944	Duration	1	116.155	3.048	0.101
	Residual	15	38.109		

Table 6.10 Results of power analysis for selected performance parameters, α =0.05. A two-sample z-test uses the means and standard deviations derived from the ANCOVA.

Parameter	Combined sample size required to have 0.80 power of detecting a difference
Final dry weight	18
Consumption	64
Assimilation	58
Frass (AD)	2942
Growth (ECD)	32

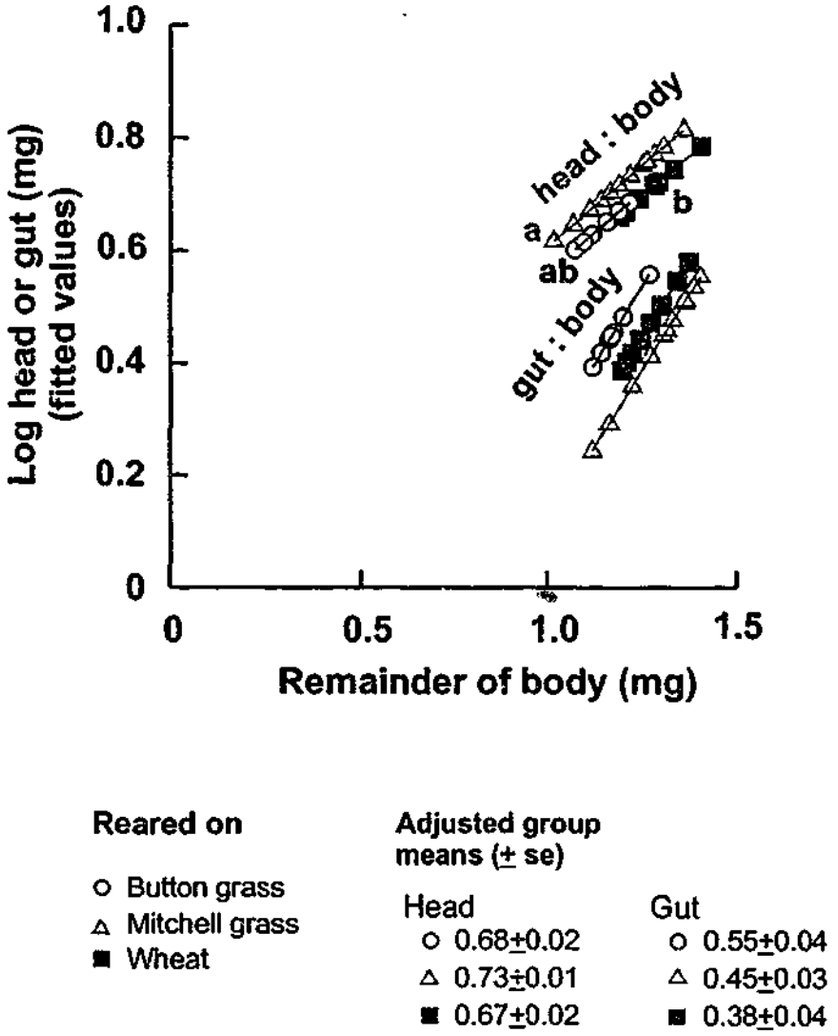


Fig. 6.1 ANCOVA-adjusted size of head and gut relative to the rest of the body of freshly moulted Instar V (timed to within 4 h) nymphs raised on the three treatment diets. For the head mass, lines with different letters are significantly different ($P < 0.05$).

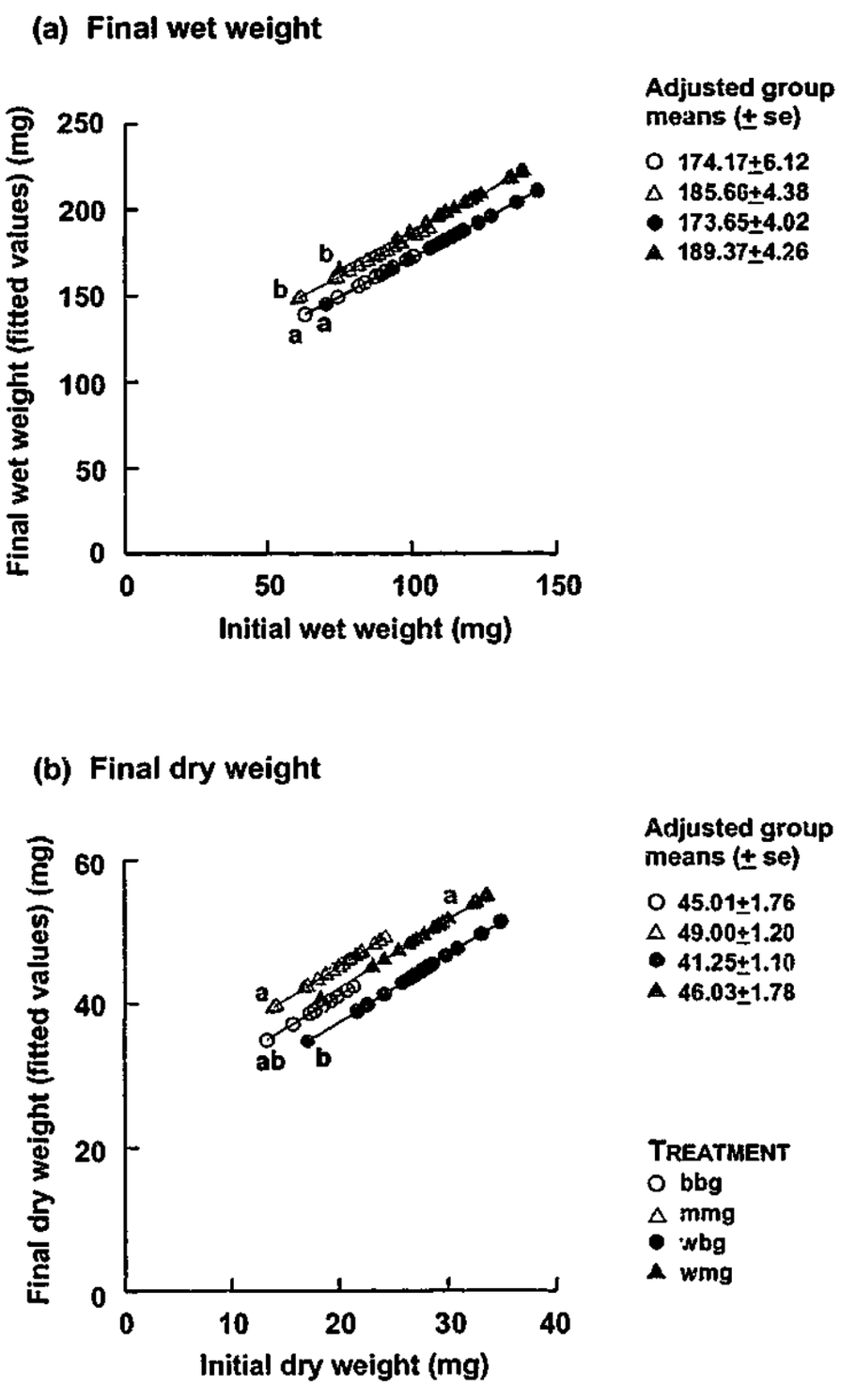
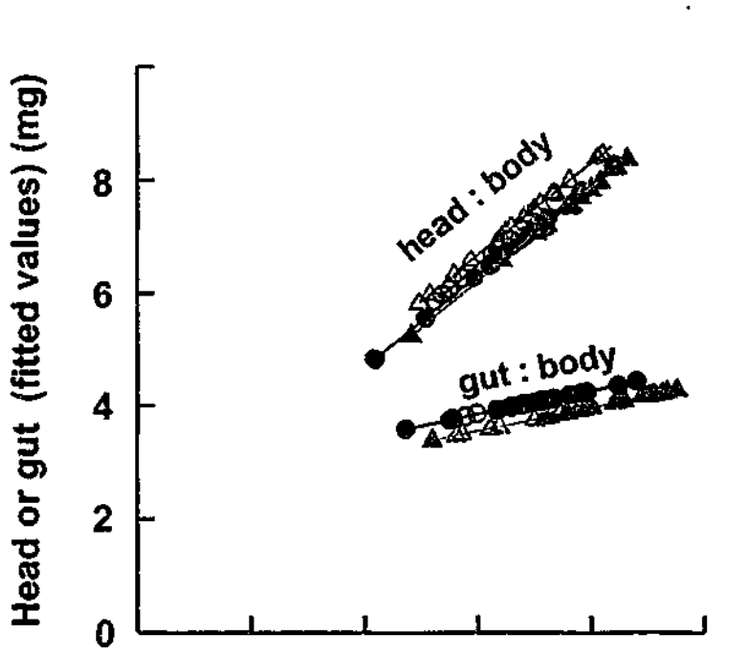


Fig. 6.2 ANCOVA-adjusted (initial weight) (a) final fresh weight and (b) final dry weight for each treatment. Lines with different letters are significantly different ($P < 0.05$).



TREATMENT	Adjusted group means (± se)	
	Head	Gut
○ bbg	○ 0.85±0.01	○ 0.63±0.04
△ mmg	△ 0.87±0.01	△ 0.58±0.03
● wbg	● 0.85±0.01	● 0.61±0.03
▲ wmg	▲ 0.84±0.01	▲ 0.56±0.03

Fig. 6.3 Ratio of head and gut to the remainder of the body of the locusts at the completion of Instar V for the four treatments. Lines with different letters are significantly different ($P < 0.05$).

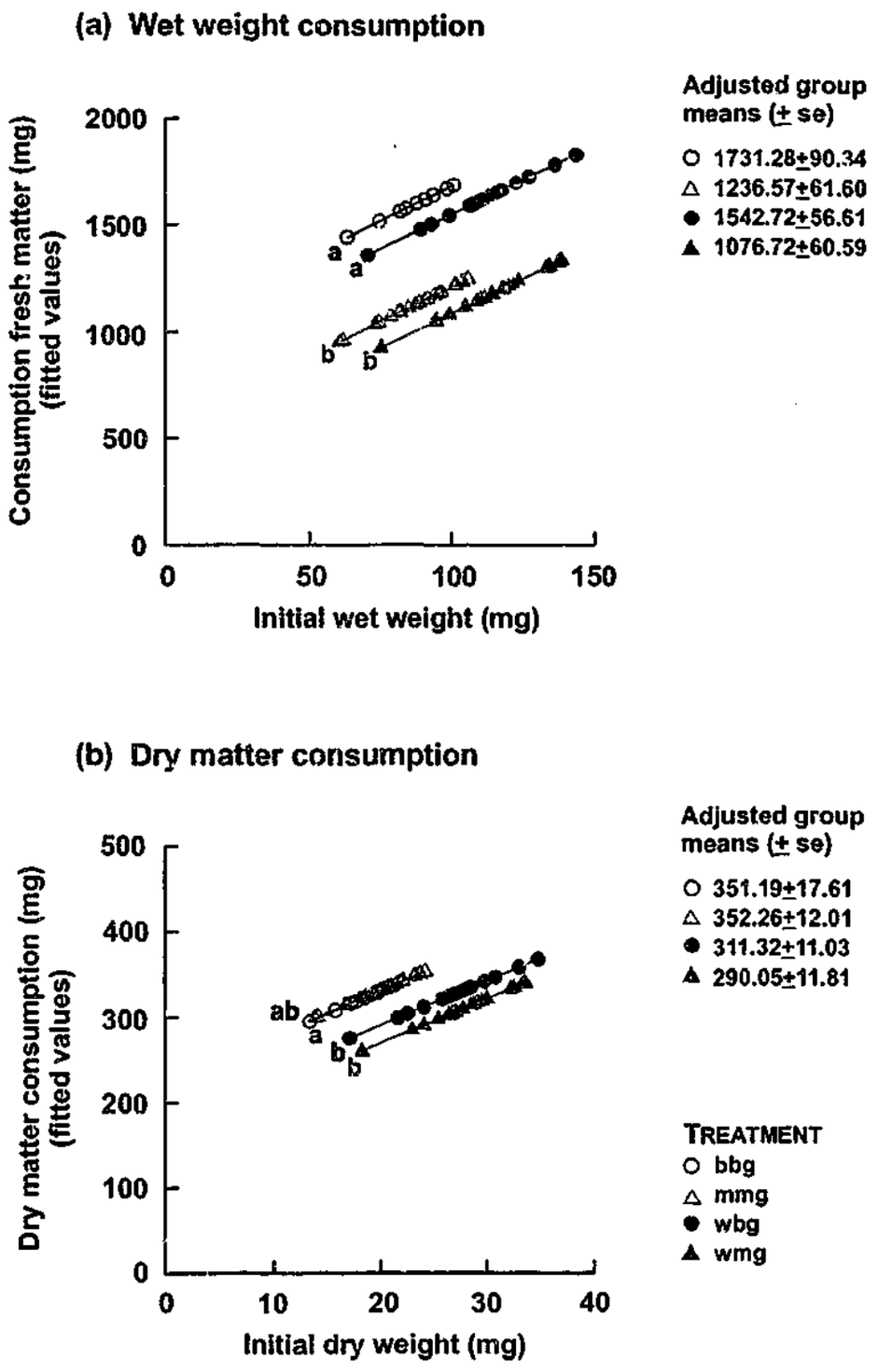


Fig. 6.4 ANCOVA adjusted (initial weight) total consumption of (a) fresh matter and (b) dry matter for each treatment. Lines with different letters are significantly different ($P < 0.05$).

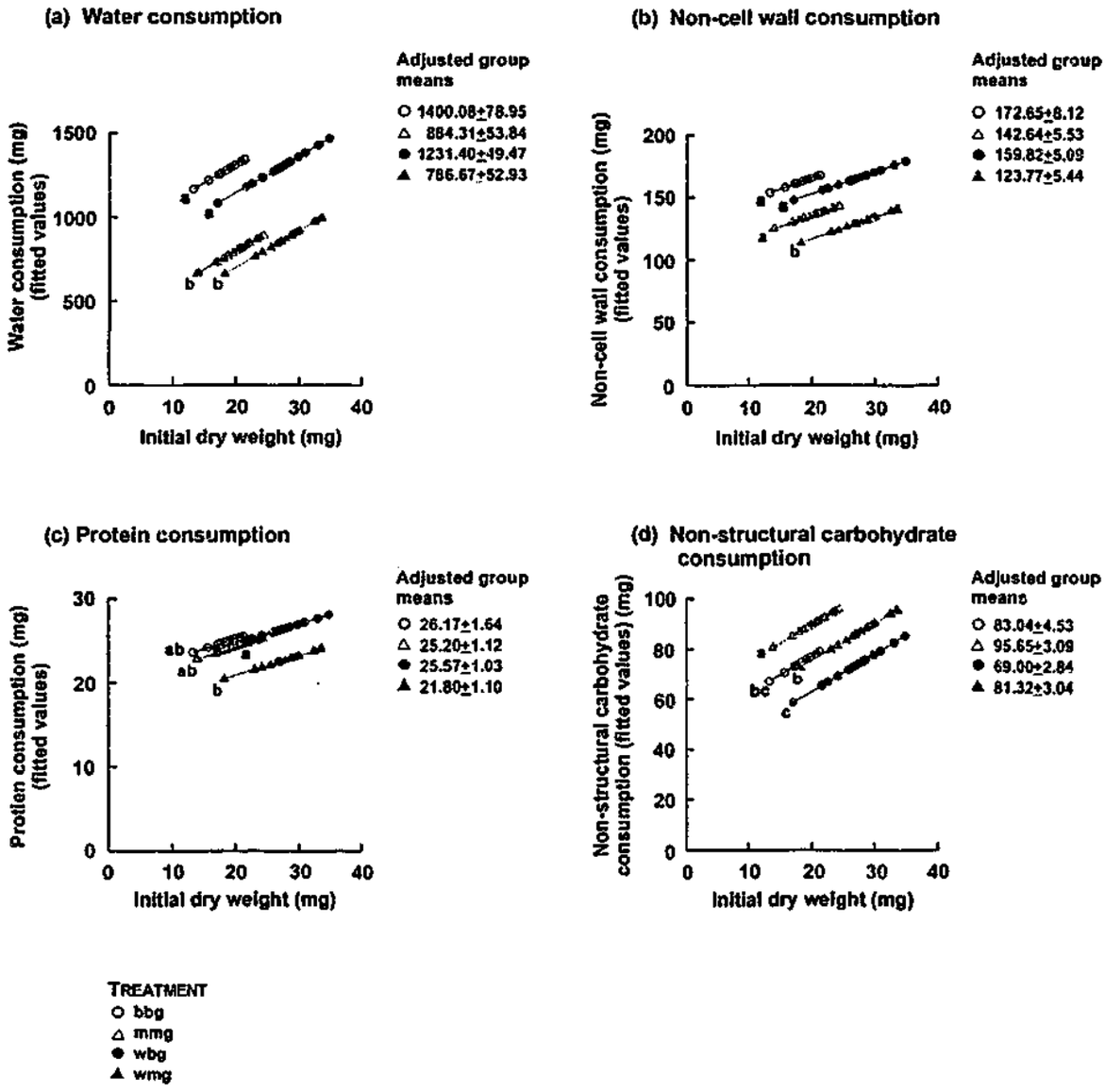


Fig. 6.5 ANCOVA adjusted (initial dry weight) total consumption of (a) water, (b) non-cell wall material, (c) protein and (d) non-structural carbohydrate for each treatment. Lines with different letters are significantly different ($P < 0.05$).

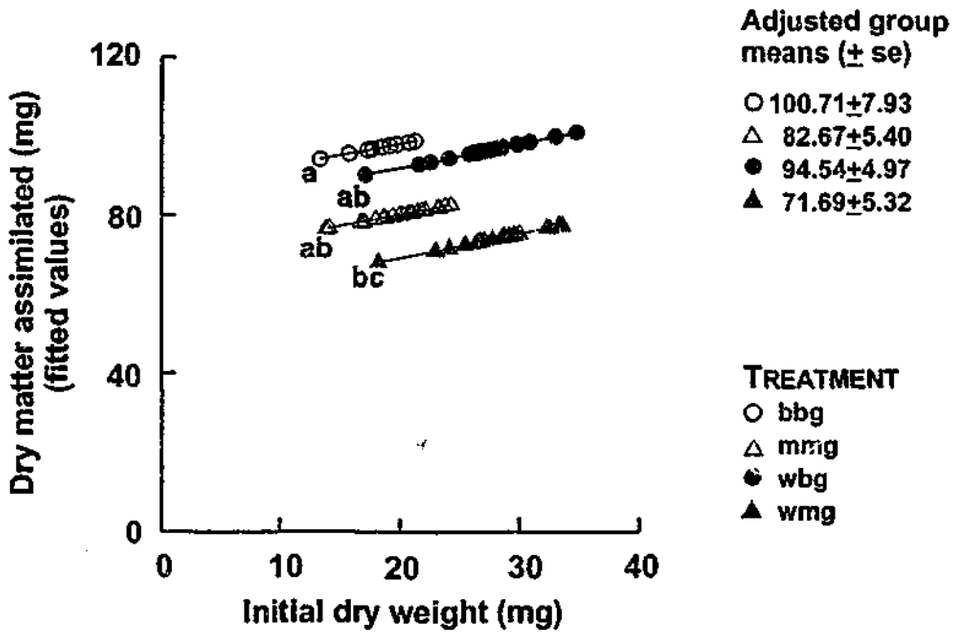


Fig. 6.6 ANCOVA adjusted (initial dry weight) dry matter assimilation (diet) for each treatment. Lines with different letters are significantly different ($P < 0.05$).

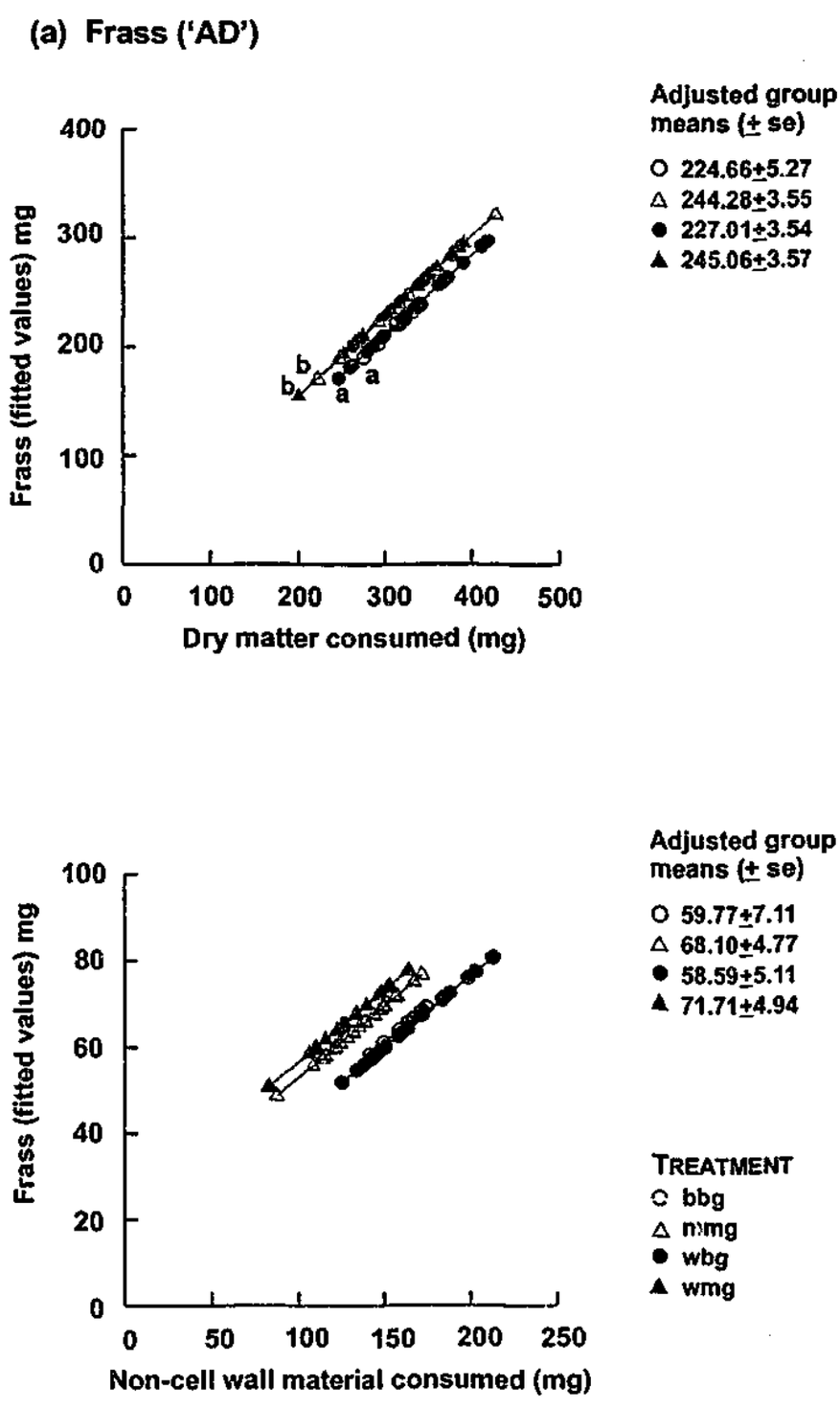


Fig. 6.7 'Utilization plot' of ANCOVA of (a) frass against dry matter consumption and (b) non-cell wall frass against non-cell wall consumption for each treatment. Lines with different letters are significantly different ($P < 0.05$).

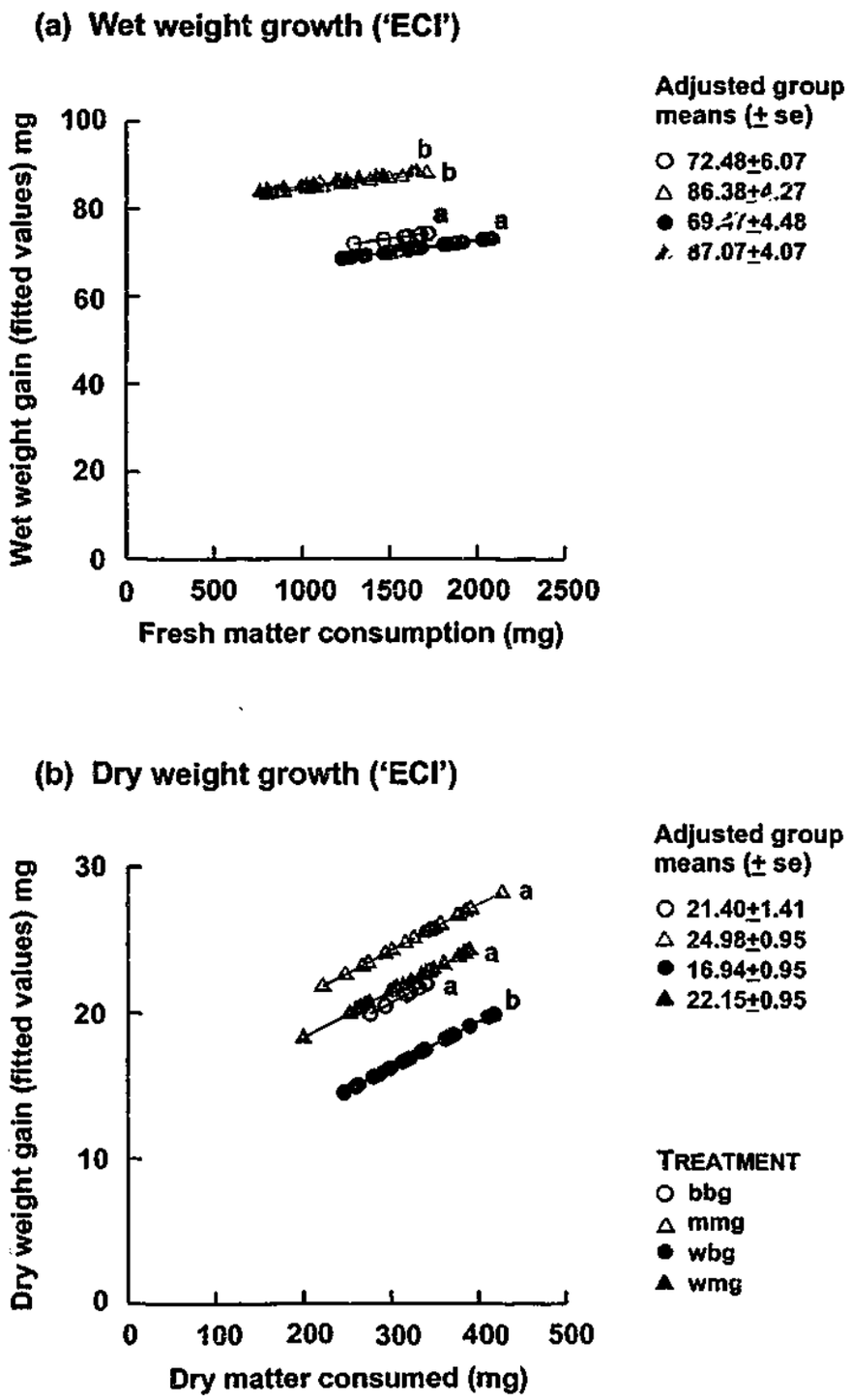


Fig. 6.8 'Utilization plot' of ANCOVA of (a) wet weight gain against fresh weight consumption and (b) dry weight gain against dry matter consumption for each treatment. Lines with different letters are significantly different ($P < 0.05$).

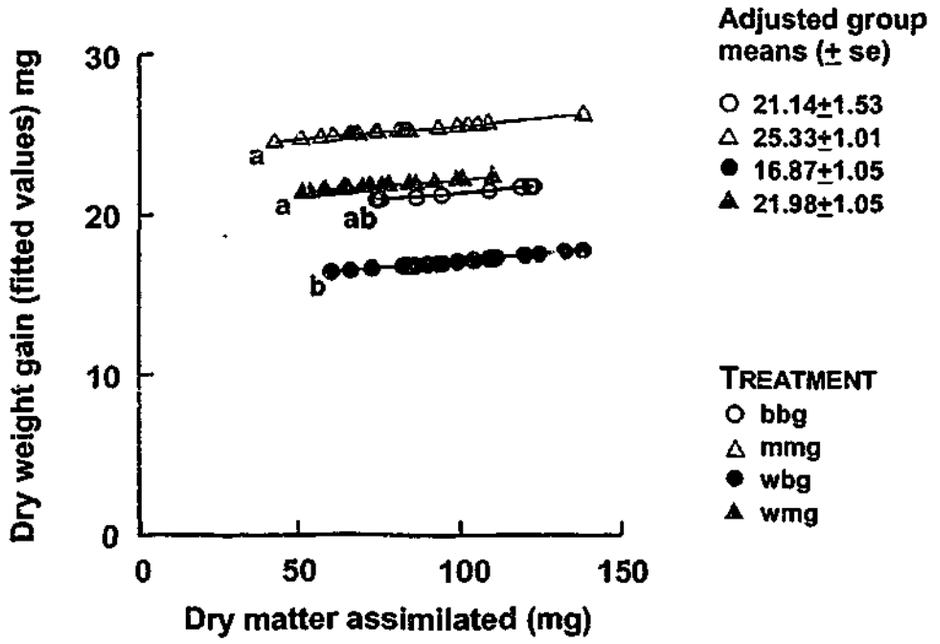


Fig. 6.9 'Utilization plot' of ANCOVA of dry weight gain against dry matter assimilation for each treatment ('ECD'). Lines with different letters are significantly different ($P < 0.05$).

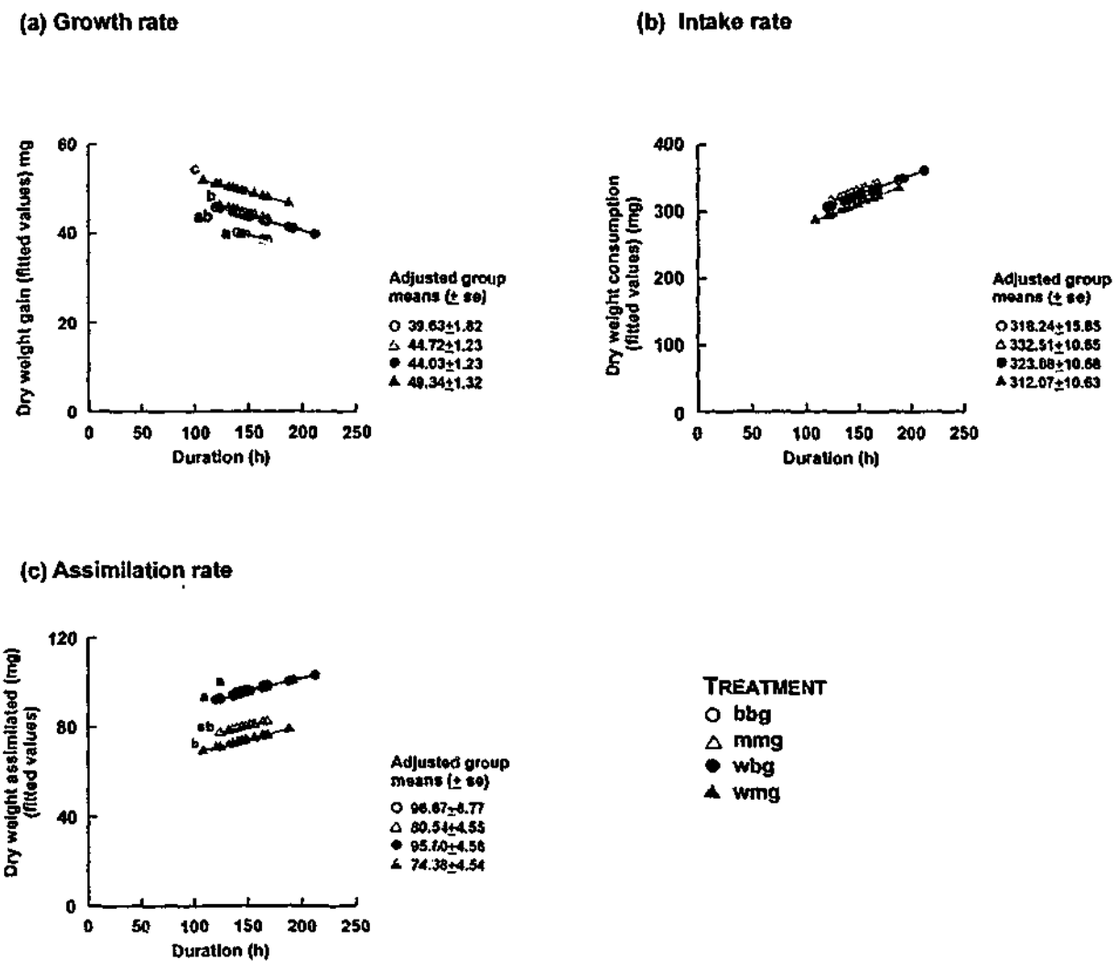


Fig. 6.10 Rate of (a) growth, (b) consumption and, (c) assimilation for nymphs feeding on either Button grass or Mitchell grass reared on either the treatment diets or wheat. Lines with different letters are significantly different ($P < 0.05$).

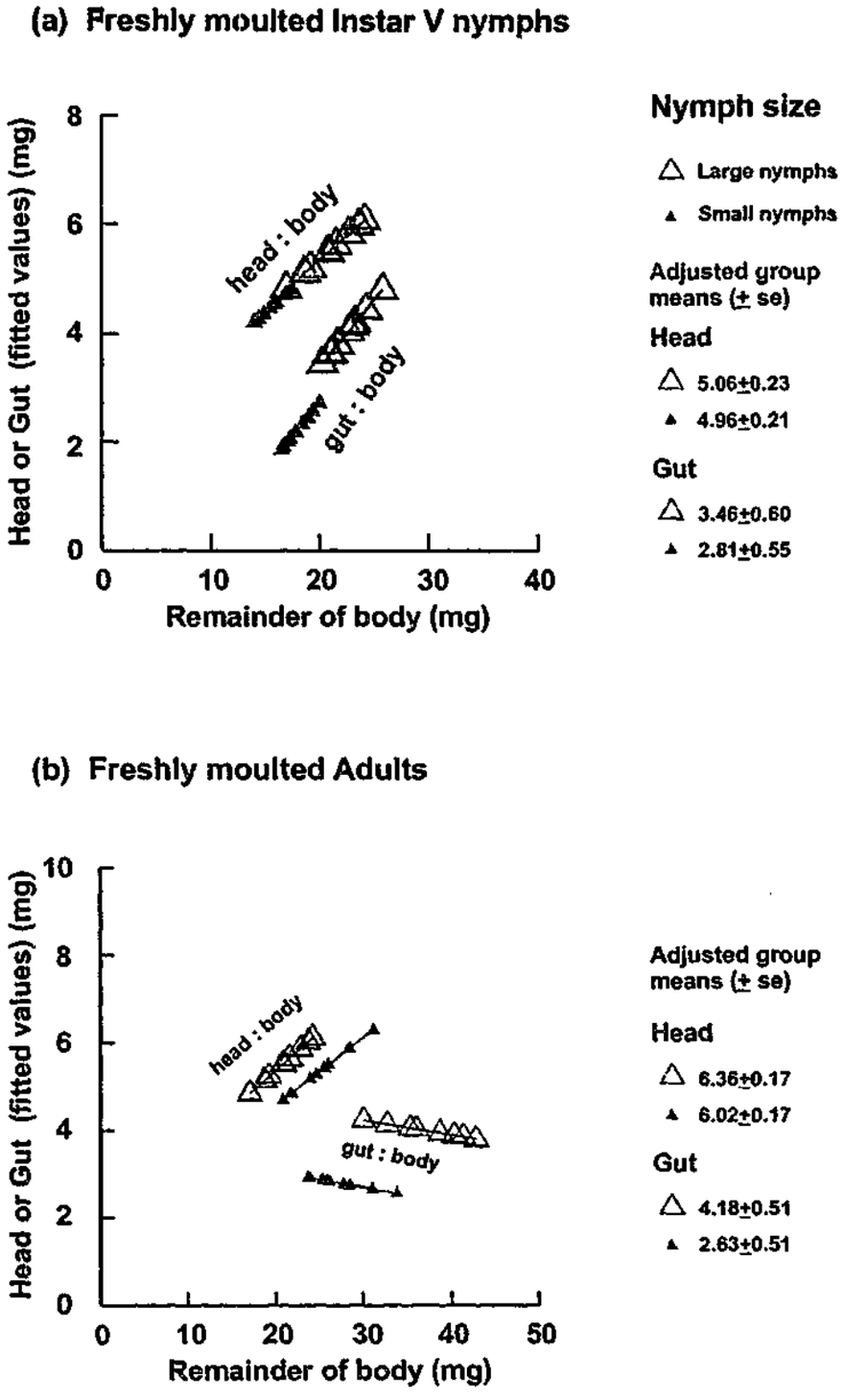


Fig. 6.11 Ratio of head and gut to the remainder of the body of the locusts (a) at the commencement of Instar V and (b) at the completion of Instar V for the large and small nymphs. Lines were not different significantly different ($P < 0.05$).

CHAPTER 7. THE EFFECT OF GRASS ON LOCUST GROWTH

SUMMARY

1. The effect of plant structure on locust perform ar
   it was observed that the two grasses differed i n t
   higher SLA with more water per unit dry mat er
   hypothesized that Mitchell grass may have be en
   prevented nutrient assimilation at the same ra :e i

2. When water was provided *ad lib.* dry matter a ssi
   nutrients were digested and at an increased ra :e.
   difference in growth or instar duration, althou gh
   significantly increased.

3. Removal of the need for the locusts to mastic ate
   diet, significantly increased the amount of dr / m
   significantly decreased the costs associated w ith
   More dry matter was assimilated because the gr
   digestible and significantly more was consum ed

4. Nymphs fed the powdered diet grew at almo t tv
   whole fresh diet (water provided *ad lib.*), co sur
   assimilated dry matter twice as quickly.

5. Ground Mitchell grass was significantly m re c
   grass, and this was not due to differences ir am
   both diets, equal amounts were consumed a nd a
   powdered Button grass ingested significant y m
   difference in the final dry weight of nymph s fe
   nymphs feeding on Button grass had signif can
   wet weight. Therefore, plant structure has he p
   capacity of a locust to access nutrients.

nts assimilated minus the nutrients 'lost' as costs in
unt of nutrients assim lated is the product of
ency. Diet chemistry l argely affects the amount of
is evidence to sugges ingestive processing also
Bennack 1981; Boys 1 981; Chapman 1964; Raupp
th (and associated mus culature) can limit the rate at
mammals occurred wh n consuming diets with
properties (Bezzobs and Sanson 1997). The effect of
een directly investigat d in orthopterans.

ructure on dietary util zation by locusts was
on grass and Mitchell grass differ in their structure.
r unit dry matter, signi icantly larger surface area
ss, although within th dry matter fraction the
soluble carbohydrate vary very little (Chapter 4).
es the amount of wate r a plant can contain. Dietary
tin and Van't Hof 198), but it also regulates
pson 1987; Ben Halir a *et al.* 1983; Bernays and
; Raubenheimer and C ade 1994; Roessingh *et al.*
sts (Paul *et al.* 1992; Scriber 1977; Slansky Jr. and
88; Van'T Hof and Ma rtin 1989). Orthopterans
by mechanically frac uring the cell wall with the
wn about how the diff erent morphologies of
ure, reduced nutrient a ssimilation has been recorded
ns of mandible morph ology was fed to a
ay be due to inadequa e processing by worn
cause the diet is struct ured in such a way that it
mandibles (Bernays 1 991; Caswell and Reed 1976;

determined not only b y the amount of nutrients
sing efficiency. Proc ssing leaf material has been
ive. For example, the metabolic rate of the leaf-

cutting ant was 31 times higher when leaf cutting (Roces and Lighton 1995) and a locust when feeding on an artificial diet increased its' respiration rate 3-4 fold over that at resting or walking (Gouveia *et al.* 2000). Grasshoppers increased the force used to fracture food in response to the incompressibility of the diet (Seath 1977), which suggests that there will be an increased metabolic cost. Metabolic costs will be increased with the amount of chewing to reduce particle size to facilitate nutrient release. For example, *Manduca sexta* fed for significantly longer on tobacco than on an artificial diet to consume similar amounts of dry matter (Reynolds *et al.* 1986). Chewing took up about 30% of the meal time for *Locusta* feeding on seedling wheat (Simpson *et al.* 1988b) and Bernays (1991) reported that a grasshopper consuming a very tough rainforest palm chewed up to 20 times between successive bites.

Mitchell grass was significantly tougher than Button grass (Chapter 5). However, there were no differences in the efficiency with which assimilated dry matter was converted to locust biomass recorded for Instar V nymphs (Chapter 4). Nymphs feeding on Mitchell grass had reduced performance that appeared to be due to reduced consumption rather than differences in post-ingestive processing. Disregarding the cell wall (indigestible) component, Instar V nymphs feeding on these two grasses digested the remaining fraction equally. It was hypothesized that the reduced consumption of Mitchell grass was due to an increased intermeal duration resulting from increased concentration of nutrients in the haemolymph or slower leakage of nutrients from Mitchell grass, or a combination of both. When Mitchell grass with a reduced protein concentration was fed to the nymphs, consumption was the same as that of Button grass, suggesting that the ratio of protein and water may be regulating intake (Chapter 6).

The aim of this study was to investigate the effect of plant structure on locust nutrient assimilation and subsequent growth. The locusts were allowed to regulate water independently of diet by the provision of additional water with the Mitchell grass diet, and the effect of processing the diets was removed by grinding both grasses.

MATERIALS AND METHODS

Experimental animals

Locust stocks were reared as described in Chapter 2. The experiments described here followed those described in Chapter 6 and the colony now consisted of approximately 90% third generation field-caught grasshoppers (Griffith District, NSW) with the remainder from the long-term colony maintained at Monash University.

Experimental design

In this experiment, 15 locusts reared on wheat were randomly allocated to each of the following treatments within 4h of moulting to Instar V:

Fresh diet (either Button grass or Mitchell grass)

Fresh Mitchell grass + water

Dried whole Mitchell grass + water

Powdered Mitchell grass + water

The dried whole Mitchell grass treatment was included to act as a control for the powdered Mitchell grass treatment to detect whether drying the grass altered it nutritionally for the insect. However, it also introduced other problems that were unforeseen at the time and these will be discussed below.

Originally it was planned to include a powdered Button grass comparison but due to a misjudgement in the planting of Button grass there appeared to be insufficient Button grass to do this. However, on the completion of the trials there was sufficient Button grass remaining, so a final trial was performed using 15 freshly moulted Instar V nymphs fed on either powdered Button or Mitchell Grass with water provided *ad lib.*

Fresh diets were provided as previously described (Chapter 2). Four days before the commencement of the experiment, sufficient Mitchell grass for the powdered and dried Mitchell grass treatments for the entire experiment was harvested. The leaf blades were detached in the same way they would have been if offered fresh. The

sample was halved and after being freeze-dried, one half was offered to the locusts as dried whole blades and the other half was milled (Spex® freezer/mill) to a fine powder to form the powdered meal. Powdered Button grass used in the second trial was produced in the same way. The remains of the powdered Mitchell grass were used in the second trial. The dried whole blades were offered in the same manner as the fresh blades, but the water vial in the base was empty and only used to hold the blades in a similar manner to that of the fresh blades, in case this affected feeding. The powdered material was placed into a lid detached from an eppendorf tube and placed on the base of the feeding container.

Ad lib. water was offered to the locusts where stated by an inverted water-filled 10 ml vial. The locusts accessed the water through a rubber lid with a 2 mm hole enlarged slightly with a cross cut. The hole was small enough so that surface tension held the water in the vial but big enough so that the locusts could drink.

Two comparisons were made:

(1) To test the effect of extra water, fresh Mitchell grass and fresh Mitchell grass with water provided *ad lib.* were offered.

(2) To investigate the effect of plant structure, (a) fresh, dried, and powdered Mitchell grass each with water provided *ad lib.* and (b) fresh Button grass and fresh Mitchell grass, and powdered Button grass and powdered Mitchell grass with water provided *ad lib.*

Ten freshly moulted locusts were harvested randomly throughout the experiment to determine mass allocation to body parts and water content so the initial dry weight of the locusts could be estimated.

Plant analysis

The control portions of the fresh diets collected daily for the duration of the experiment were analyzed for cell wall material, protein and non-structural carbohydrates as previously described in Chapter 2. The chemical constituents of the powdered and dried whole Mitchell grass blades were analyzed from four randomly selected samples from the original fresh sample. It was assumed that since the

powdered and whole blades came from the same original sample the chemical constituents would be the same.

Data analysis

The plant chemical constituents were compared over the duration of the experiment (16 days for Button grass and 17 days for Mitchell grass) by the use of scatter plots and one-way ANOVA. One-way ANOVA was used to check if, by chance, the chemistry of the grasses offered in the different treatments varied as expected from measurements of samples collected daily (as per Chapter 4). For the powdered diets, ANOVA was performed using the four values derived from the replicates sub-sampled from the original sample.

Intake of total dry matter, water and protein was corrected as outlined in Chapter 2. ANOVA and ANCOVA were used to determine whether there was a significant effect of dietary treatment on each of the measured parameters, using initial weight or instar duration, where appropriate, as the covariate. Insect performance across the four treatments was compared using ANOVA and ANCOVA as for Chapter 4. Box plots were used to check for normality and homogeneity of variances across the treatments. Scatterplots were used to ensure that the relationship between the covariate and the dependent variable was linear for each treatment and no variables were transformed. When significant differences were found between treatments, *post hoc* testing of adjusted means was performed using pairwise comparisons with *P*-values corrected for multiple testing with the sequential Holm method using the MULTI.exe programme (version 2, written by Barry W. Brown and Kathy Russell, 1996).

RESULTS

Diet chemistry

For all parameters there was no difference between the fresh Mitchell grass offered to the nymphs without extra water and that offered with extra water (Table 7.1). As previously recorded, fresh Button grass had significantly more water and significantly less cell wall material than Mitchell grass and within the dry matter the amount of protein and non-structural carbohydrate was the same for both grasses (Table 7.1). The powdered and dried Mitchell grass diet was the same for each day and therefore there was no variation around the mean (derived for four subsamples) (Table 7.2). In terms of cell wall, protein and non-structural carbohydrates, the powdered Button grass did not differ from the powdered Mitchell grass diet (Table 7.2). While a significant difference was found between the amounts of cell wall in each grass, the difference was small (*c.* 1%) and it is highly unlikely that this will be of biological significance, rather a result of the high precision of the tests used. A dried Mitchell grass blade required over twice as much work to fracture than a fresh Mitchell grass blade (Fig. 7.1).

Locust performance

The initial weight of the nymphs used in each treatment was the same ($F_{6, 98} = 0.399$, $P = 0.878$). The instar duration was the same for all treatments (Fig. 7.2). Survival was higher on the Button grass diets than when feeding on the Mitchell grass diets (Table 7.3). Nymphs consuming Mitchell grass with added water had the lowest survival. The responses of the locusts to the various treatments were analyzed together (Table 7.4) and the results have been reported for the question of interest below.

Addition of water

Mitchell grass compared to Mitchell grass with free water provided ad. lib.

Nymphs were observed to drink the provided water, although the amount ingested could not be quantified. Addition of water with the fresh Mitchell grass blades had no effect on instar duration (Fig. 7.2) or dry and fresh weight gain (Fig. 7.3). The major chemical constituents of the Mitchell grass fed to both treatments were identical, and

as consumption was the same (Fig. 7.4) there was no difference in terms of protein, carbohydrate and cell wall ingested (Fig. 7.5). Due to increased digestibility of the non-cell wall fraction of the grass blades (Fig. 7.6) the locusts with additional water assimilated more dry matter (Fig. 7.7). The efficiency of converting both assimilate and ingesta to growth was the same for both treatments (Fig. 7.8). However, although it was not significant, the costs of converting assimilate to biomass was higher for nymphs with free water, as on both diets nymphs accumulated the same amount of dry matter and consumed equal amount of nutrients. Significantly more nutrients were assimilated when free water was provided and at a faster rate (Fig. 7.9).

Fresh Mitchell grass with added water compared to dried Mitchell grass and powdered Mitchell grass

The nymphs consuming the powdered diet gained significantly more fresh and dry weight than those consuming fresh and dried Mitchell grass with additional water (Fig. 7.3). Locusts offered the dried and powdered treatments consumed significantly more dry matter than those feeding on the fresh grass (Fig. 7.4), resulting in significantly more non-cell wall dry matter, protein and non-structural carbohydrates being ingested by these nymphs (Fig. 7.5). Although the nymphs on the dried Mitchell grass diet ingested more non-cell wall material compared to those feeding on fresh Mitchell grass, equivalent amounts of protein were ingested (Fig. 7.5a, b). Nymphs consuming the powdered diet compared to those on the other diets assimilated significantly more dry matter, while the nymphs feeding on the dried Mitchell grass assimilated significantly more than those feeding on the fresh diet treatment (Fig. 7.7). Fresh and dried Mitchell grasses were equally digestible and were significantly less digestible than the powdered diet (Fig. 7.6). Locusts consuming the powdered diet were significantly more efficient at converting assimilate and intake to growth compared to those consuming dried and fresh grass blades (Fig. 7.8). Nymphs consuming fresh Mitchell grass were able to convert both ingesta and assimilate to biomass significantly more efficiently than those consuming the dried grass blades. This resulted in the nymphs feeding on powdered Mitchell grass gaining weight significantly faster as they were able to ingest and assimilate nutrients significantly faster than nymphs feeding on the other forms of Mitchell grass (Fig. 7.9).

Fresh Button grass and Mitchell grass

Locusts consuming fresh Button grass and Mitchell grass, completed Instar V in the same time (Fig. 7.2) and gained the same amount of biomass (Fig. 7.3) as they consumed the same amount of dry matter (Fig. 7.4) and although nymphs feeding on Mitchell grass ingested significantly less non-cell wall matter and water (Table 7.1, Fig. 7.5a) both grasses were digested equally (Fig. 7.6) resulting in the same amount of dry matter being assimilated (Fig. 7.7). There was no difference in the efficiency with which the nymphs were able to convert either assimilate or ingesta to growth (Fig. 7.8).

Powdered Button grass and Mitchell grass

There was no significant difference in the duration of Instar V (Button grass: 7.8 ± 0.3 days; Mitchell grass: 8.3 ± 0.3 days, $F_{1, 28} = 1.215$, $P = 0.280$) or in the initial dry weight (Button grass, 25.0 ± 0.8 mg; Mitchell grass 25.0 ± 0.8 mg). Regardless of diet, nymphs gained the same amount of dry weight (Table 7.5), however, nymphs feeding on powdered Button grass had a higher ratio of water to dry matter, resulting in their final wet weight being significantly heavier than that of locusts consuming Mitchell grass (Table 7.5). Although powdered Mitchell grass was more digestible than powdered Button grass, even after the cell wall was accounted for (Table 7.5), there was no difference in the amount of dry matter consumed and assimilated or the efficiency with which it was converted to body mass (Table 7.5). The nymphs fed Button grass consumed more protein than those feeding on Mitchell grass (Table 7.5). There was no difference in allocation to digestive capacity between nymphs feeding on any of the diets (Fig. 7.10).

For both the trials using powdered Mitchell grass diet, the same powdered Mitchell grass was used and the initial weights of nymphs were the same ($P = 0.977$). However, in the second trial, the instar duration was significantly longer (2.10 days, $P < 0.001$) and the freshly moulted adults were significantly lighter in weight (8.23 mg, $P < 0.001$). In both trials there was no difference in the digestibility of the powdered Mitchell grass and the same amount of dry matter was assimilated. The difference in growth was due to the significantly lower efficiency of converting both intake ($P = 0.003$) and assimilates ($P < 0.001$) to biomass in the second trial.

DISCUSSION

Anatomical characteristics of the grass blades affected the amount and rate of growth by the locust. The anatomy of the grass blade determines the accessibility of nutrients *per se* and the amount of water per unit dry weight. Removal of the anatomical structure of the leaf blades by grinding resulted in increased growth through altered pre- and post-ingestive mechanisms. Increased growth resulted from increased intake and nutrient assimilation combined with decreased metabolic costs. Additional water increased nutrient assimilation, but also probably increased costs associated with converting dry matter to biomass. Grinding the diets increased the amount of non-cell wall dry matter digested, over 200% more from Mitchell grass and 167% increase for Button grass, while for nymphs provided with *ad lib.* water while feeding on fresh Mitchell grass, non-cell wall diet digestibility increased only 136 %.

Water

The addition of free water to nymphs offered fresh Mitchell grass allowed these nymphs to regulate their water intake independently of their diet, if water was lacking. While the actual amount of water ingested could not be determined, the nymphs were observed drinking. However, the addition of free water resulted in increased assimilation of the Mitchell grass diet due to increased digestibility, although growth was not increased because of increased metabolic costs. Increased diet digestibility associated with increased dietary water content was recorded for lepidopterans (Martin and Van't Hof 1988; Slansky Jr. and Wheeler 1991). Lewis and Bernays (1985) recorded significantly more weight gain by *Schistocerca gregaria* nymphs consuming the drier of the diets offered due to increased efficiency with which ingested food was converted to body mass. Timmins *et al.* (1988) found that growth and consumption for *Manduca sexta* declined along with the efficiency with which nutrients were converted to biomass when the diet contained excessive water.

The increased water intake did not result in an increase in food consumption as has been previously observed (Ben Halima *et al.* 1983; Paul *et al.* 1992; Raubenheimer and Gade 1994 and results from Chapters 4 and 5). Not all dietary water is absorbed (Baines *et al.* 1973; Dow 1981). Therefore, a better approach may have been to

include a trial with Button grass and added water, or to inject a known amount of water into the haemolymph at set time intervals. Although Button grass has more water associated with it, it was not ascertained how much of this was absorbed or passed out with the frass.

Removal of the effects of leaf structure by grinding

The locusts do not digest cell wall, and while it is not known what percentage of Mitchell grass cell contents is available, Instar V nymphs digested *c.* 44% of the cell contents. When the Mitchell grass was ground, the digestibility doubled to *c.* 90%. Removing the costs of food processing almost doubled the efficiency with which both assimilate and ingesta were converted to locust biomass. The 'traditional' ratio analysis (ANOVA on ECD) suggests that these nymphs were the most inefficient. However, when growth and assimilation were standardized (mean=1, sd=1), thus removing scale related differences, and ratio analysis applied, the pattern was the same as that generated by the ANCOVA. So although the nymphs consuming powdered Mitchell grass assimilated more dry matter and appeared to grow proportionally less this suggests that the ANCOVA results are correct. The increased utilization efficiency, together with the increased intake and digestibility of the powdered diets, resulted in the weight of the freshly moulted adults being significantly heavier. These adults were still smaller than those reared in the colony on wheat and bran, suggesting that either Button grass and Mitchell grass are nutritionally inferior compared with wheat or behaviourally induced restrictive feeding regimes invoked by the introduction to a new diet are occurring (Grabstein and Scriber 1982; Schoonhoven and Meerman 1978). Populations of field-collected adults ranged in size between both extremes encountered in the laboratory.

It is assumed that when the locusts were consuming the powdered diets they ingested the plant constituents in the ratios measured, i.e. the locusts were unable to selectively remove particles from the mixture. As for the fresh diets, the powdered diets were provided *ad lib.*, but only in sufficient quantities to ensure there was less than *c.* 5% remaining each day.

In the first of the two trials that used the powdered Mitchell grass, the final dry (and wet) biomass gain was larger and the instar duration was significantly shorter. Nymphs in the first trial consumed significantly more Mitchell grass but the amount

assimilated did not differ, rather increased growth resulted from higher efficiency of converting assimilates to biomass. The reduced efficiency recorded in the second trial resulted from a similar intake but increased instar duration (*c.* 2 days).

The exact cause of the observed differences between the two trials using powdered Mitchell grass is unknown. The experiments will be discussed separately and conclusions will be drawn regarding the two grasses in the context of the trials performed. Differences in life history parameters were also found previously for Instar V nymphs consuming Button grass or Mitchell grass (Chapter 4, Chapter 6 and this chapter). Potentially this could be due to changes to the powdered Mitchell grass due to decomposition by bacterial agents, effects of previous dietary history (Stockhoff 1992) or different selection pressures imposed by laboratory rearing conditions. The former is unlikely as the powder (which was dry) was stored in an airtight container within a desiccator during the trials, and stored at –20°C between the two trials. If the powdered grass deteriorated over time a negative relationship would be predicted in both experiments between growth and the time. However, no pattern was detected (scatterplot and regression analysis of growth, corrected for initial body weight, against time since the experiment commenced) over the time frame of either experiment (24 days and 14 days respectively). There was a strong effect of the previous diet on subsequent growth rates of instars when they were switched between diets of different nitrogen levels (Stockhoff 1992). Previous dietary history affected both the assimilation and growth efficiency. Although I attempted to standardize the conditions under which the wheat was grown, a glasshouse is subjected to the vagaries of the climatic conditions at the time and the source of the wheat seed was not constant. Wheat blades were grown from seed with only water added, if the seeds contained more nitrogen this could have altered the nymphal rearing diet. The nymphs used in the second trial involving the powdered Mitchell grass were 1–2 generations older than those used in the first trial.

The observed differences in locust performance on the powdered and whole diets did not appear to be due to any effects of drying the diet. Although, weight gain was reduced on whole dried blade diet compared to the powdered diet this appeared to be mostly due to increased metabolic costs of consuming the dried whole Mitchell grass. Even though the leaves were dry, the effect of cellular structure was still present. Equivalent digestibility of the dried blades and fresh blades (with free water) was

observed. The intake of both dried diets was equal, although due to reduced digestibility of the whole blades more powdered diet was assimilated. The increased metabolic costs could be due to the significantly increased energy required to fracture the dried compared to the fresh grass blades.

Button grass versus Mitchell grass

Instar V nymphs consuming Button grass gained more weight as they ingested and assimilated more dry matter and although Mitchell grass was significantly tougher than Button grass, no differences in the costs associated with converting assimilate to biomass were recorded. Button grass was less digestible than Mitchell grass when the effect of cellular structure was removed by grinding the grasses. When the diets were consumed fresh, differences in digestibility of the whole leaf blade were due to differences in cell wall quantities of the two grasses. However, when the grasses were powdered, while both grasses were significantly more digestible than when fed fresh, Mitchell grass was significantly more digestible when measured both in its entirety and when the indigestible cell wall was corrected for. This suggests that when fed whole, Button grass was more digestible. Locusts may extract nutrients more easily from fresh Button grass, as the increased water associated with the dry matter facilitates nutrient assimilation and/or more cells were fractured during the initial processing in the oral cavity. However, digestibility may have been the same but the reduction observed may result from increased egestion of surplus nutrients (Zanotto *et al.* 1993; Zanotto *et al.* 1994).

Addition of free water with the Mitchell grass diet increased its digestibility making it more digestible than Button grass, mirroring the results for the powdered diets. Nymphs consuming the powdered diets were able to regulate dietary water. There was not a comparable trial with fresh diets (no Button grass plus water treatment was included), so it is not known whether the locusts could have increased digestibility of fresh Button grass.

The lower digestibility of powdered Button grass, even when the cell wall component was accounted for, could be due not only to reduced digestion of exogenous matter but to increased endogenous matter. Significantly more protein was ingested by nymphs feeding on Button grass (fresh or powdered) due to differences in the chemistry of the two diets. Excess protein is voided post-absorptively in the frass as

uric acid and other nitrogen-products e.g. lysine (Zanotto *et al.* 1993; Zanotto *et al.* 1994). There is also some evidence to suggest that when a diet is nutritionally unbalanced, selective uptake can occur through changes to the cells lining the gut (Zudaire *et al.* 1998). However, the extra protein ingested does not account for the increased frass produced by nymphs on powdered Button grass.

Generally, the efficiency with which Button grass (total intake and assimilate) was converted to biomass was the same or higher than for Mitchell grass. When the grasses were powdered no difference was found in the costs to convert assimilate to growth, suggesting that the increased energy required to fracture Mitchell grass may be having a slight effect on processing costs. When locusts were fed whole dried Mitchell grass blades, which required twice as much work to fracture as fresh Mitchell grass blades, the efficiency with which they converted it to biomass decreased. Although more dry matter was assimilated than for the fresh Mitchell grass diet (with free water), growth was still reduced.

The significantly higher gain in wet weight by the nymphs fed powdered Button grass appeared to be due to them having more water per gram dry matter compared with the nymphs fed powdered Mitchell grass. This appeared to be a trend that nymphs feeding on Button grass had a slightly higher ratio of water to dry matter. Previously it was thought that this was because fresh Button grass has a significantly higher amount of water per unit dry matter. However, why this trend also occurred when the nymphs were allowed to control their own water intake is not known as it has been argued that haemolymph nutrient concentration may influence intake (Ben Halima *et al.* 1983). This suggests that any plant factor that leads to a unfavourable water balance within in an insect could potentially be an antiherbivore defence.

Typically, intake is regulated by diet quality and nutrient requirements. Several studies have shown that increased food intake is associated with a decrease in diet quality (e.g. McGinnis and Kasting 1967; Simpson and Abisgold 1985; Simpson and Raubenheimer 1993b; Slansky Jr. 1993; Timmins *et al.* 1988). One study (Yang and Joern 1994b) suggested that, as in mammals (Demment and Van Soest 1985), grasshoppers were able to alter their gut capacity to increase nutrient transfer when dietary nutrients were limiting. However, in a very small animal the capacity to do this may be limited (Weeks 1996). The Australian plague locust did not demonstrate

any ability to alter gut capacity in association with diet quality. Previously (Chapter 6), when fed Mitchell grass compared to wheat, nymphs had a relatively bigger head, which was thought to be because of increased toughness of the diet. However, when the toughness effect of the diet was removed by grinding, no differences in the relative head sizes were observed. Nymphs consuming the powdered diets ingested larger amounts and while it is not known if equivalent dry weight mouthfuls were ingested on fresh and powdered diets, it may suggest that mandible muscle may play some role in causing the relative increase in head size.

The chemical and physical properties of the diet interrelate with volumetric factors. Drying and grinding reduce the volume a diet occupies. Meal size is controlled by the integration of numerous exogenous and endogenous factors. Negative feed-back during a meal comes from volumetric feed-back from the gut (Bernays and Chapman 1973a; Bernays and Simpson 1982; Roessingh and Simpson 1984; Simpson 1983b) and rapid changes in blood osmolarity and nutrient composition (Abisgold and Simpson 1987; Bernays and Chapman 1974a; Simpson and Raubenheimer 1993a). It was predicted that ground meals could be larger due to reduced volumetric constraints. Haemolymph nutrient concentration and osmolarity might increase faster on a ground diet due to both more nutrients per gram dry matter ingested being digested. However, the increase in accessibility of ingested nutrients might allow more rapid processing by gut enzymes, which would result in the termination of a meal. When the meal is ground it may increase feeding due to the release of phagostimulants such as sugars (Barton Browne *et al.* 1975a) that are normally enclosed within the cells.

Intake over an instar is a product of the size and number of meals. Previously, it was hypothesized that the concentration of nutrients in the haemolymph may be responsible for the increased intermeal duration recorded for later instar nymphs consuming Mitchell grass (Chapter 4). Such findings have been recorded in other studies (Abisgold and Simpson 1987; Ben Halima *et al.* 1983; Bernays and Chapman 1974a; Paul *et al.* 1992; Raubenheimer and Gade 1994; Roessingh *et al.* 1985) However, nymphs consuming both the powdered diets and those on fresh Mitchell grass with free water would have had a higher concentration of nutrients in the haemolymph. Nymphs consuming the powdered diets consumed and assimilated more than when feeding on the fresh diet. This suggests that intermeal duration may

be influenced by the rate and thus duration of nutrient transfer from the gut to the haemolymph. It has been hypothesized that a locust's nutritional strategy is to extract the maximum amount of nutrients from their diet (Raubenheimer and Simpson 1998), i.e. maximizing diet digestibility ('efficiency') is favoured over maximizing assimilation rate ('power') (Raubenheimer and Simpson 1996). Thus it appears that digestibility of a diet controls gut passage rate, suggesting a locust cannot increase its intake to maximize nutrient assimilation when diet quality is reduced.

It has been argued that leaf structural properties have the potential to act as antiherbivore defences (Sanson *et al.* 2001). This could be achieved by increasing the difficulty of extracting leaf pieces from leaf material with enhanced biomechanical properties such as toughness (Bernays and Chapman 1970; Hehn and Grafius 1949; Nichols-Orians and Schultz 1990; Williams 1954) and/or the way nutrients are compartmentalized (Caswell and Reed 1975; Caswell and Reed 1976), or the physical structure of the entire leaf blade, i.e. insect gape width can limit access to thick leaves (Bernays 1991; Casher 1996). This study has demonstrated that biomechanical properties may also affect the quantity of nutrients able to be ingested as well as the cost of (1) fracturing the plant and (2) the degree of mastication required to release nutrients.

TABLES AND FIGURES

Table 7.1 Chemical analysis of the three treatment diets, Button grass, Mitchell grass and Mitchell grass that was used with added water. Results were averaged over the duration the locusts were fed. Different letters across a row represent significant differences ($P < 0.05$).

Treatment	Button grass	Mitchell grass	Mitchell grass + water	
No. of replicates	16	17	18	
Water (g g^{-1} dry weight)	4.82±0.12[a]	2.35±0.08[b]	2.36±0.07[b]	$F_{2,48} = 238.243$ $P < 0.001$
Cell wall material (% dry weight)	51.75±0.84	54.41±0.85	54.06±0.87	$F_{2,48} = 2.742$ $P = 0.075$
Protein (% dry weight)	9.61±0.28	9.59±0.28	9.70±0.29	$F_{2,48} = 0.050$ $P = 0.951$
Non-structural carbohydrates (% dry weight)	22.79±.0.55	24.17±0.81	24.05±0.77	$F_{2,48} = 1.074$ $P = 0.350$
Ratio protein : non-struct. carbohydrates (g g^{-1})	1:2.41±0.10	1:2.57±0.13	1:2.53±0.13	$F_{2,48} = 0.492$ $P = 0.615$

Table 7.2 Chemical properties of the dried and powdered Mitchell grass and the powdered Button grass diets.

	Button grass	**Mitchell grass**	
Cell wall material (% dry weight)	52.61 ± 0.12	53.98 ± 0.14	$F_{1,3} = 52.636$, $P = 0.005$
Protein (% dry weight)	9.45 ± 0.73	8.02 ± 1.26	$F_{1,3} = 0.977$, $P = 0.361$
Non-structural carbohydrates (% dry weight)	27.81 ± 0.55	26.13 ± 0.88	$F_{1,3} = 2.627$, $P = 0.166$
Ratio protein: non-struct. carbohydrate ($g\ g^{-1}$)	1:3.12 ± 0.23	1:3.26 ± 0.36	$F_{1,3} = 0.105$, $P = 0.759$

Table 7.3 Percentage survival of Instar V Australian plague locust nymphs feeding on fresh Button grass or Mitchell grass, fresh Mitchell grass plus water, dried Mitchell grass plus water, powdered Mitchell grass or Button grass plus water.

	Button grass (%)	**Mitchell grass (%)**
Fresh diet	81.0	78.9
Fresh Mitchell grass + water		48.5
Dried Mitchell grass		77.3
Powdered Mitchell grass		88.9
Powdered diet	78.6	59.3

Table 7.4 Results of ANCOVA of performance measures of the locusts feeding on the five treatment diets, fresh Button grass and Mitchell grass, fresh Mitchell grass with *ad lib.* water, dried whole Mitchell grass blades and powdered dried Mitchell grass blades.

Type of analysis	Source of variation	df	MS	F	P
Final wet weight	Treatment	4	3216.466	11.396	< 0.001
Interaction	Initial wet weight.	1	3654.803	12.949	0.001
$P = 0.150$	Residual	69	282.237		
Final dry weight	Treatment	4	323.639	16.271	< 0.001
Interaction	Initial dry weight.	1	235.434	11.837	0.001
$P = 0.288$	Residual	69	19.890		
Final wt. gut	Treatment	4	1.307	1.508	0.209
Interaction	Remainder of body.	1	7.867	9.081	0.004
$P = 0.779$	Residual	69	0.866		
Final wt. head	Treatment	4	0.054	0.209	0.933
Interaction	Remainder of body	1	13.304	51.738	< 0.001
$P = 0.695$	Residual	69	0.257		
Consumption: total dry matter					
Interaction	Treatment	4	21050.573	12.614	< 0.001
$P = 0.301$	Initial dry weight	1	15758.858	9.443	0.001
	Residual	69	1668.810		
Consumption: non-cell wall material					
Interaction	Treatment	4	5390.071	17.166	< 0.001
$P = 0.276$	Initial dry weight	1	2508.136	7.988	0.006
	Residual	69	314.002		

Type of analysis	Source of variation	df	MS	F	P
Consumption: protein					
Interaction	Treatment	4	72.388	4.188	0.004
P =0.377	Initial dry weight	1	134.019	7.754	0.007
	Residual	69	17.283		
Consumption: non-structural carbohydrates					
Interaction	Treatment	4	2554.982	30.634	< 0.001
P =0.259	Initial dry weight	1	905.411	10.856	0.002
	Residual	69	83.404		
Assimilation					
Interaction	Treatment	4	17699.563	42.920	< 0.001
P =0.389	Initial dry weight	1	968.350	2.348	0.130
	Residual	69	412.381		
Frass (AD)					
Interaction	Treatment	4	5331.158	22.513	< 0.001
P =0.634	Consumption	1	61221.328	258.529	< 0.001
	Residual	69	236.807		
Non-cell wall frass (AD)					
Interaction	Treatment	4	5132.609	22.821	< 0.001
P =0.612	Non-C.W. consumption	1	1429.509	6.356	0.014
	Residual	69	224.903		
Dry weight growth (ECI)					
Interaction	Treatment	4	236.823	13.308	< 0.001
P =0.144	Consumption	1	154.294	8.671	0.004
	Residual	69	17.795		
Dry weight growth (ECD)					
Interaction	Treatment	4	182.717	9.306	< 0.001
P =0.623	Assimilation	1	27.434	1.397	0.241
	Residual	69	19.634		

Type of analysis	Source of variation	df	MS	F	P
Growth rate					
Interaction	Treatment	4	293.626	14.958	< 0.001
P =0.809	Duration	1	27.683	1.410	0.239
	Residual	69	19.630		
Consumption rate					
Interaction	Treatment	4	2.172×10^4	11.513	< 0.001
P =0.741	Duration	1	740.439	0.393	0.533
	Residual	69	1886.468		
Assimilation rate					
Interaction	Treatment	4	1.729×10^4	40.604	< 0.001
P =0.702	Duration	1	33.841	0.080	0.779
	Residual	69	425.925		

Table 7.5 ANCOVA-adjusted means for the locust performance parameters for Instar V nymphs consuming powdered Button grass and Mitchell grass. * significantly different ($P < 0.05$) (statistics are given in Table 7.6).

	Button grass	**Mitchell grass**	***P***
Wet weight final	209.13 ± 6.72	187.52 ± 6.72	*
Dry weight final	43.21 ± 1.23	40.68 ± 1.23	
Consumption: dry weight	284.01 ± 10.24	278.38 ± 10.24	
Consumption: non-cell wall	134.58 ± 4.77	128.11 ± 4.77	
Consumption: protein	26.85 ± 0.88	22.33 ± 0.88	*
Assimilation	111.53 ± 5.66	123.03 ± 5.66	
Frass (AD)	170.955 ± 3.24	156.88 ± 3.24	*
Non-cell wall frass (AD)	22.98 ± 3.25	5.14 ± 3.25	*
Growth (ECI)	18.09 ± 1.14	15.81 ± 1.14	
Growth (ECD)	18.35 ± 1.25	15.55 ± 1.25	
Growth rate	17.82 ± 1.13	16.08 ± 1.13	
Intake rate	286.16 ± 10.90	276.24 ± 10.90	
Assimilation rate	113.20 ± 5.69	121.36 ± 5.69	

Table 7.6 Results of ANCOVA of performance measures of the locusts feeding on powdered Button grass and Mitchell grass.

Type of analysis	Source of variation	df	MS	F	P
Final wet weight	Treatment	1	3500.512	5.176	0.031
Interaction	Initial wet weight.	1	6803.575	10.060	0.004
$P = 0.631$	Residual	27	676.279		
Final dry weight	Treatment	1	48.207	2.115	0.157
Interaction	Initial dry weight.	1	302.083	13.254	0.001
$P = 0.974$	Residual	27	22.792		
Final log wt. gut	Treatment	1	0.012	0.502	0.485
Interaction	Log remainder of body.	1	0.005	0.198	0.660lo
$P = 0.4799$	Residual	27	0.025		
Final log wt. head	Treatment	1	0.001	0.266	0.610
Interaction	Log remainder of body	1	0.072	17.306	< 0.001
$P = 0.987$	Residual	27	0.004		
Consumption: total dry matter					
Interaction	Treatment	1	237.186	0.151	0.71
$P = 0.149$	Initial dry weight	1	7433.814	4.727	0.039
	Residual	27	1572.661		
Consumption: non-cell wall material					
Interaction	Treatment	1	314.717	0.924	0.345
$P = 0.139$	Initial dry weight	1	1655.472	4.859	0.036
	Residual	27	340.737		

Type of analysis	Source of variation	df	MS	F	P
Consumption: protein					
Interaction	Treatment	1	153.396	13.189	0.001
P =0.099	Initial dry weight	1	63.578	5.466	0.027
	Residual	27	11.631		
Consumption: non-structural carbohydrates					
Interaction	Treatment	1	336.470	3.012	0.094
P =0.126	Initial dry weight	1	562.566	5.036	0.033
	Residual	27	111.711		
Assimilation					
Interaction	Treatment	1	992.336	2.065	0.162
P =0.158	Initial dry weight	1	1626.495	3.385	0.077
	Residual	27	480.526		
Frass (AD)					
Interaction	Treatment	1	1475.406	9.398	0.005
P =0.486	Consumption	1	14781.799	94.154	< 0.001
	Residual	27	156.996		
Non-cell wall frass (AD)					
Interaction	Treatment	1	2316.704	18.842	0.001
P =0.561	Non-C.W. consumption	1	5.172	0.033	0.857
	Residual	27	156.088		
Dry weight growth (ECI)					
Interaction	Treatment	1	38.780	2.009	0.168
P =0.767	Consumption	1	94.269	4.882	0.036
	Residual	27	19.308		
Dry weight growth (ECD)					
Interaction	Treatment	1	54.881	2.437	0.130
P =0.980	Assimilation	1	7.439	0.330	0.570
	Residual	27	22.524		

Type of analysis	Source of variation	df	MS	F	P
Growth rate					
Interaction	Treatment	1	21.944	1.167	0.290
P =0.584	Duration	1	107.751	5.729	0.024
	Residual	27	18.808		
Consumption rate					
Interaction	Treatment	1	707.438	0.405	0.530
P =0.719	Duration	1	2778.055	1.592	0.218
	Residual	27	1745.097		
Assimilation rate					
Interaction	Treatment	1	478.624	1.008	0.314
P =0.451	Duration	1	1779.314	3.747	0.063
	Residual	27	474.866		

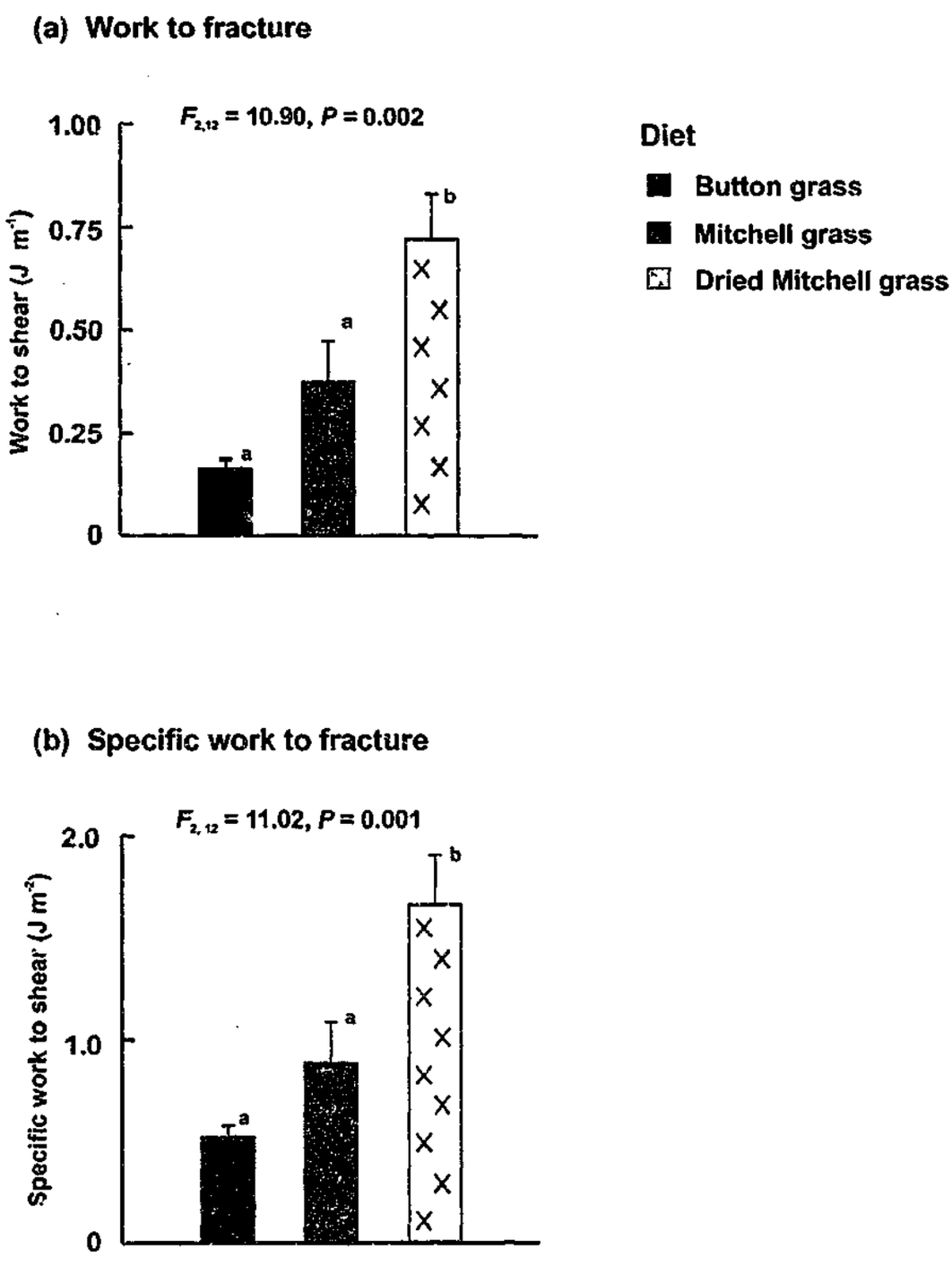


Fig. 7.1 Biomechanical parameters of both grasses; (a) work to shear (J m^{-1}), and (b) specific work to shear (J m^{-2}). Bars with different letters are significantly ($P < 0.05$) different. The F and P values given are for ANOVA, $n = 5$.

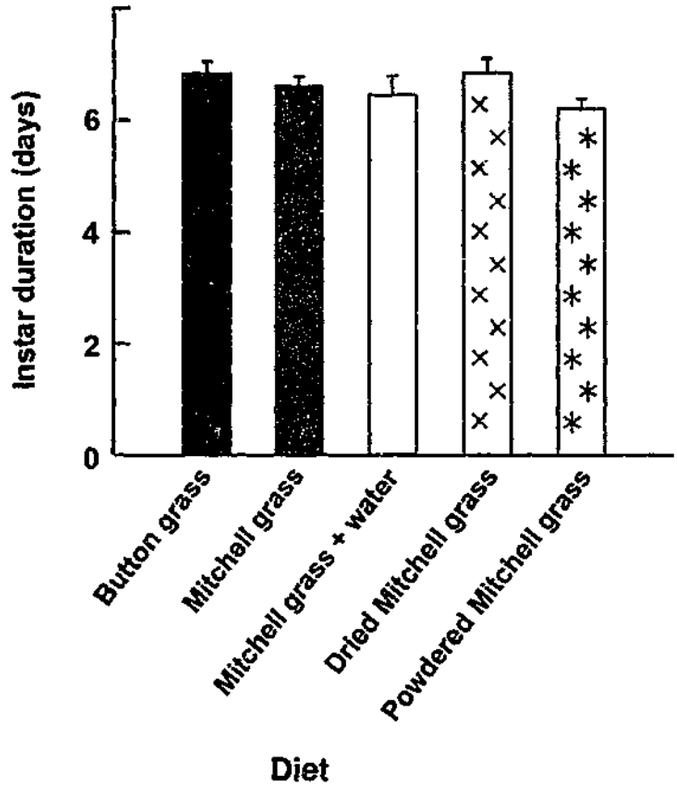


Fig. 7.2 Instar V duration of Australian Plague Locust nymphs fed on five different diets. The five diets, results shown from left to right were, fresh Button grass, fresh Mitchell grass, fresh Mitchell grass plus water, dried Mitchell grass plus water and powdered dried Mitchell grass plus water. There was no significant difference ($P < 0.05$) between treatments.

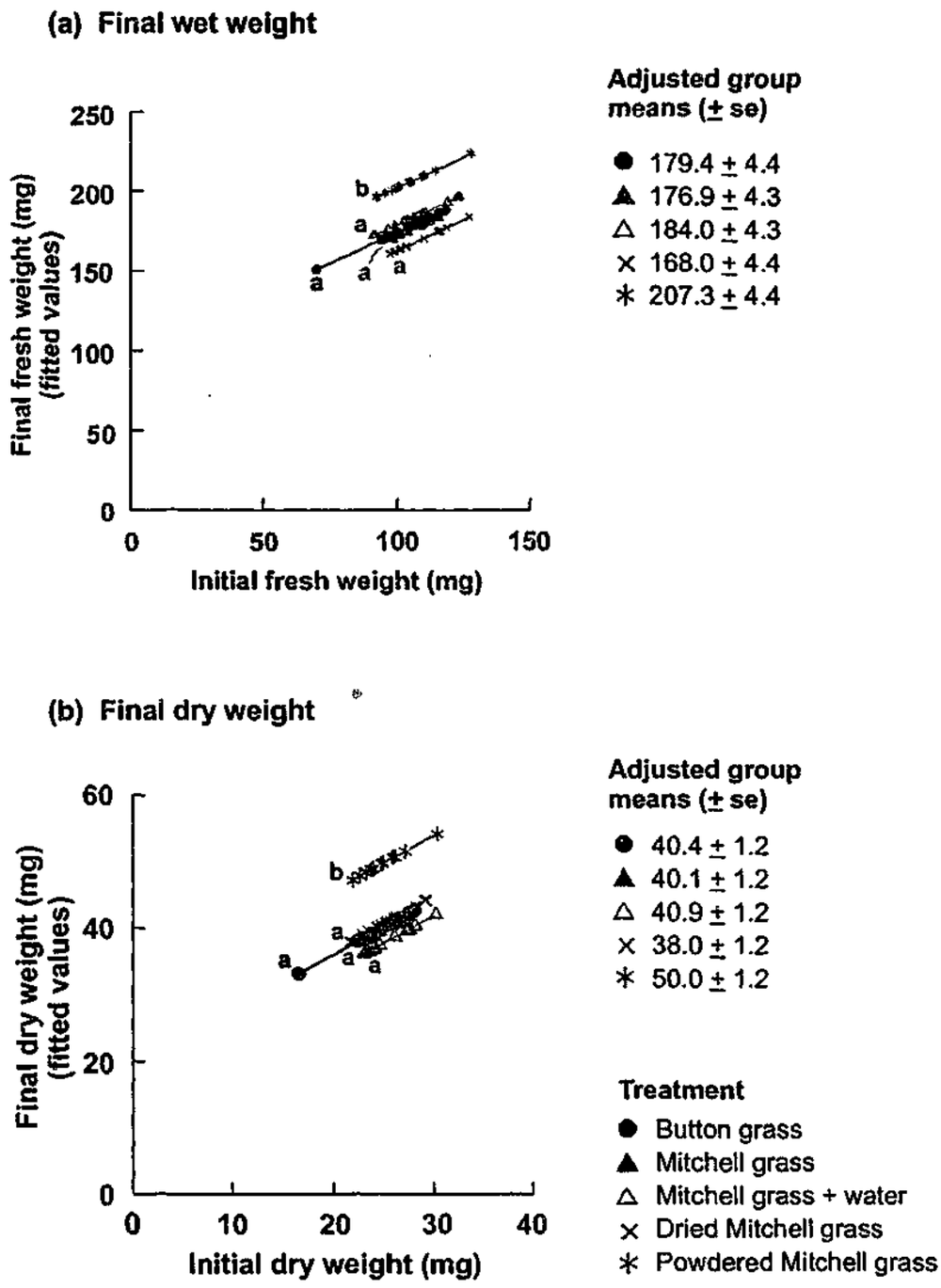


Fig. 7.3 Final fresh weight (ANCOVA fitted values) for nymphs feeding on each treatment diet. Lines with different letters have significantly different ($P < 0.05$) adjusted group means.

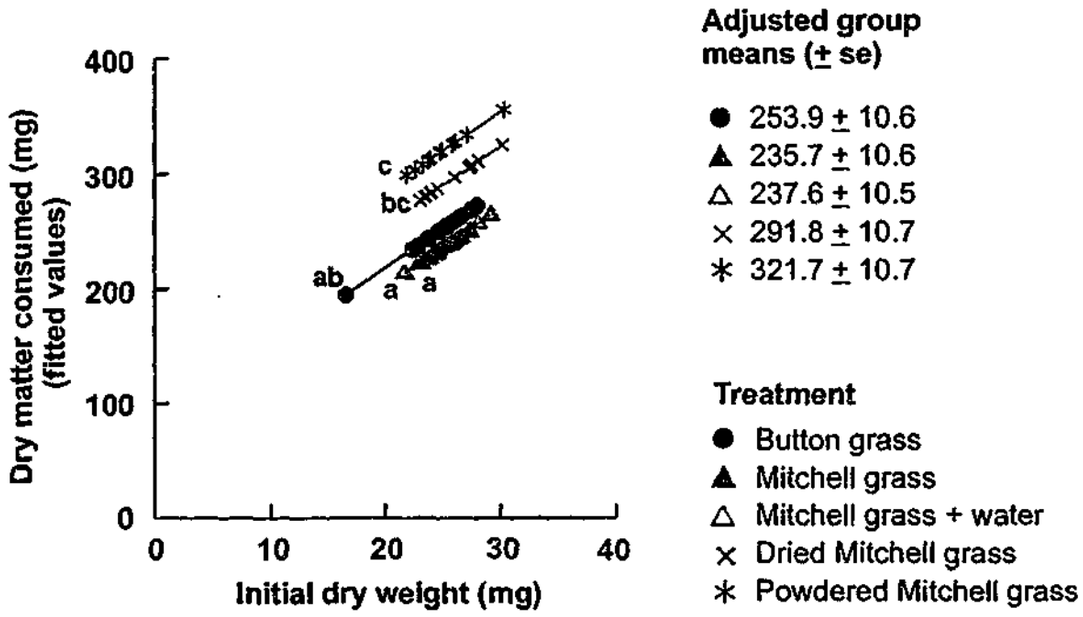


Fig. 7.4 Total dry matter intake (ANCOVA fitted values) for nymphs feeding on each treatment diet. Lines with different letters have significantly different ($P < 0.05$) adjusted group means.

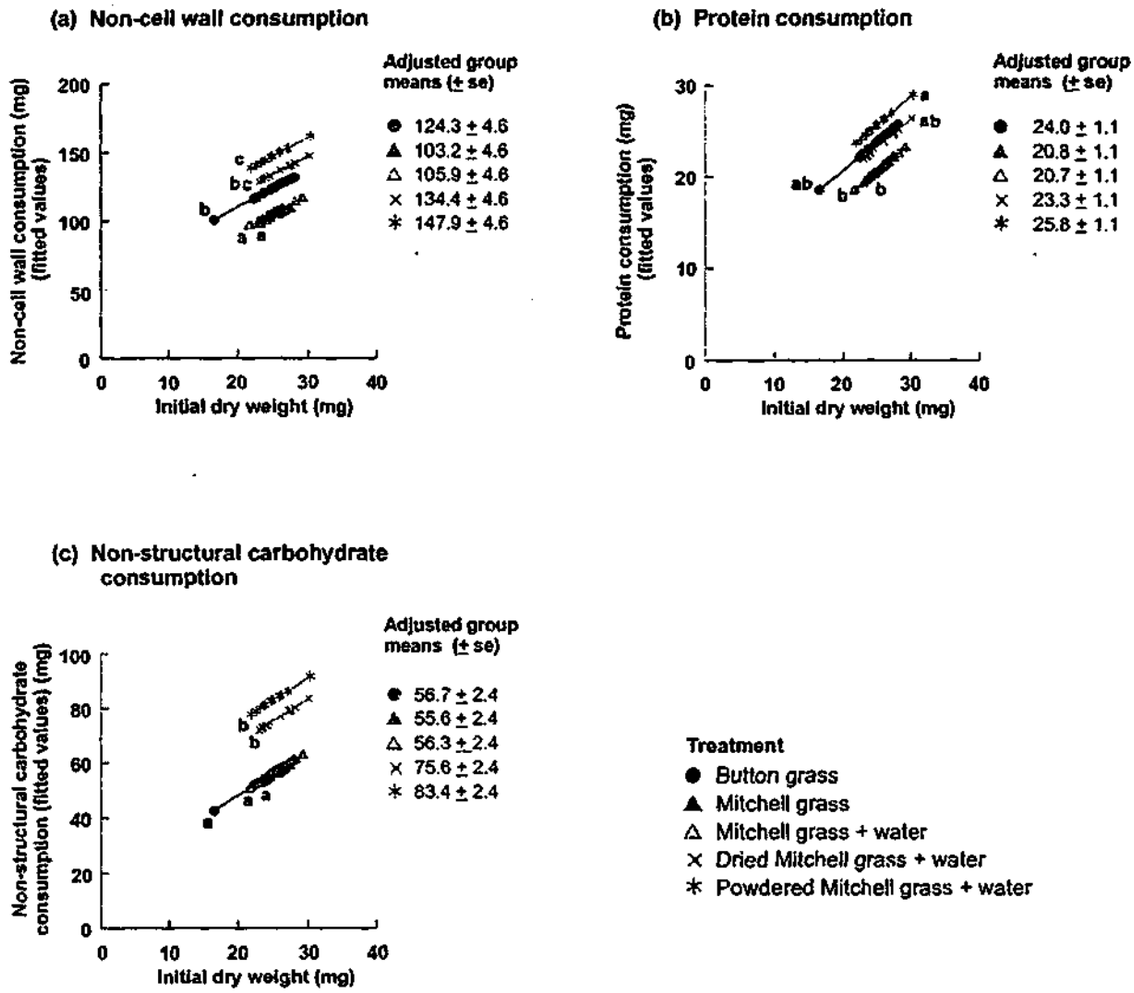


Fig. 7.5 Total intake (a) non-cell wall dry matter, (b) protein intake, and (c) carbohydrate intake (ANCOVA fitted values) for nymphs feeding on each treatment diet. Lines with different letters have significantly different ($P < 0.05$) adjusted group means.

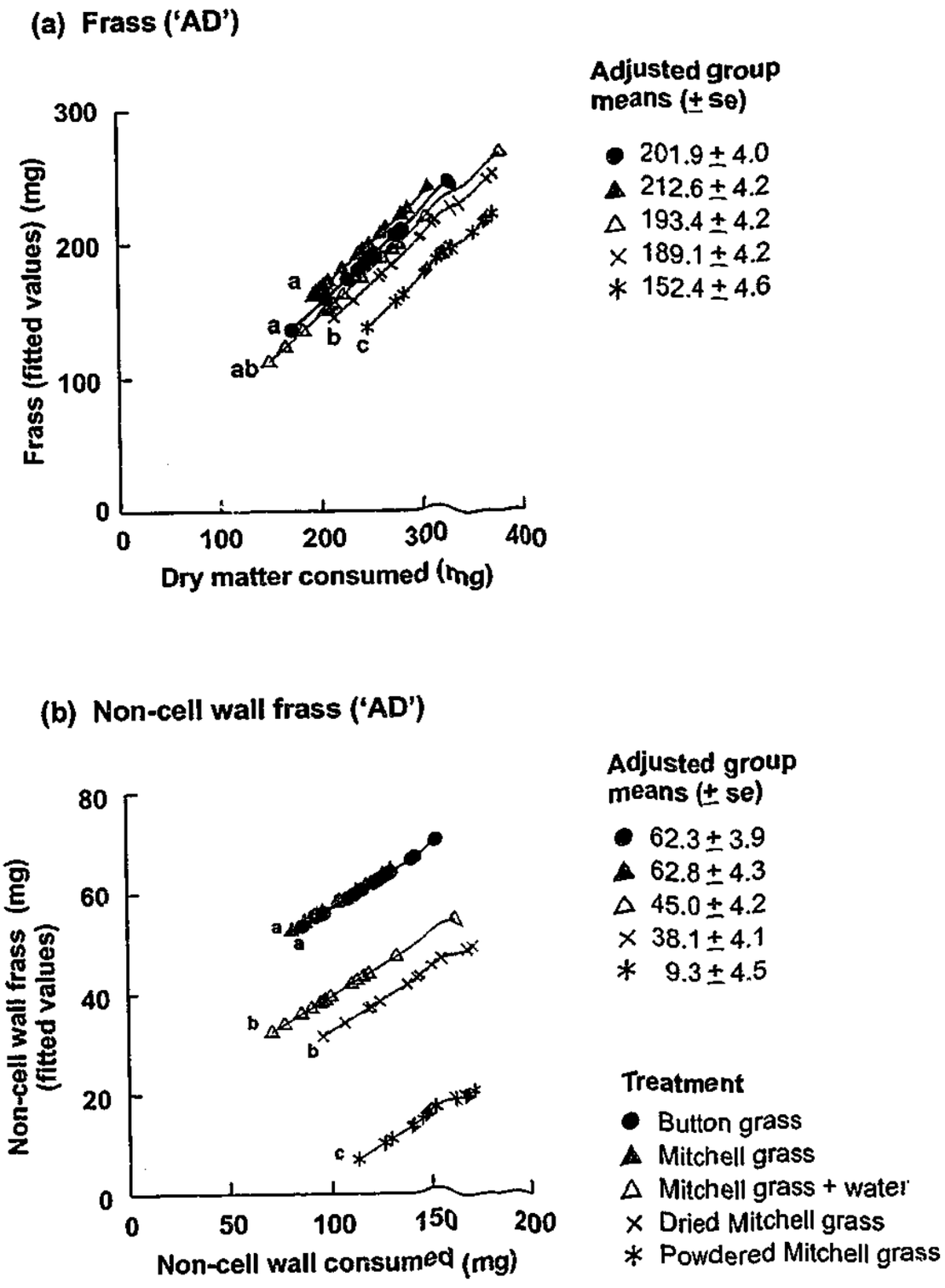


Fig. 7.6 Utilization plots of A) total frass and B) non-cell wall frass (ANCOVA fitted values) on total intake and non-cell wall intake respectively for each treatment. Lines with different letters have significantly different ($P < 0.05$) adjusted group means.

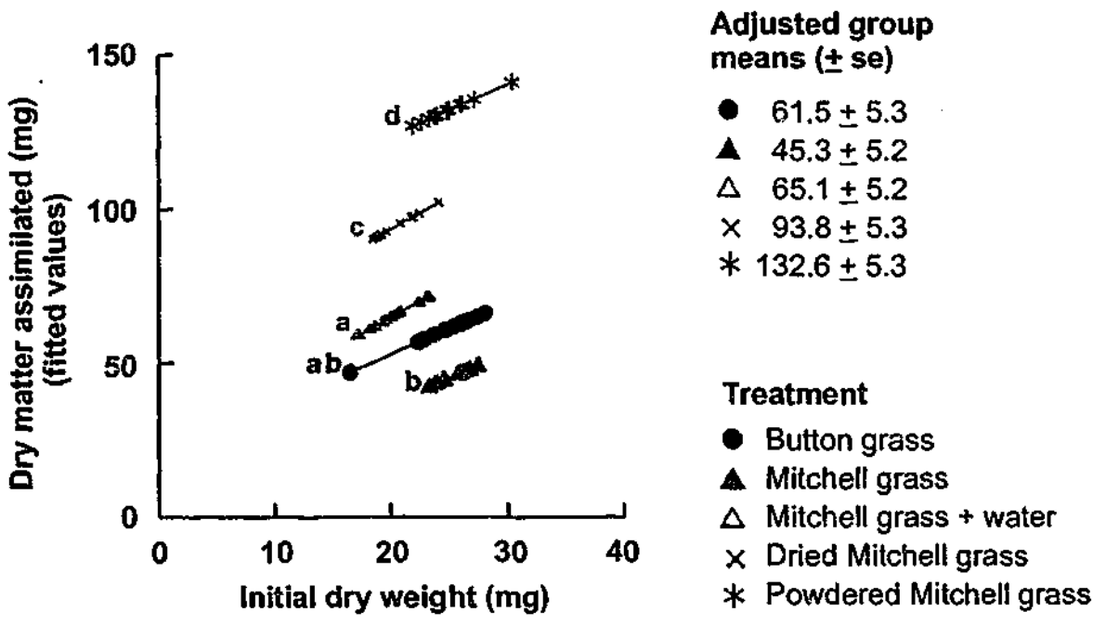


Fig. 7.7 Dry matter assimilation (ANCOVA fitted values) by nymphs feeding on each treatment diet. Lines with different letters have significantly different ($P < 0.05$) adjusted group means.

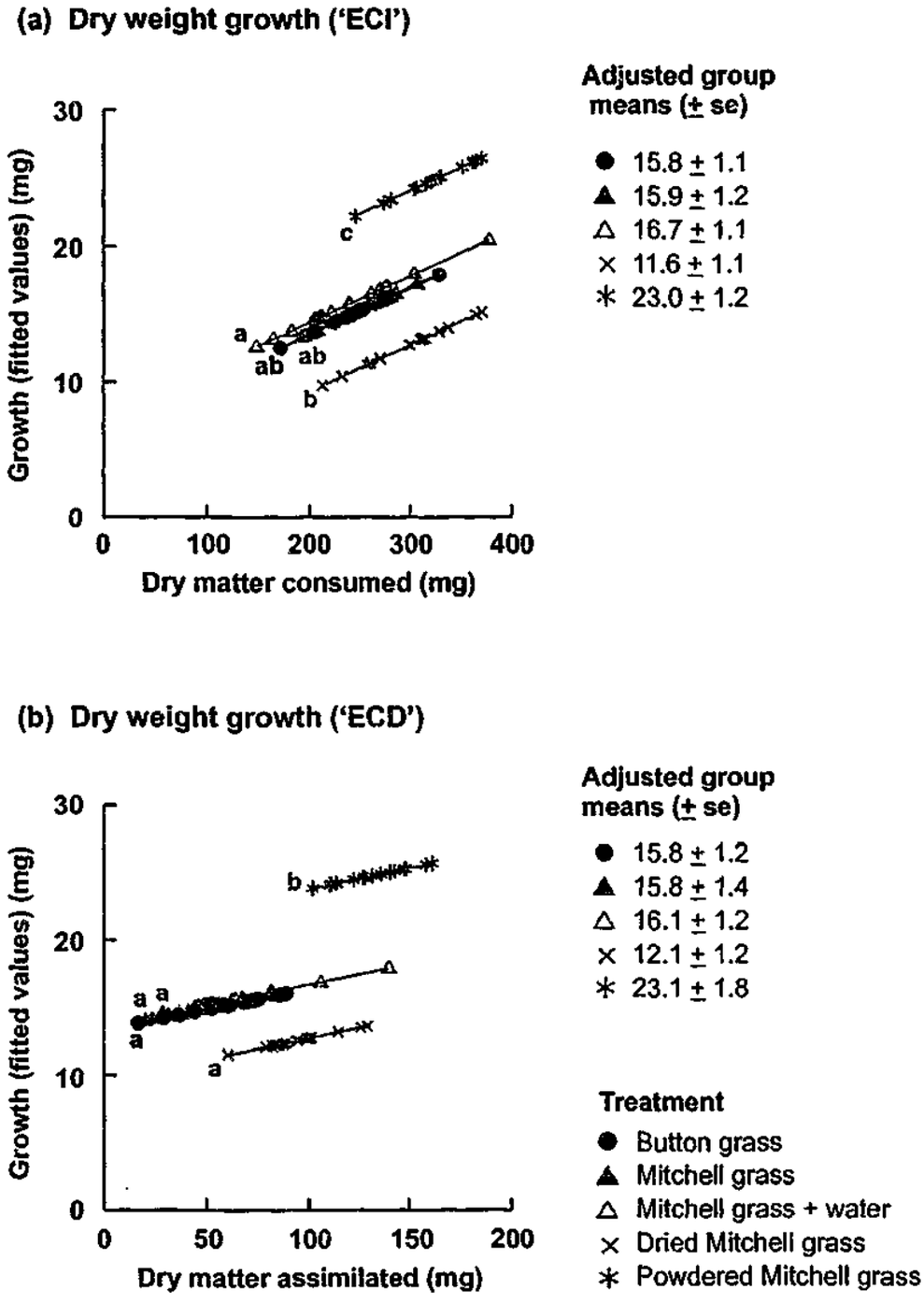


Fig. 7.8 Utilization plots of a) total dry matter intake and b) total dry matter assimilated (ANCOVA fitted values) on growth for nymphs feeding on each treatment diet. Lines with different letters have significantly different ($P < 0.05$) adjusted group means.

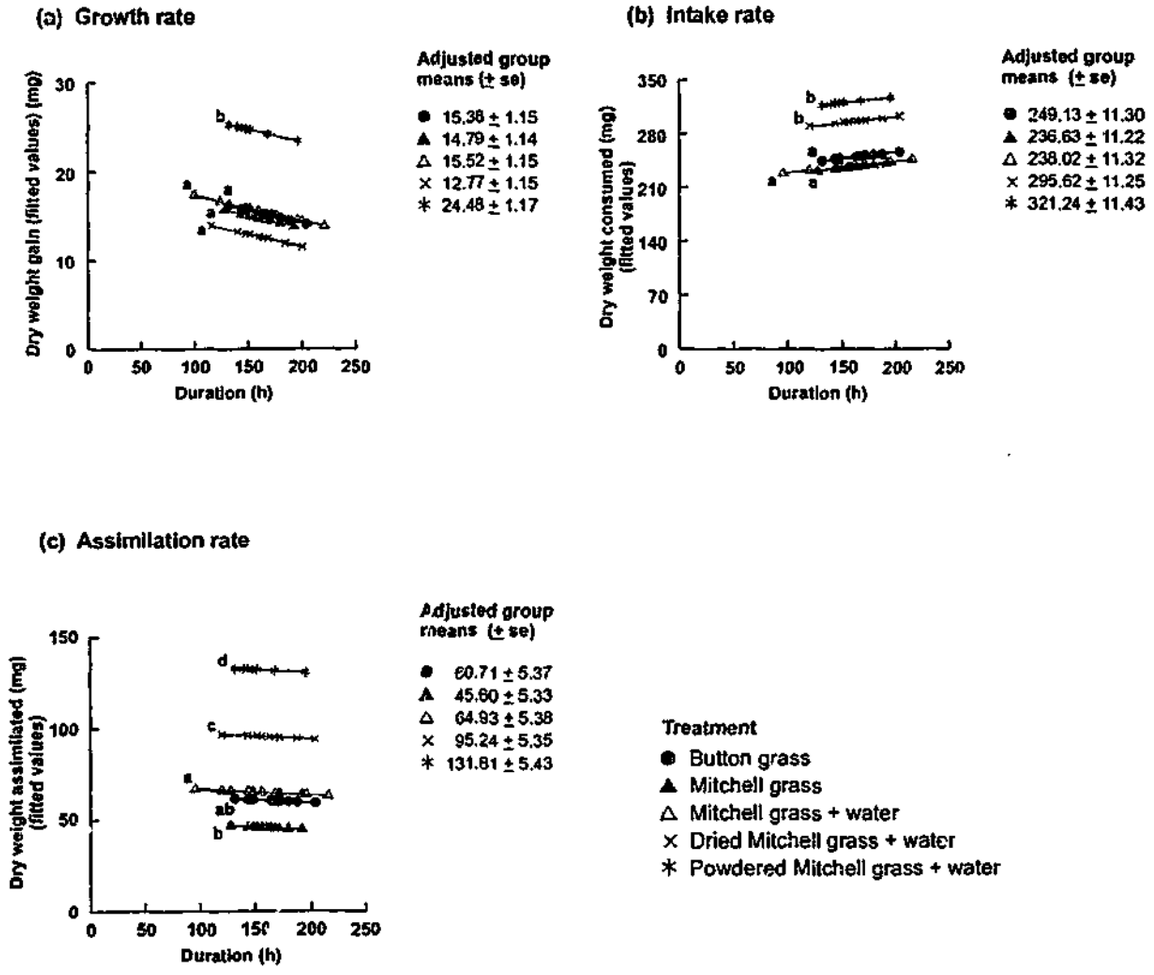


Fig. 7.9 Rate of (a) growth, (b) intake and (c) assimilation by Instar V nymphs feeding on the five different diets. Lines with different letters have significantly different ($P < 0.05$) adjusted group means.

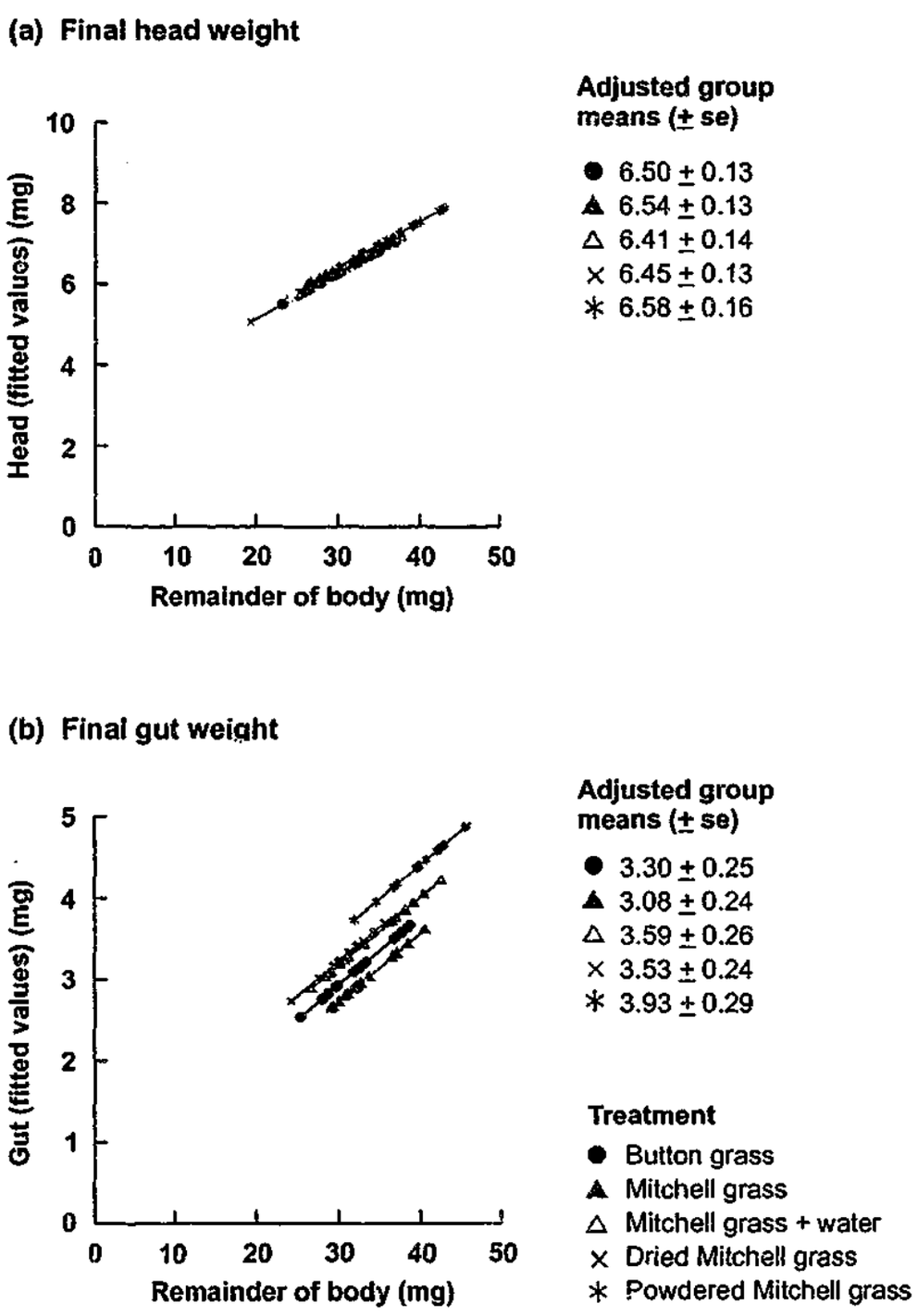


Fig. 7.10 Relationship between (a) head and (b) gut mass (ANCOVA fitted values) and the remainder of the body for each treatment. Lines with different letters have significantly different ($P < 0.05$) adjusted group means.

CHAPTER 8. GENERAL DISCUSSION

The majority of studies of locust nutritional physiology have been undertaken using artificial diets. Studies using whole leaf diets have generally not measured the digestibility of the different constituents of the plants, rather they report locust performance as survival and growth or in terms of dietary utilization. This study has focussed on digestibility of natural diets. By determining the chemical constituents of each grass diet and then comparing dietary utilization, growth and development of the Australian plague locust on both grasses, I have shown that existing general models could not be used to predict locust performance.

The Australian plague locust develops in a nutritional environment that deteriorates probably more rapidly than for the majority of insects. The success of Australian plague locusts is dependent on their ability to develop to the winged adult dispersal stage on the plant growth that may result from only a single rainfall event (Hunter *et al.* 2001). The plants that grow following rain generally produce seeds and dry off rapidly (< 8 weeks). As the soil dries, potential food plants both decline in quality and diversity until only Mitchell grass remains. In summer, when temperatures are favourable to locust development, the plants that respond to rain on Mitchell grasslands are predominantly perennial Mitchell grass and annual Button grass.

It was predicted from previous research using artificial diets, that the locusts would utilize these grasses equally, as they contained similar amounts of the major chemical constituents, (i.e. cell wall, protein and non-structural carbohydrate) that appear to most influence dietary intake (McGinnis and Kasting 1967; Simpson and Raubenheimer 1995b). However, while the early instars were able to digest and grow on either diet equally, the later instars consuming Mitchell grass experienced reduced growth and increased instar duration. This pattern was not repeated in experiments the following year and possible reasons for this are discussed below. However, for all nymphs consuming Button grass, they developed faster with higher survival rates compared to those consuming Mitchell grass, because they were able to assimilate significantly more dry matter. This appeared to be largely due to increased consumption.

The later instars consuming Mitchell grass experienced reduced growth and increased instar durations as they consumed and assimilated less dry matter. This was because the intermeal duration was longer for nymphs on Mitchell grass than Button grass. The two grasses differed biomechanically and anatomically. For the annual Button grass, SLA and water per unit dry matter was *c.* twice that of the perennial Mitchell grass. Button grass cells were on average bigger than those in Mitchell grass, i.e. the nutrients were in a few large 'packages' in Button grass and many small 'packages' in Mitchell grass.

The nutrient state of the haemolymph appears to control intermeal duration (Abisgold and Simpson 1987; Simpson and Raubenheimer 1993a). Since meals of equivalent dry weight were consumed, equal amounts of protein and carbohydrate were ingested. The increased intermeal duration for the older locusts feeding on Mitchell grass could be due to (1) increased concentration of nutrients *per se*; (2) a specific nutrient preventing the haemolymph returning to a pre-meal state as quickly as occurred with nymphs consuming Button grass; or (3) nutrients being released more slowly, which would also prolong the post-feeding state of the haemolymph. However, the intermeal duration declined with increasing age, contrary to what would be predicted from the presumed increase in haemolymph concentration with age. The haemolymph may be more dilute in the younger nymphs as more water per unit dry matter was recorded in the early instars (Chapter 4), which appeared to be due to body size constraints (Chapter 6) and/or due to endocrine differences with age (Johnson and Hill 1975; Morgan and Poole 1976). Juvenile hormone declines with age and increased amounts of juvenile hormone have been linked to increased water retention in grasshoppers (Beenakkers and Van Den Broek 1974). As meal size increased linearly with increasing body weight it is thought that the concentration of nutrients in the haemolymph following a meal would thus be higher the older the nymph. Therefore, it is thought that the concentration of nutrients or osmolarity of the haemolymph inhibiting gut emptying must alter with age (body size).

Mitchell grass required more work to fracture than Button grass, not just because the Mitchell grass blades were thicker, but also because of differences in cellular structure. However, no differences were observed in the efficiency of the locusts of the same age converting assimilate to body mass. Locusts appeared to minimize the energy and force required to excise leaf tissue by making the majority of the fractures

between the vascular bundles only, severing the tougher vascular bundles at the start and end of each 'strip'.

Locusts were unable to digest cell wall (Chapter 3), indicating that cell wall acts as a mechanical barrier to nutrient acquisition. Removal of the cell wall barrier effect enabled the locusts to double the digestibility of the non-cell wall fraction of both diets. In the powdered form, the non-cell wall fraction of Mitchell grass was found to be more digestible than the non-cell wall fraction of Button grass, suggesting that Button grass had a higher proportion of indigestible constituents. However, when the grass blades were consumed whole, no differences were found in digestibility of the non-cell wall component of both grasses. As the proportion of nutrients available for digestion was less in whole Button grass, Button grass was actually more digestible, since a larger proportion of available nutrients were assimilated.

By grinding the diet and thereby removing the need for the locusts to fracture the cells, the amount of dry matter assimilated was increased, and the costs associated with converting this to biomass were decreased. This resulted in nymphs growing at almost twice the rate of nymphs fed whole fresh diet and consuming dry matter *c.* 33% faster and assimilating dry matter twice as quickly. When water was provided *ad lib.* dry matter assimilation increased 145%. Additionally, destruction of the cell wall increased nutrient assimilation by almost 300% from that of fresh Mitchell grass.

Effect of age and body size on dietary utilization

For later instar nymphs that were larger, intake per gram of body mass was proportionately higher and digestibility of the cell contents was lower with assimilation efficiency remaining the same. In an attempt to investigate the effect of insect size *per se* on dietary utilization different sized Instar V nymphs were fed Mitchell grass. With increasing instar number, water per unit dry matter decreased (Chapter 4). This appeared to be an effect of size as it was also observed between the small and large Instar V nymphs (Chapter 6). Mitchell grass was used, as previously this had been shown to be the 'poorer' resource and thus it was predicted any differences might be more easily observed with this diet. However, the locusts responded differently to Mitchell grass in these trials (discussed below). Being bigger allows nymphs to consume and assimilate nutrients at a significantly faster rate. Meal size was correlated with size (Chapter 5) and from observation of locusts

consuming grass blades it was predicted that having bigger mandibles increases the width of the strip excised, and with a bigger gut, meal size can be larger.

With increasing age, the digestibility of both grasses decreased. However, this trend was not observed with the different sized Instar V locusts. This may be because the differences in size of the Instar V nymphs were just not enough to affect these parameters. Consequently, as instars got older, development per unit body mass was slower. The implications of this are unclear, although this could lead to localized extinctions in a nutritionally declining environment.

As the mandibles increase in size it was hypothesized that proportionately fewer cells would be fractured per bite (Chapter 5). Exactly how the mandibles occluded and functioned was not determined. However, dietary processing per unit dry matter increased with age, and Mitchell grass was chewed more than Button grass which meant that per unit dry matter both grasses were processed (bites + chews) equivalently. As Mitchell grass has lower SLA, more dry matter is consumed per unit area suggesting the locusts maybe able to adjust dietary processing. Whether the decreased digestibility of the diets by the later instar nymphs was due to proportionately fewer cells being fractured by the bigger mandibles or some other factor affecting nutrient transfer, e.g. constraints imposed by increased gut size or the regulation of enzyme production, remains to be determined. Instar duration increased with instar number (Chapter 4). The later instars were able to increase total dry matter assimilation, as they were able to increase the assimilation rate by taking meals more frequently (Chapter 5) and more of them (meal size for Instar II nymphs was *c.* twice that as a proportion of total intake compared to Instar V nymphs).

Comparisons of locust performance on Button and Mitchell grass

The three experiments where nymphs were reared on wheat until the beginning of Instar V then fed on either Button grass or Mitchell grass (Chapters 4, 5, 6 and 7) did not result in exactly the same outcome. Specifically, the increased instar duration and reduced growth originally recorded for nymphs consuming Mitchell grass that appeared to be due to reduced consumption (Chapter 4), was not observed the following year (Chapter 6 and 7). The patterns reported in Chapters 4 and 5 were also observed previously in pilot trials (*unpub. data*). The results reported in Chapters 4, 6, and 7 were combined and analyzed to determine the effect of the two

grasses on the locust. Although the initial dry weight was the same, the locust responses (development rate, final dry weight, diet intake and assimilation) varied significantly with experiment. The diets varied *per se*, and between experiments, in terms of protein, non-structural carbohydrates, water content and cell wall material (Fig. 8.1). The amount of protein varied between experiments but not between diets. Mitchell grass had significantly more cell wall and non-structural carbohydrates and significantly less water per unit dry matter than Button grass. While, the amount of cell wall material differed between the diets, the difference was small (a range of 49-58% of the total dry matter) and previous work using artificial diets suggests that this would not be of biological significance (McGinnis and Kasting 1967; Simpson and Raubenheimer 1993b).

Instar duration (Fig. 8.2a), the final dry weight (Fig. 8.2b) and growth rate (Fig. 8.3a) varied significantly between experiments but not between diets. However, there was a significant interaction between diet and experiment. Nymphs feeding on Button grass consumed (Fig. 8.2c) and assimilated (Fig. 8.2d) more dry matter and at a faster rate (Fig. 8.3b, c) than nymphs on Mitchell grass. Due to these differences and the nature of the diets, there was a higher intake of non-cell wall dry matter, protein and water for nymphs on Button grass (Fig. 8.4a, b, c). The amount of non-structural carbohydrates consumed varied between experiments but not between diets (Fig. 8.4d). Button grass was more digestible than Mitchell grass (Fig. 8.5a) because it generally had less cell wall and when this was corrected for, there was no difference in the digestibility of the cell contents (Fig. 8.5b). The efficiency with which the nymphs converted intake and assimilate to biomass was not affected by diet but varied significantly with experiment (Fig. 8.5c, d). The nymphs reported in Chapter 6 were able to grow more efficiently than those in Chapters 4 and 7 and as they consumed and assimilated more dry matter their final weight was significantly higher. It is thought the locust nutritional regulatory systems are fairly robust (Simpson *pers. comm.*) which suggests the differences in locust responses may be due to changes in diet chemistry. However, there are other possible reasons for the differences in locust responses to the grasses (discussed later).

The intake target for Australian plague locusts over an instar is not known. Even if it were, the requirements within an instar are likely to vary, as has been found for other species (Simpson 1982a). Therefore, an intake target that has been averaged over an instar may not give a true indication of the value of that diet to the insect at any one point in time. When a cockroach was fed a diet that was nutritionally 'optimal' for an instar (determined from self selection) it performed poorly, since the insect was unable to regulate throughout the instar for the variations in nutrients required (Cohen *et al.* 1987). So while the diets varied in this study, with each experiment, the cause of the differences in locust response could be their ability to compensate, or their compensation varied in response to disproportionate changes in nutrient concentrations. The ANCOVA results suggested that the Instar V nymphs maintained a fairly consistent protein intake while non-structural carbohydrate intake reflected its ratio to protein in the diet. For nymphs feeding on either diet the protein intake only varied by *c.* 11% across the three experiments while the non-structural carbohydrate intake varied 28% for nymphs on Button grass and 55% for nymphs on Mitchell grass (calculated as the spread of adjusted ANCOVA means divided by their average). Although nymphs on Button grass consumed more protein, this may just reflect the slightly lower protein digestibility that was found previously (Chapter 4). The concentration of protein in the grasses correlated negatively with the concentration of non-structural carbohydrates (Fig. 8.6a). This also held true when both grasses were analyzed separately, although the relationship was slightly different (Fig. 8.6b). However, the nature of the correlation of protein with water and cell wall material varied with grass species (Fig. 8.6b).

Hierarchical partitioning was used to quantify the 'independent' correlation of each component of diet chemistry (cell wall material, protein, non-structural carbohydrates and water) with insect growth, development time, intake and amounts assimilated (Chevan and Sutherland 1991; Quinn and Keough 2002). Combining experiments and diet suggested that variation in intake was explained most by the protein concentration of the plants being consumed (Table 8.1, Fig. 8.7). However, subsequent growth and instar duration appeared to be best described by the amount of non-structural carbohydrates consumed (Table 8.1, Fig. 8.8). Intake was negatively correlated with the concentration of protein in the diet (Fig. 8.7). Thus it appeared that the locusts maintained a consistent consumption of protein per instar as described

earlier (Fig. 8.4c). For both grasses, the concentration of protein and non-structural carbohydrates were negatively correlated (Fig. 8.6). Increased non-structural carbohydrate intake occurred when the locusts increased consumption due to reduced protein concentration in the grasses (Fig. 8.8). Instar duration was slightly negatively correlated with the concentration of non-structural carbohydrates in the grasses (Table 8.1).

As intake was best explained by the protein concentration of the diet, and for each grass the chemical components correlated differently with protein (Fig. 8.7) different amounts of non-protein nutrients were consumed. Thus, for Button grass total intake decreased with increasing cell wall material in the dry matter while for Mitchell grass the trend was the opposite (Fig. 8.6).

If dietary protein concentration controls intake, the ratio of protein to carbohydrates is also important as this may influence the amount of growth and the rate of development (Fig. 8.9). The protein:carbohydrate ratio where growth was highest was much higher than that previously recorded for other locusts (Chambers *et al.* 1995; Simpson and Raubenheimer 1993b), and growth correlated positively with non-cell wall carbohydrates and negatively with protein (Fig. 8.8). Dry matter assimilation was best described by the amount of water rather than protein consumed (Table 8.2, Fig. 8.9, 8.10). When locusts increased their water consumption, dry matter assimilation also increased as recorded in Chapter 7.

If protein is controlling intake, then it will also be controlling the intermeal duration. This has been demonstrated in *Locusta migratoria* where it was observed that protein concentration influenced the intermeal interval and for which dilutions to the carbohydrate fraction were not compensated (Simpson and Abisgold 1985). This may be because increased carbohydrates did not alter haemolymph osmolarity (Simpson and Abisgold 1985) as it appears carbohydrates are rapidly removed from the haemolymph (Turunen 1985). Feeding rate appears not to be related to differences in the rate of passage of food through the gut and its effect on inhibitory volumetric feedback (Simpson and Abisgold 1985); rather haemolymph factors control gut emptying.

Discussion of the different results from the three experiments

The exact cause of the observed differences between the three trials where nymphs were reared on wheat until the start of Instar V and then fed either of the treatment diets is unknown. Potential reasons for the different results found between the three feeding trials are (1) slight differences in the treatment diets, as described below, (2) differences in nymph rearing conditions, abiotic and/or nutritional factors, and (3) nymphs selected from a different population.

The experiments reported in Chapters 4 and 5 were conducted in a different controlled temperature room from those reported in Chapters 6 and 7, due to demolition of the rooms used initially. The temperature conditions were the same for all experiments and the temperature was monitored with data loggers, which did not suggest that conditions varied. However, in the 'new' constant temperature rooms a minimum humidity was maintained at 30% RH. Gregg (1981) found that humidity did not affect rates of Australian plague locust development and survival except at low (< *c.* 20 °C daily average) temperatures combined with humidities at either extreme. So it is not thought that changes to abiotic conditions, if any, were responsible for the observed differences in locust performance. Previous dietary history has been shown to affect both assimilation and growth efficiencies (Stockhoff 1992) and although I attempted to standardize the conditions under which the wheat was grown I did not measure the environmental conditions over which the diet was grown. The wheat blades were grown from seed with only water added, and seed nitrogen could have altered the wheat diet. The experiments took place at approximately the same time each year and although climatic conditions would have varied, day length, that can promote changes in leaf chemistry (Hinks and Olfert 1992), would have been similar each year. Although I did not measure the chemistry of the wheat blades, the initial dry weight of Instar V nymphs did not differ significantly between each experiment suggesting the wheat was fairly similar for each experiment.

The nymphs used in the trials reported in Chapters 4 and 5 were reared from nymphs collected from *c.* 4-5 different locations and reared for 12 months (*c.* 7 generations) on wheat before they were used, whereas the nymphs used in Chapters 6 and 7 were *c.* 90% second and third generation respectively, of field-caught grasshoppers from

one location (Griffith District, NSW) with the other 10% from the long-term colony. It is not known if the selection pressures for laboratory-reared insects were responsible for the nymphs responding differently to the diets. It has been observed that laboratory cultures differ from field insects in their responses to insecticides (Hunter *pers. comm.*).

Australian plague locusts and Mitchell grasslands

Button grass appears to be relatively 'undefended'. Locusts may consume more Button grass since the higher content of water may increase the proportion and rate of protein assimilated, leading to shorter intermeal durations. However, consumption of the same dry weight of both grasses resulted in Button grass losing significantly more photosynthetic area. Under the experimental conditions, Button grass appeared able to convert inorganic carbon to organic matter at three times the rate of Mitchell grass (*unpub. data*). Therefore, for a typical meal by a locust, Button grass would lose twice the leaf area of Mitchell grass, but the remaining leaf tissue can photosynthesise three times faster. Also, intake was negatively correlated with plant protein concentration, i.e. locusts compensated for reduced nutrients by increasing consumption. Plants can either divert resources from their growth and reproduction into 'defence', or use resources to replace lost tissue (Herms and Mattson 1992). Which strategy a plant 'adopts' is a function of the cost of leaf replacement versus the likelihood of being removed by herbivores. The cost of manufacturing a leaf is related to its life span (Chabot and Hicks 1982), the size and shape of that leaf (i.e. % photosynthetic area it represents) (Mooney and Gulmon 1982) and available resources (Coley *et al.* 1985). Although Mitchell grass is a perennial, its growing season is only slightly longer than that of Button grass, and it needs to assimilate sufficient nutrients to survive the dormant 'dry' period, the length of which is highly unpredictable (Hunter *et al.* 2001). Button grass appears to have adopted the strategy of rapid growth and replacement of tissue if it is lost. Mitchell grass appears to grow more slowly as it allocates more to below-ground than above-ground biomass (*pers. obs.*). Given the annual habit of Button grass it needs to grow rapidly to complete its lifecycle. Therefore, rapid growth may have been selected over expensive leaf construction, with consequent effects on its nutritional quality for a locust.

In the arid and semi-arid interior where Mitchell grasslands are found, rainfall events are highly variable, both spatially and temporally. Both the grasses and locust have developed behavioural mechanisms to resist the stress of low rainfall. Rainfall is the best predictor of locust outbreaks (Hunter *et al.* 2001). It has been observed that the longer Button grass remains green, the more likely plagues are to develop and this has been attributed to the extended period of Button grass availability (Hunter *pers. comm.*; Symmons and McCulloch 1980). However, interpretation of this pattern is confounded by the fact that the longer Button grass remains greener, the longer Mitchell grass will also remain green. In addition, higher soil moisture has been correlated with increased plant quality (nitrogen and water) (Phelps and Gregg 1991). Therefore, it is not clear whether the controlling factor of plaguing is due to the extended period of Button grass availability, or the extended period of Mitchell grass availability, the enhanced nutritional quality of both grasses or some combination of these. This is consistent with some limited field-based and laboratory data that showed no difference in preference for either species (*unpub. data*).

Plant anatomical structure appears to have the potential to inhibit nutrient assimilation. Plant protein concentration appeared to control intake, with other nutrients assimilated being a product of this. However, further investigation is needed to fully understand how the rate of nutrient transfer across the gut wall is influenced by the interaction of cellular structure and protoplasm nutrient content. The control a locust has over gut emptying rate also requires further investigation because if this is a passive consequence of the rate of nutrient transfer across the gut wall, this is a potential plant resistance trait. Reductions in nutrient assimilation can lead to a supernumerary larval instar (e.g. Safranek and Williams 1984), which for any insect that is attempting to exploit a temporary resource and is able to move in the adult stage to a more suitable environment (e.g. lepidopterans, orthopterans and coleopterans), could lead to localized extinction.

Australian plague locusts develop in an environment in which the nutritional quality decreases and their ability to assimilate nutrients diminishes. If decreasing digestibility is a result of increase in size, then females that are bigger and develop more slowly are more likely to be affected by reductions in diet quality.

Digestive strategies and nutritional ecology

Compensation can maximize net nutrient assimilation when an animal has limited opportunity to select among diets due to patchiness of the environment, limited mobility and/or food resources are scarce. Rate limiting processes in the allocation of nutrients to growth are (1) intake (nutrient flow from the environment to the gut), (2) assimilation (nutrient flow from the gut to the haemolymph), and (3) metabolic costs. Compensation is achieved by altering either pre- or post-ingestive processes. Evidence that insects can actively alter pre-ingestive mechanisms such as switching diets (Cohen *et al.* 1987; Lee *et al.* 2002; Simmonds *et al.* 1992; Simpson *et al.* 1988a; Simpson *et al.* 1990) or adjusting food intake (McGinnis and Kasting 1967; Slansky and Wheeler 1991) is strong. Whether insects actively regulate post-ingestive processes or passively respond to the nature of the diet is more difficult to determine (Raubenheimer 1992; Simpson and Simpson 1990; Yang 1993).

The total amount of nutrients an insect can assimilate is the product of the amount of food ingested and its digestibility. The rate of nutrient assimilation is generally thought to be regulated by the food retention time (Batzli *et al.* 1994; Raubenheimer and Simpson 1996; Raubenheimer and Simpson 1998; Yang and Joern 1994c), which in turn influences intake. The rate of nutrient assimilation has been hypothesized to be sigmoidal function when an organism consumes food in discrete meals (Fig. 8.11). A sigmoidal curve is also predicted for processes that are limited by enzymatic reactions and this curve shape has been recorded in locusts for protein and carbohydrate assimilation from the gut to the haemolymph (Raubenheimer and Simpson 1998). The rate of assimilation of a whole meal reaches its maximum where a tangent to the curve, forced through the origin, has the highest slope ('power' = slope of the line P). Maximum accumulated power over successive meals is achieved when the gut is voided at t_1 and a new meal taken. The maximum amount of nutrients per meal ('maximum efficiency' = slope of line E) are assimilated if the food is retained until t_2. Voiding the gut at t_1 rather than t_2 means that potentially available nutrients are 'wasted'. The lower the gradient of lines P and E, the greater the difference between t_1 and t_2 (Raubenheimer and Simpson 1996; Raubenheimer and Simpson 1998; Reynolds and Nottingham 1985; Sibly 1981; Slansky and Feeny 1977)

It has been argued that diet digestibility is not actively controlled by the insect, rather it is a result of 'changes in the interactive digestive tactics to maintain an optimal digestion rate' (Yang 1993). However, it has not been demonstrated whether a locust can maintain an optimal assimilation rate, nor does this model include metabolic costs. Thus, for a locust to maximize nutrient assimilation, it needs to maximize nutrient acquisition and minimize costs. This may be why insects tend to reduce intake when faced with diets where the nutrients are extremely dilute. Although increased intake provides more nutrients, the costs to do this may be too high for the reductions in assimilation that may occur with rapid gut passage rates (McGinnis and Kasting 1967; Timmins *et al.* 1988).

Locusts feed in discrete bouts (meals). Thus the ingestion of a meal is followed by a period without feeding (intermeal duration) which is fairly easy to distinguish (reviewed by Simpson and Raubenheimer 2000). Typically a locust begins feeding and continues until negative feedback from food consumption results in the cessation of feeding (Fig. 8.12). As food is pushed into the foregut, it distends the gut, which stimulates stretch receptors and triggers the release of various hormones (Mordue 1969; Simpson and Bernays 1983; Simpson and Raubenheimer 2000). The cessation of feeding results from input from the stretch receptors (Bernays and Chapman 1973a; Bernays and Chapman 1974b; Roessingh and Simpson 1984), reduced chemosensory input from the mouthpart sensilla (Bernays and Chapman 1972b; Bernays *et al.* 1972) and rapid changes in haemolymph osmolarity and nutrient concentration (Abisgold and Simpson 1987; Bernays and Chapman 1974a; Bernays and Chapman 1974b) (Fig. 8.12 step 1). Most digestion occurs in the crop (Simpson and Bernays 1983) with enzymes being 'pumped' into there from the caeca. Fluids pass back down the gut very quickly (Baines *et al.* 1973; Simpson 1983b). The absorbed nutrients increases the osmolarity and nutrient concentration of the haemolymph that directly inhibits feeding by acting on the mouthpart sensilla and indirectly through the central nervous system (Fig. 8.12 step 2). Haemolymph osmolarity and concentration of specific nutrients declines as the absorbed nutrients are processed by the fat body and allocated to growth, other life activities or excreted through the malpighian tubules. Thus, the rate at which this occurs will affect the movement of a meal through the gut (Fig. 8.12 step 3).

The foregut and hindgut have musculature that can move food along the gut. However, it is thought the midgut musculature is too weak to do this and food is primarily propelled through this section by the ingestion of the next meal (Fig. 8.12 step 4 and 5) (Anderson and Cochrane 1977; Baines 1979; Chapman 1985a). Egestion itself can stimulate feeding (Simpson 1983a; Simpson and Ludlow 1986). Thus it has been hypothesized that the rate at which the crop empties determines the intake rate (Bernays and Chapman 1974b).

It appears that locusts maximize efficiency rather than void the gut earlier in order to maximize digestion rate (Raubenheimer and Simpson 1998; Zanotto *et al.* 1993; Zanotto *et al.* 1994). Thus the rate of nutrient assimilation (Fig. 8.13) may be a result of factors controlling the emptying rate of the foregut (Abisgold and Simpson 1987). Where the digestibility of diets are equivalent, nutrients will be accumulated at a faster rate the quicker they can be transferred from the gut to the haemolymph (Fig. 8.13). Equivalent amounts of Button grass and Mitchell grass were assimilated per meal (Chapter 4) but the intermeal duration was longer (Chapter 5).

If a locust cannot maximize assimilation rate directly by controlling when it voids food from the gut, it may be able to manipulate this indirectly via the amount it processes the food or by phenotypic plasticity (Yang and Joern 1994b). For *Locusta migratoria*, meal duration has been recorded as generally being very constant but meal size is highly variable even when restricted to a single diet (Simpson 1982b; Simpson and Bernays 1983; Simpson *et al.* 1988a), suggesting that meals are ingested at different rates. Whether this means that locusts are able to vary how they chew or process a meal remains to be determined.

The cellular structure of the diet influenced locust growth by reducing both the amount and rate of available nutrient assimilation. The cellular anatomical structure determines the cell size and the amount of water present. The rate of food intake and assimilation was increased when (1) extra water was consumed, and (2) the cellular structure was removed (Chapter 7). Thus nutrient assimilation may be limited by the rate nutrients are transferred from the gut to the haemolymph. The rate of decline resulting from negative feedback from gut stretch receptors does not appear to be related to differences in the rate of passage of food through the gut (Simpson and

Abisgold 1985). Instead, food retention time appeared to be a consequence of haemolymph osmolarity and concentration of specific nutrients.

It was hypothesized that the longer intermeal duration of nymphs feeding on Mitchell grass in Chapter 4 may have been due to either the nutrients being released and absorbed more slowly and/or the reduced water that was consumed with Mitchell grass (Fig. 8.13). This would have resulted in the nutrient concentration in the haemolymph being (1) at elevated levels for longer, or (2) higher for nymphs feeding on Mitchell grass compared to those on Button grass. Therefore, the limiting step may be the ability of the fat body to metabolise nutrients and thus reduce the negative feedback preventing gut emptying and subsequent feeding. The latter is unlikely as nymphs on the powdered diets consumed more nutrients and at a faster rate, suggesting that nutrients are rapidly removed from the haemolymph (Fig. 8.14). Also, when the nymphs were fed on the powdered form of both diets and allowed to maintain their own water balance, nymphs consuming Button grass assimilated significantly more wet weight due to them having more water per unit dry matter. However, while these nymphs (with increased water and thus more dilute haemolymph) consumed more protein, overall nutrient assimilation and rate of assimilation was no different to nymphs consuming powdered Mitchell grass with less water per unit dry matter. This again suggests that intake rates are controlled by factors influencing uptake of nutrients by the haemolymph, rather than the rate of nutrient removal from the haemolymph.

It has been argued that when the nutrient constituents of the diet are present in differing proportions, the amount assimilated will be determined by the time the diet remains in the gut (Fig. 8.15) (Raubenheimer and Simpson 1996; Raubenheimer and Simpson 1998). In a diet where nutrient X, Y and Z are present in the proportion of 1:2:2.25, if gut emptying rate is controlled by the cessation of assimilation of nutrient X, then assimilation of all of nutrient A_X at t_1 will result in an equivalent amount of nutrient Y being assimilated (A_Y, Fig. 8.15). However, at t_1 only A_Z of nutrient Z will be assimilated (Fig. 8.15). This assumes all nutrients ingested can be assimilated.

The two grasses have equivalent amounts of protein on a dry weight basis but differing amounts of non-structural carbohydrates (Chapter 4). For the same sized meal, Instar V nymphs feeding on Button grass should assimilate equivalent amounts

of protein and non-structural carbohydrates although they are present in the plant in the ratio of 1:2.0 (Fig. 8.16). Mitchell grass had protein:non-structural carbohydrates in the ratio of 1:2.2 (Chapter 4). The intermeal duration was longer for Instar V nymphs feeding on Mitchell grass than on Button grass (Chapter 5). Nymphs feeding on both diets digested the same proportion of protein (Chapter 4) but this was achieved more quickly from Button grass (t_1) than Mitchell grass (t_2) (Fig. 8.16).

The pattern indicated in Fig. 8.16 assumes that the amount of protein and carbohydrate available for assimilation and that the rate of assimilation is the same (equal digestibility of the non-cell wall component was measured for both grasses (Chapter 4)). If this occurs then this would result in nymphs on Mitchell grass assimilating (1) less than *c.* 30% of the non-structural carbohydrates ($AM_{g_{cho}}$) ingested ($IM_{g_{cho}}$), and (2) more non-structural carbohydrate ($AM_{g_{cho}}$) than would have been assimilated (A_c) per meal if the gut had been voided at the same time as for Button grass. From the data collected for Chapter 4, the digestibility of the non-structural carbohydrates was measured for the Instar V nymphs and it was found that *c.* 55% was digested from Button grass and *c.* 42% from Mitchell grass.[2] This is slightly higher than that predicted from the model, suggesting that carbohydrate was being assimilated faster than protein or that carbohydrate assimilation may have increased towards the end of the instar (Simpson 1983b) and the intermeal duration may have been longer during this time. Assimilation of nutrients was determined over an entire instar while the intermeal duration was determined on Day 3 of the instar. The increased time to assimilate carbohydrate may be the reason that later instar nymphs feeding on Mitchell grass took longer to develop than earlier instar nymphs.

Over an instar of the same duration the nymphs consuming the powdered diet ingested more (Chapter 7). Assuming equivalent meal sizes, nymphs consuming whole leaf blades would have had a longer intermeal duration (Fig. 8.17). The meal duration ($t_{M_{gp}}$) as illustrated in Fig. 8.17 suggests that a lower proportion of carbohydrate would have been assimilated from the powdered diet than from the

[2] Frass samples (n = 10) from Instar V nymphs consuming Button grass and Mitchell grass were analyzed as previously reported (Chapter 2) for protein and non-structural carbohydrate.

whole blade diet where the meal duration was longer (t_{Mgw}). Analysis showed that more protein was digested from the powdered Mitchell grass (*c.* 86%) than from the whole blades (*c.* 78%). A higher proportion of non-structural carbohydrates was digested from the powdered Mitchell grass (*c.* 65%) than from the whole blades (*c.* 51%)[3], even though the reverse was predicted from the protein:carbohydrate ratio (Fig. 8.17). This suggests that nutrients were rapidly assimilated from the gut and meal duration was increased due to limitations in returning the haemolymph to a pre-meal state. That is, when the gradient of the slope of assimilation is very steep, small increases in intermeal duration will result in increased assimilation of the non-limiting nutrients in larger proportions than the limiting nutrient. Again, it suggests that non-structural carbohydrates are assimilated at a faster rate than protein or this model does not account for differences in assimilation within an instar.

The amount of nutrients ingested correlated with the amount and rate of insect growth (Fig. 8.18a, b). Insect growth was positively correlated with intake, while developmental rate correlated negatively with Mitchell grass intake. Intake appears to be controlled by the rate of transfer of nutrients from the gut to the haemolymph. Digestibility of a diet is influenced by (1) its anatomy and (2) the degree the cell walls are fractured. The anatomy affects the accessibility of nutrients and affects the amount of water per unit dry matter. It seems that locusts can only control the rate of assimilation through changes to absolute diet digestibility. Thus the action of the mandibles and degree to which a locust can process a diet may be critical. Older, larger nymphs were more susceptible to limitations in nutrient attainment than the early instars, although the exact reasons behind this are not known. If being smaller allows increased nutrient assimilation then there are conflicting selection pressures being applied to the locusts. That is, a larger head allows an increased rate of nutrient assimilation and this maybe important in the avoidance of predators, by reducing exposure time. In addition, a larger head accommodates bigger muscles necessary for the fracture of tough plants. However, a smaller head and mandibles may increase the efficiency with which nutrients are extracted by increasing the number of cell walls fractured.

[3] Frass samples (n = 5) from Instar V nymphs consuming whole and powdered Mitchell grass were analyzed as previously reported (Chapter 2) for protein and non-structural carbohydrate

It has been well established that locusts will switch diets to balance nutrient inequality. However, if an insect is unable to switch diets then the accessibility of protein will influence its growth and reproduction and secondly, will influence the amount of 'damage' an insect will inflict on its host plant. The rate at which protein is transferred from the gut to the haemolymph appears to control gut emptying and subsequent intake, and thus nutrient assimilation. Gut passage rate will not only affect the amount of nutrients assimilated, but also the relative proportions of the major chemical constituents. Insects restricted to a single host need to either synchronize development when plant conditions are most favourable for growth, or develop mechanisms to increase the rate of nutrient transfer across the gut wall. Thus, insects may be able to actively regulate post-ingestive processes through the degree of processing. Removing the need to fracture the cell wall decreased metabolic costs, suggesting that this could be critical in models of insect growth. Therefore, the mandibles and their ability to process the diet may play a more important role in regulating nutrient assimilation than previously thought.

TABLES AND FIGURES

Table 8.1 Individual effects from the hierarchical partitioning of r^2 for the predictor variables (ratio of water, cell wall material, protein and non-structural carbohydrates in the grasses offered to the locusts) against the response variables, instar duration, growth and intake for both grasses combined, Button grass and Mitchell grass.

	Duration	**Growth**	**Intake**
Water	0.018	0.007	0.050
Cell wall material	0.055	0.039	0.034
Protein	0.036	0.067	0.192
Non-struct. carbohydrates	0.189	0.254	0.047

Table 8.2 Individual effects from the hierarchical partitioning of r^2 for the predictor variables (water, protein and non-structural carbohydrates ingested by the locusts) against the response variables, instar duration, growth and assimilation for both grasses combined, Button grass and Mitchell grass.

	Duration	**Growth**	**Assimilation**
Water	0.052	0.076	0.214
Protein	0.015	0.082	0.152
Non-struct. carbohydrates	0.122	0.348	0.094

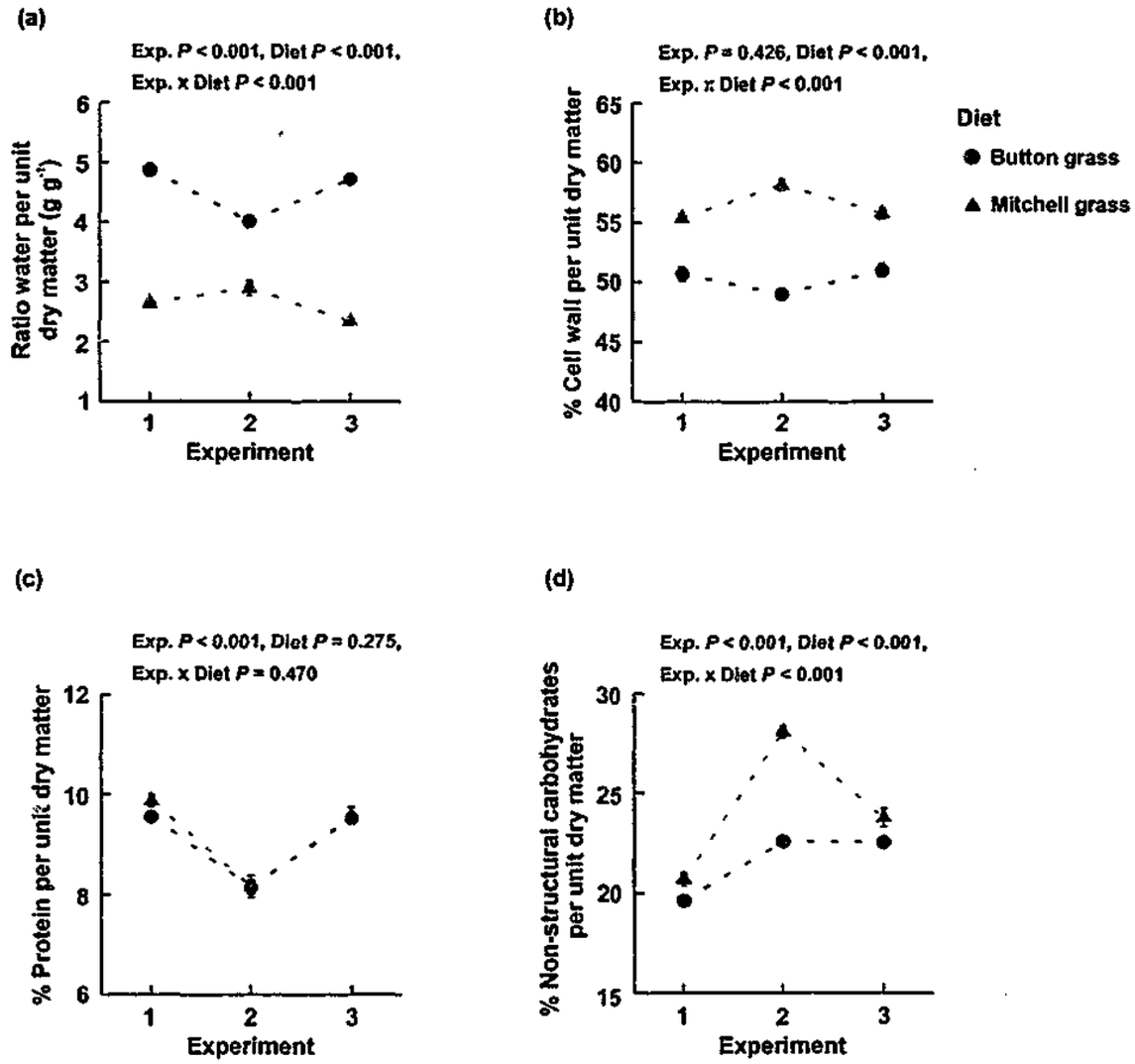


Fig. 8.1 Changes in the major constituents; (a) ratio of water per unit dry matter, (b)% cell wall, (c)% protein and (d) non-structural carbohydrates; of Button grass and Mitchell grass feed to Instar V nymphs reported in Chapters 4 and 5 (Experiment 1), Chapter 6 (Experiment 2) and Chapter 7 (Experiment 3).

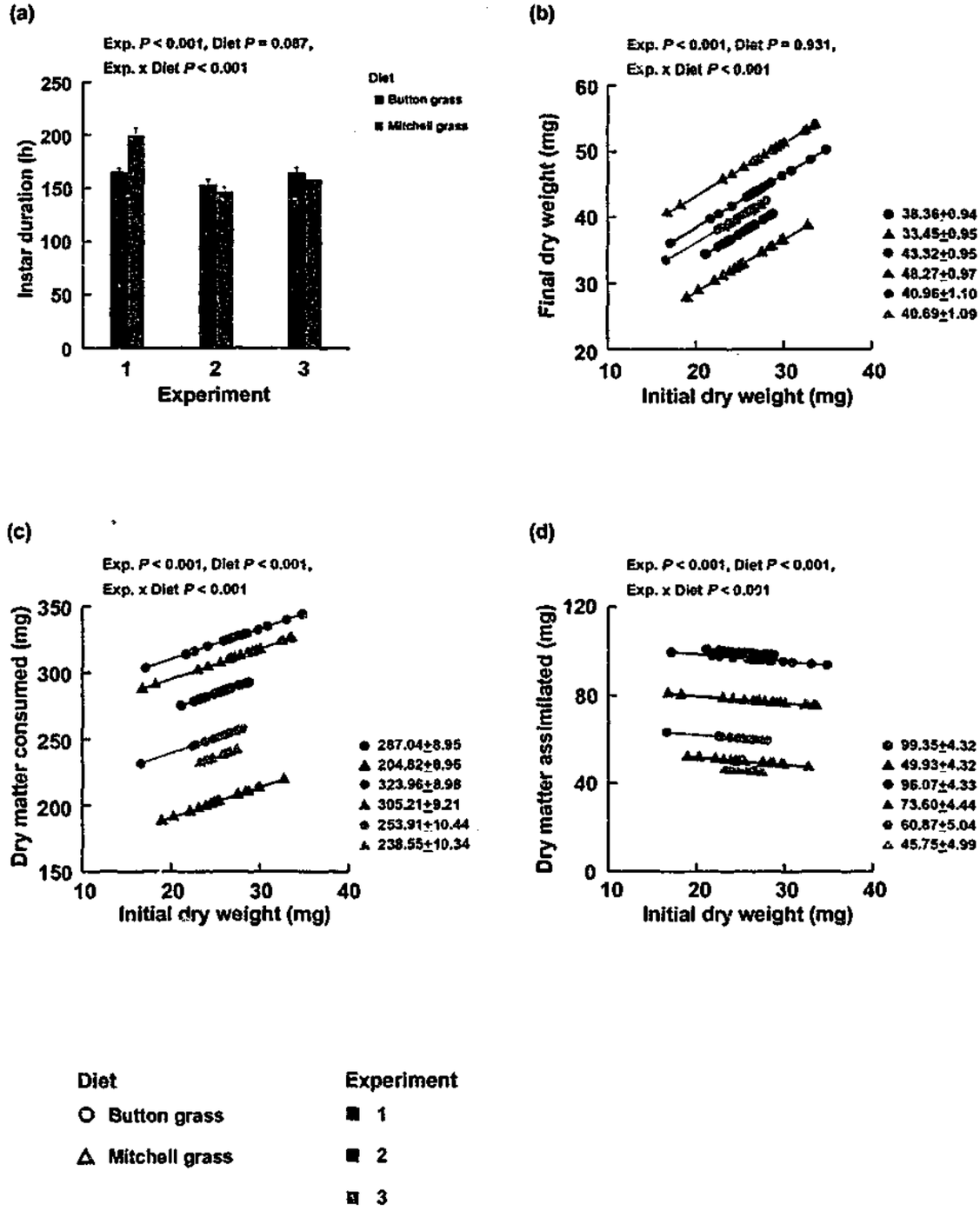


Fig. 8.2 Combined analysis of the three experiments where the nymphs were reared on wheat before being switched to either Button grass or Mitchell grass, (a) duration of Instar V, (b) final dry weight, (c) dry matter intake and (d) dry matter assimilation. Graphs (b)-(d) are the ANCOVA adjusted values with initial dry weight as the covariate. Tests for homogeneity of slopes (experiment x diet x initial dry weight) were not significant ((b) $P = 0.188$, (c) $P = 0.347$, (d) $P = 0.571$).

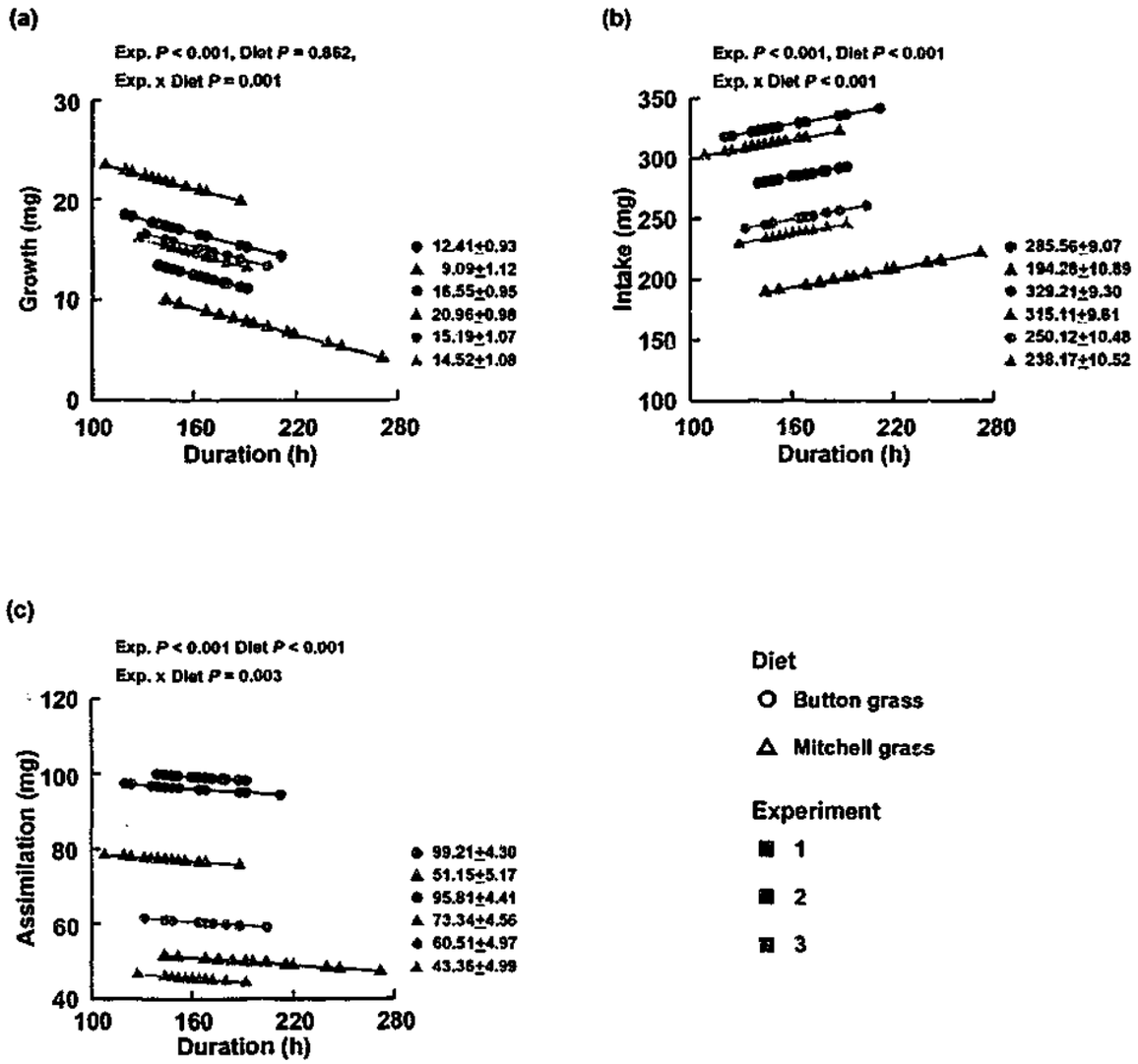


Fig. 8.3 Rates of (a) growth, (b) intake, (c) assimilation for nymphs feeding on Button grass and Mitchell grass in three trials. Tests for homogeneity of slopes (experiment x diet x initial dry weight) were not significant ((a) $P = 0.787$, (b) $P = 0.052$, (c) $P = 0.490$).

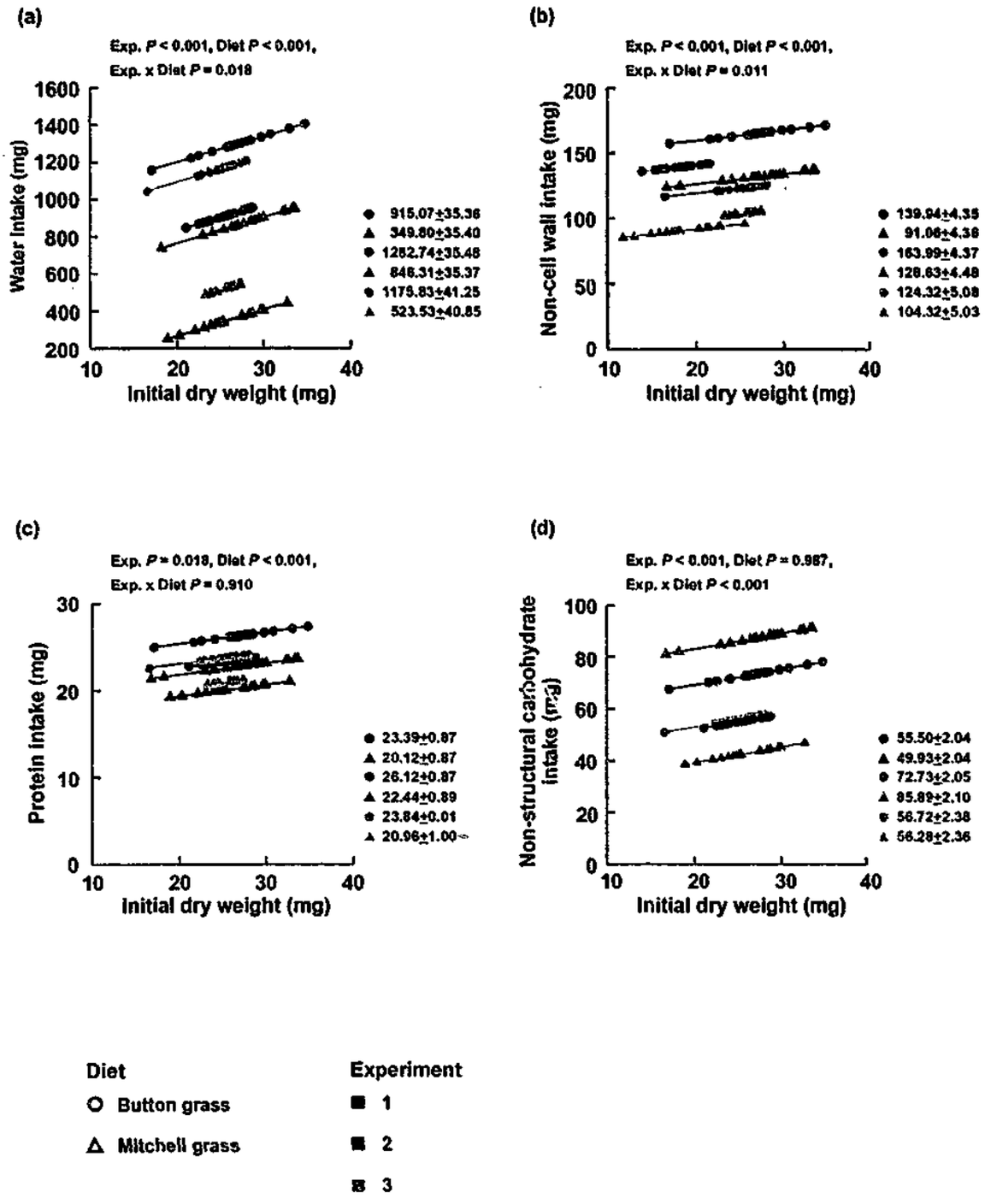


Fig. 8.4 Combined analysis of the three experiments where the nymphs were reared on wheat before being switched to either Button grass or Mitchell grass showing the ANCOVA adjusted values with initial dry weight as the covariate for intake of (a) water, (b) non-cell wall dry matter, (c) protein and (d) non-structural carbohydrates. Tests for homogeneity of slopes (experiment x diet x initial dry weight) were not significant ((a) $P = 0.772$, (b) $P = 0.253$, (c) $P = 0.314$, (d) $P = 0.500$).

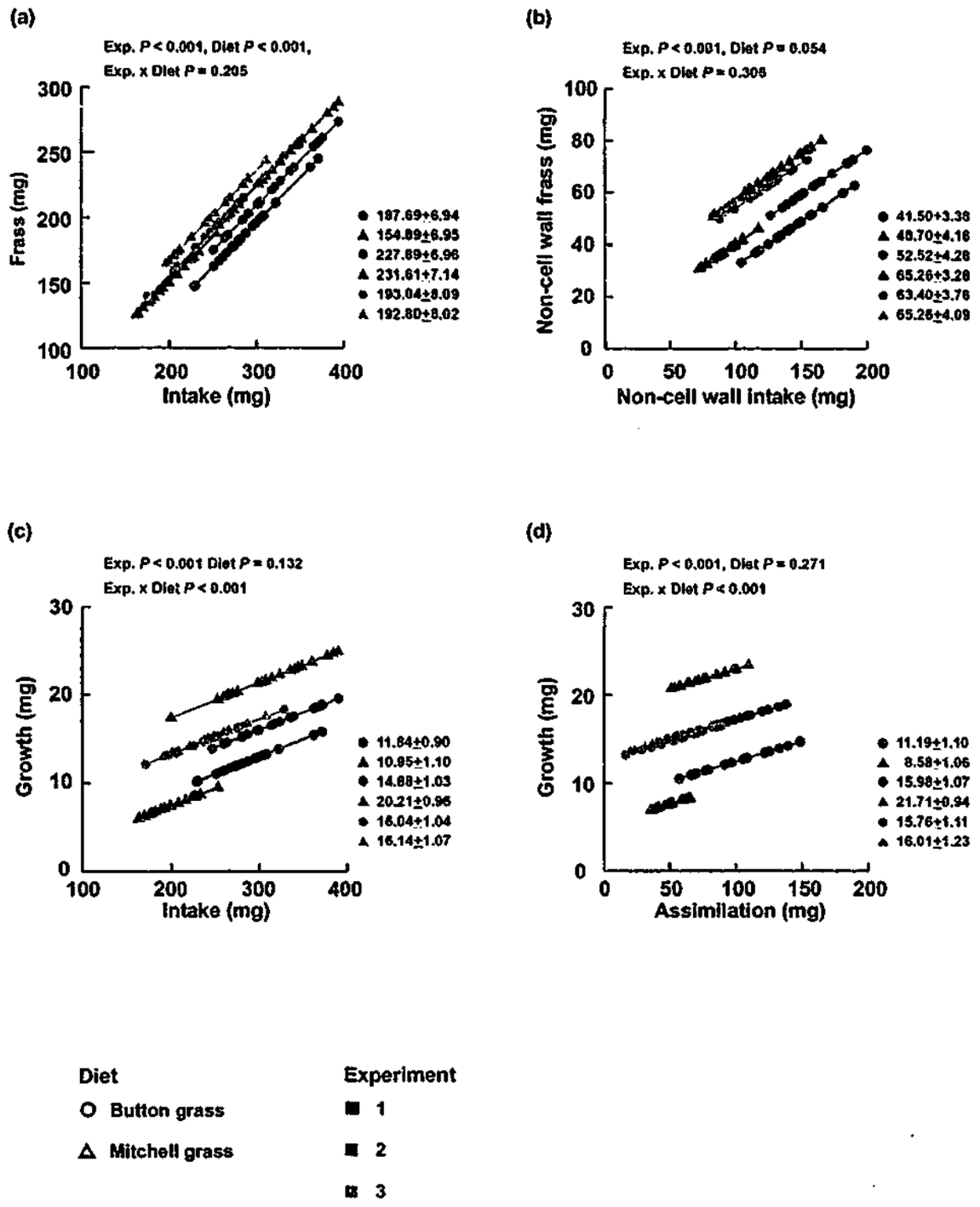


Fig. 8.5 Utilization plots of ANCOVA on (a) frass 'AD', (b) non-cell wall frass 'AD', (c) growth 'ECI',and (d) growth 'ECD'. Tests for homogeneity of slopes (experiment x diet x initial dry weight) were not significant ((a) $P = 0.427$, (b) $P = 0.587$, (c) $P = 0.650$, (d) $P = 0.622$).

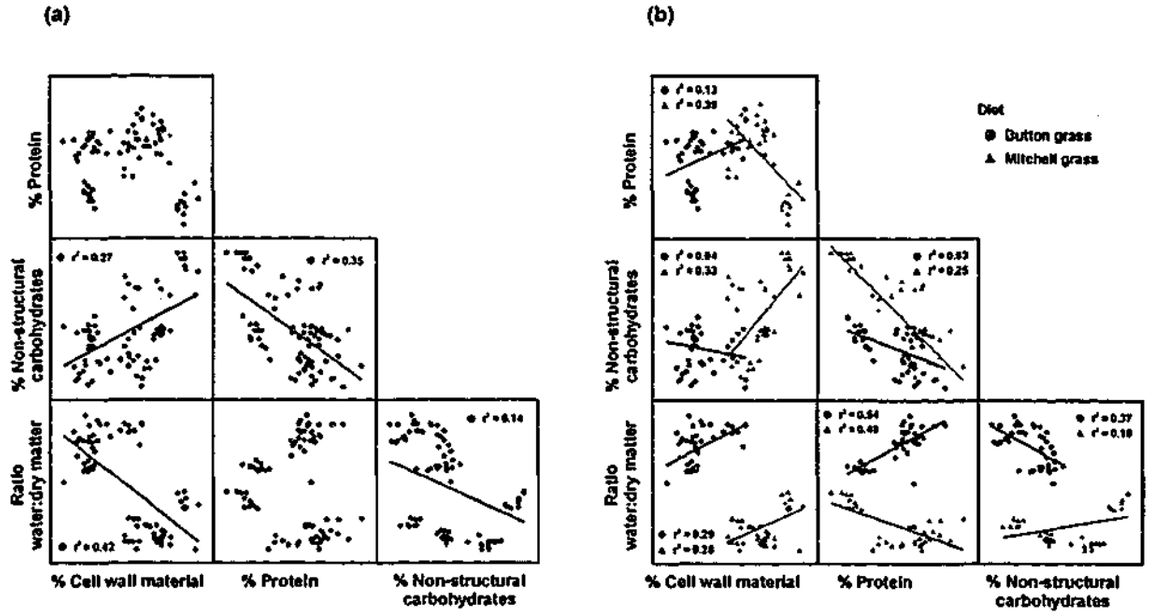


Fig. 8.6 Relationships between the major chemical constituents of the two grasses, (a) Button grass and Mitchell grass combined and (b) separate. Lines signify a significant ($P < 0.05$) relationship between the two variables being compared.

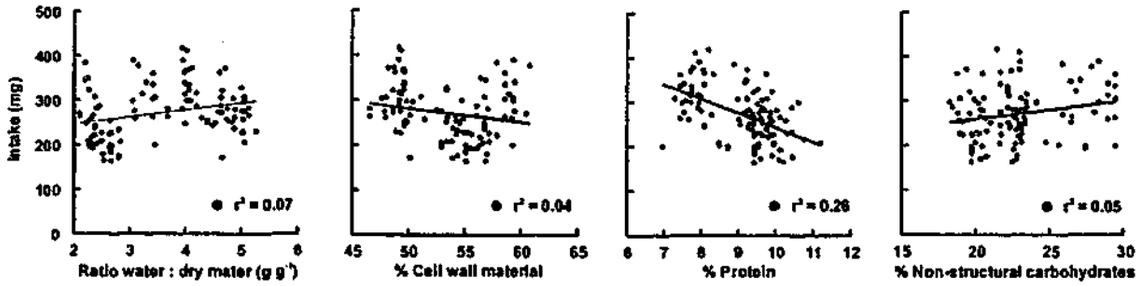


Fig. 8.7 Relationships between the major chemical constituents of the two grasses and intake. Lines signify a significant ($P < 0.05$) relationship between the two variables being compared.

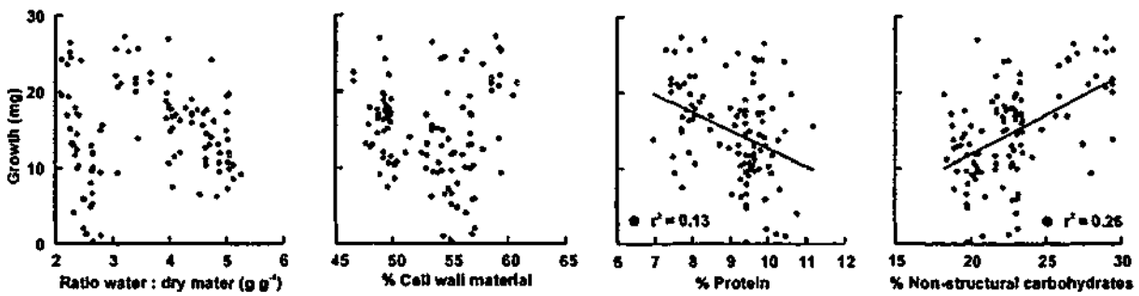


Fig. 8.8 Relationships between the major chemical constituents of the two grasses and locust growth. Lines signify a significant ($P < 0.05$) relationship between the two variables being compared.

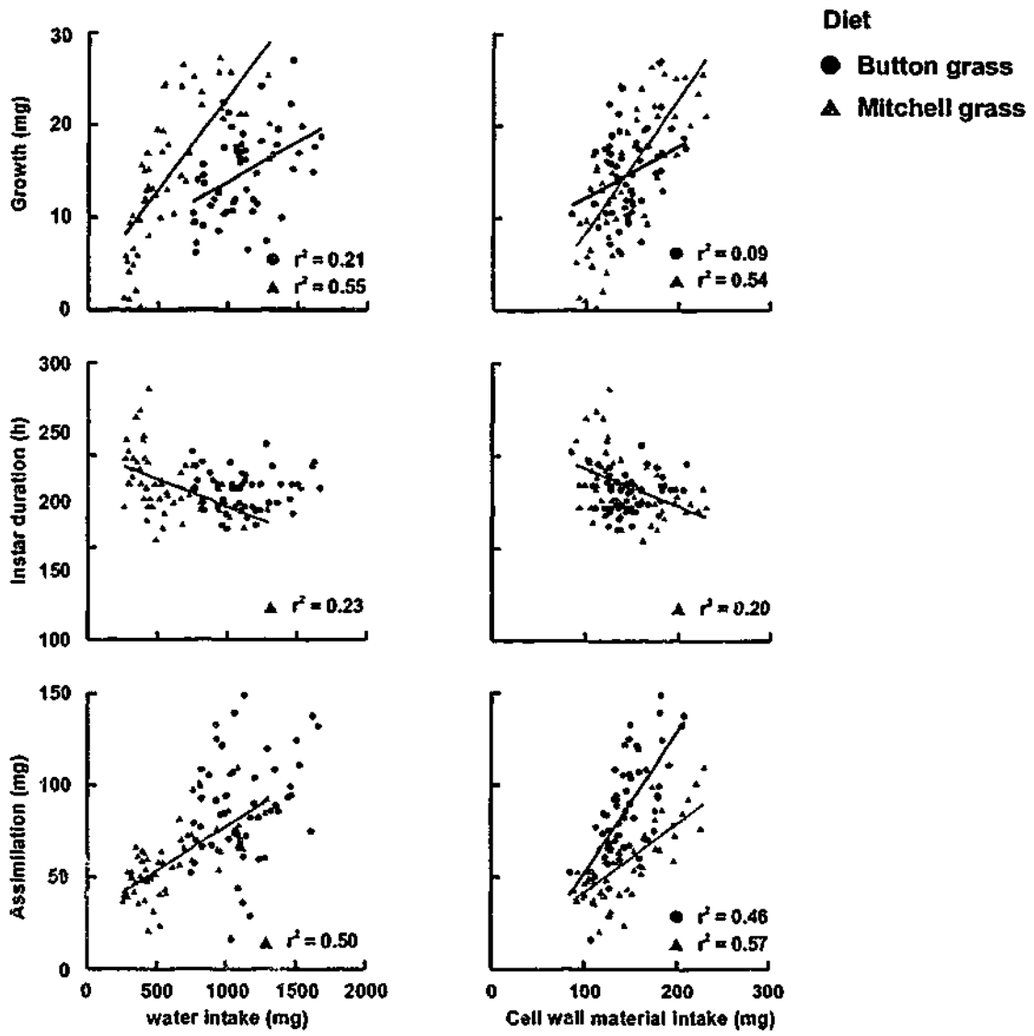


Fig. 8.9 Relationships between intake of water and cell wall material of the two grasses and locust growth, instar duration and nutrient assimilation. Lines signify a significant ($P < 0.05$) relationship between the variables being compared.

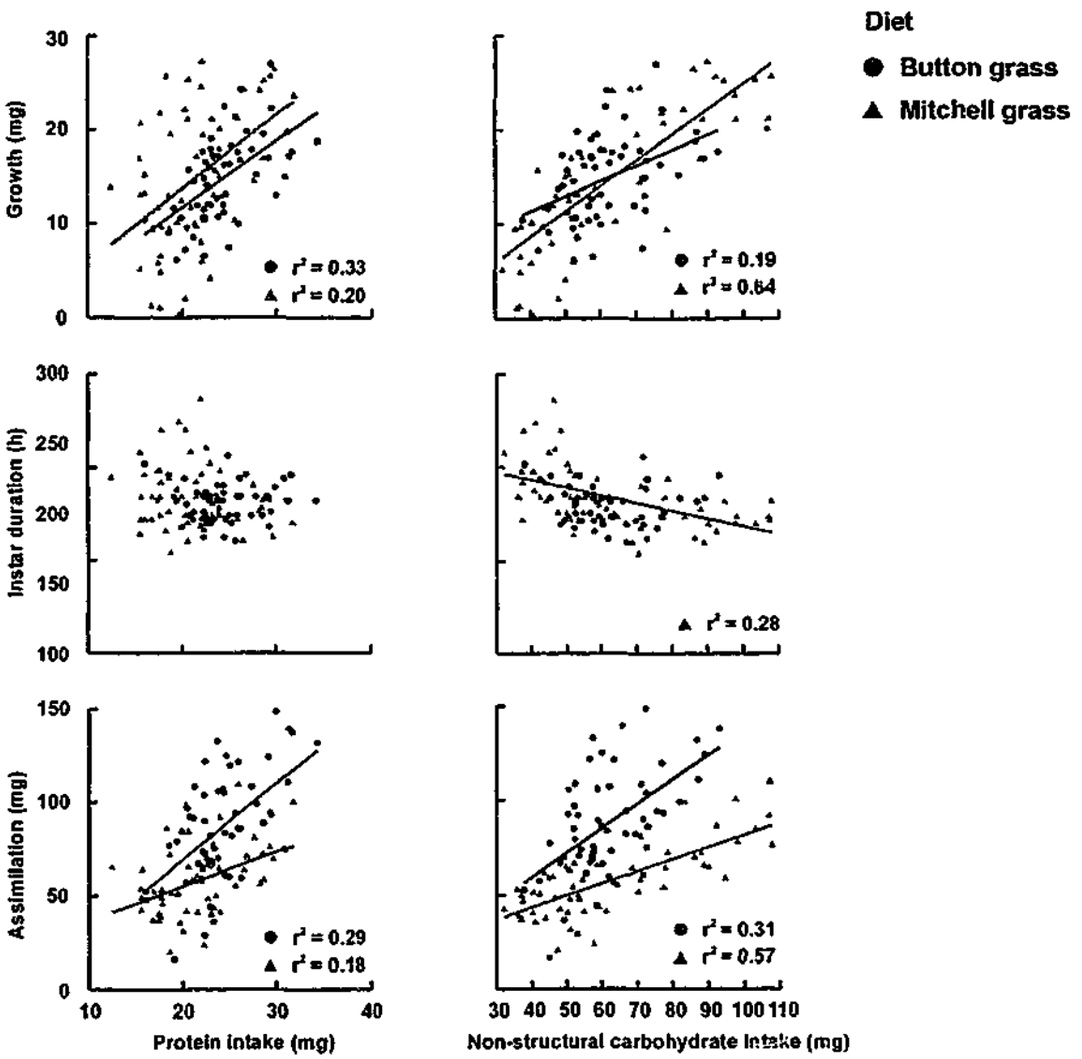


Fig. 8.10 Relationships between intake of protein and non-structural carbohydrates of the two grasses and locust growth, instar duration and nutrient assimilation. Lines signify a significant ($P < 0.05$) relationship between the variables being compared.

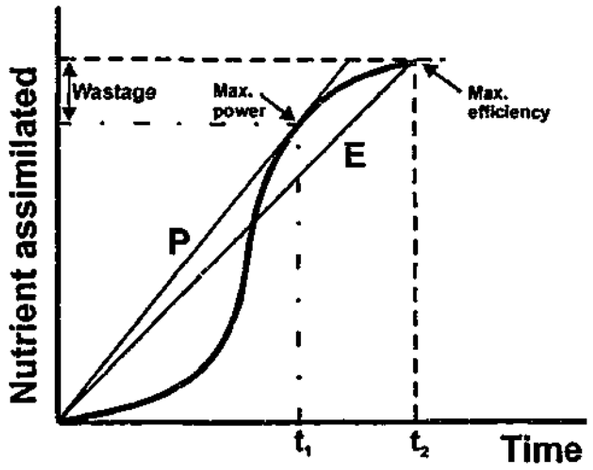


Fig. 8.11 The hypothesized relationship between time and nutrient assimilation over the course of a meal (Fig. from Raubenheimer and Simpson 1996). See text for explanation.

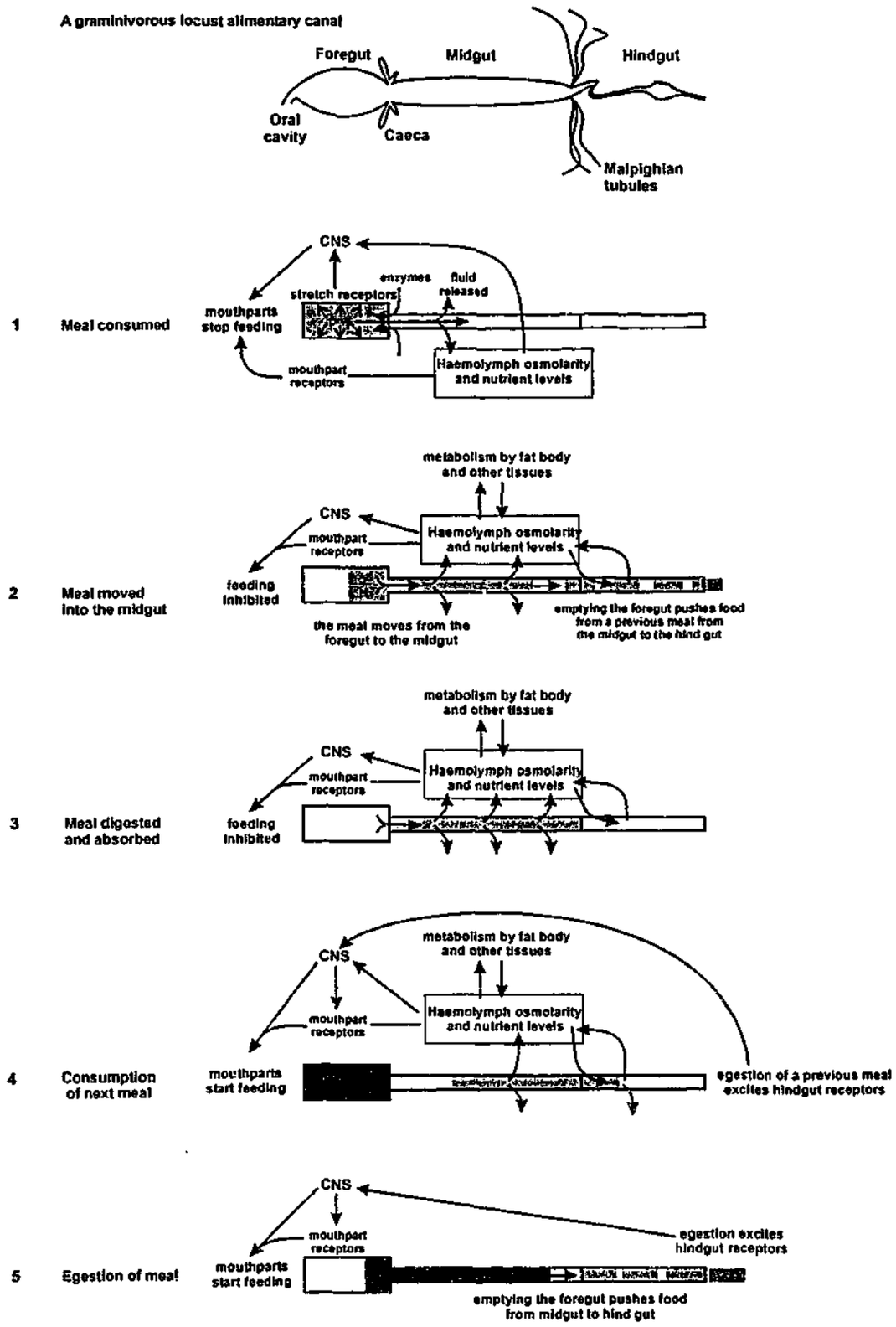


Fig. 8.12 Flow of a meal through the gut and the associated control mechanisms (*sensu* Bernays and Simpson 1982; Chapman 1988a; Jones 1981; Simpson *et al.* 1995). Refer to the text for a description.

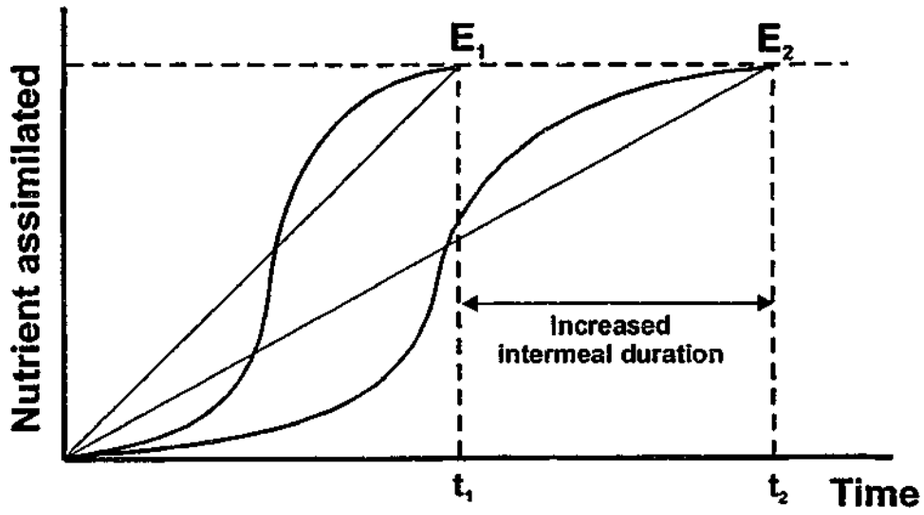


Fig. 8.13 Proposed relationship between nutrient transfer and time in diets with differing resistance to extraction of nutrients. If a locust cannot void the meal from the gut before all the available nutrients have been transferred from the gut to the haemolymph, then any factors that slow the rate of nutrient transfer will lead to increased intermeal duration, i.e. E_1 compared with E_2 (Raubenheimer and Simpson 1998).

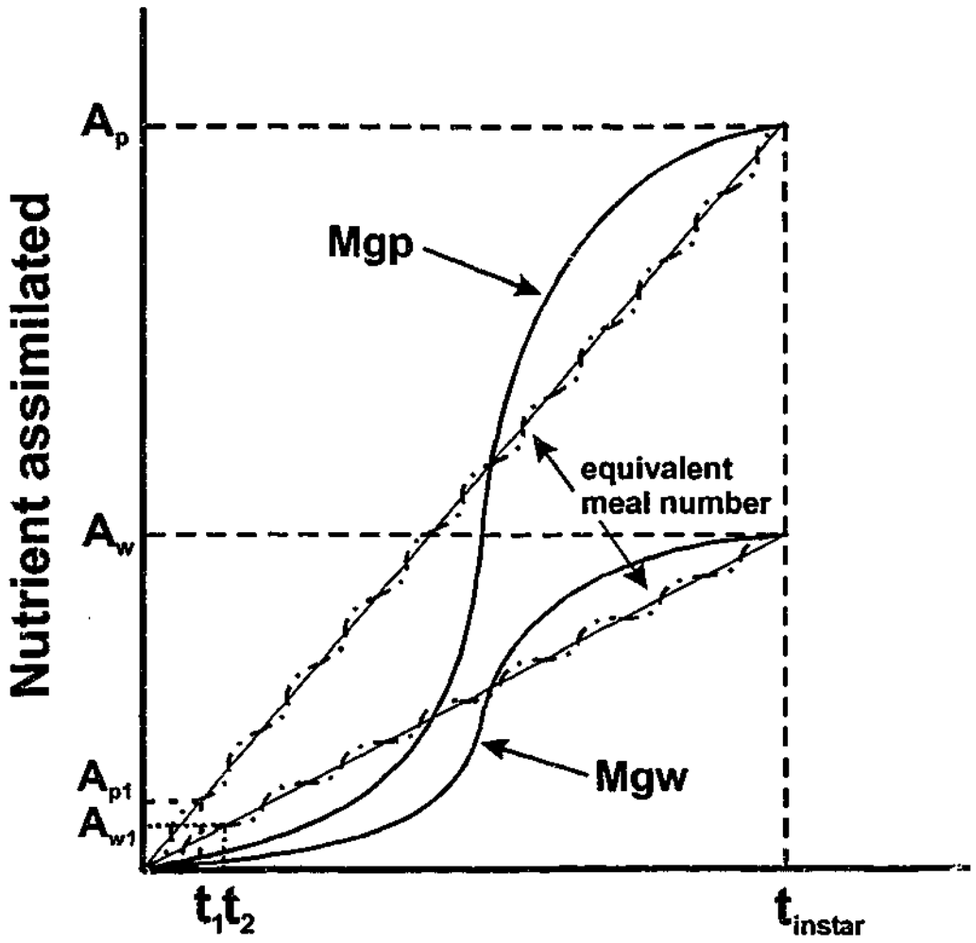


Fig. 8.14 Nutrients assimilation by nymphs over Instar V consuming a powdered Mitchell grass diet (Mgp) and whole Mitchell grass blades (Mgw). The instar duration (t_{instar}) was the same for both diets. However, the nymphs consuming Mgp assimilated A_p (*c.*132 mg dry matter) and nymphs feeding on Mgw assimilated A_w (*c.* 65 mg dry matter) (Chapter 7). Mgp was twice as digestible as Mgw (A_p vs A_w). Assuming consumption of equivalent meal sizes of both diets then the increased nutrient assimilation by nymphs feeding on Mgp resulted from both an increased diet digestibility (A_{p1}) and a faster gut passage rate per meal (t_1) compared to nymphs feeding on Mgw ($A_{w1}$and t_2). The solid lines represent the efficiency over the entire instar and the individual elements of the dashed lines represent single meals (*sensu* Raubenheimer and Simpson 1996).

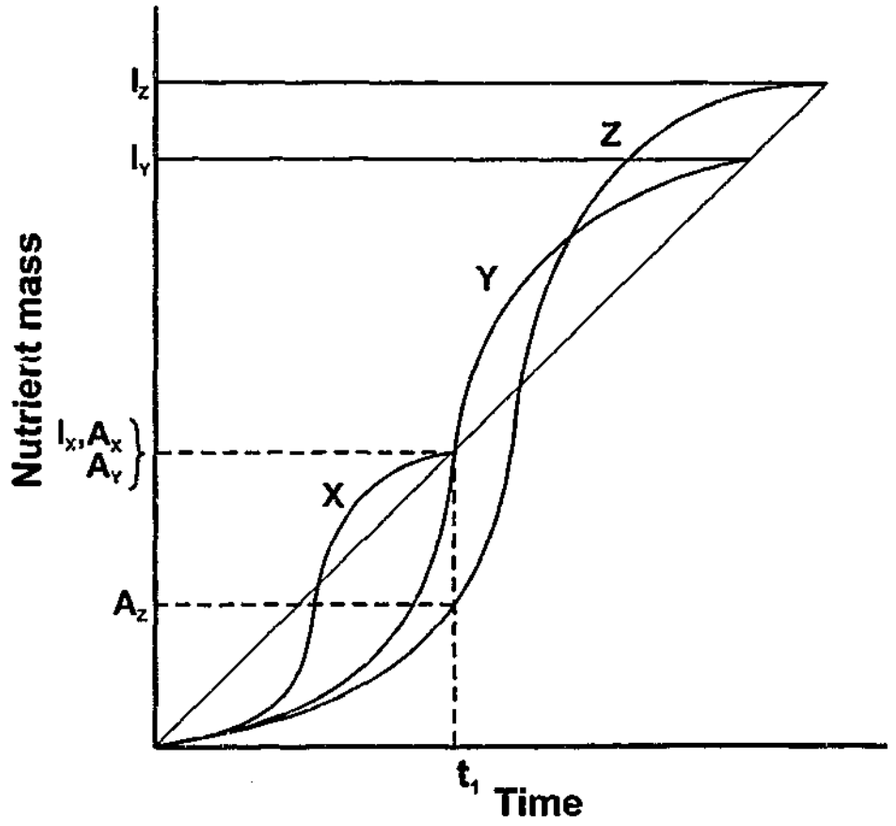


Fig. 8.15 Nutrient assimilation functions for three hypothetical nutrients, X, Y and Z, in a diet assuming equal uptake rates (*sensu* Raubenheimer and Simpson 1996). The nutrients X, Y and Z have been ingested in the ratio of 1:2:2.25 (I_X, I_Y and I_Z) respectively. Thus at t_1 the insect will have assimilated the same amount of nutrient X (A_X) as nutrient Y (A_Y) because even though only 50% of Y has been assimilated, it had double the concentration of X in the diet. However, although nutrient Z is in an even higher concentration (I_Z) than Y, the insect will assimilate absolutely less (A_Z) of it than X and Y at t_1. This model assumes that ne shape of the sigmoidal curve is the same for all nutrients. Changes in the shape of the sigmoidal curve will affect changes in the rate of assimilation.

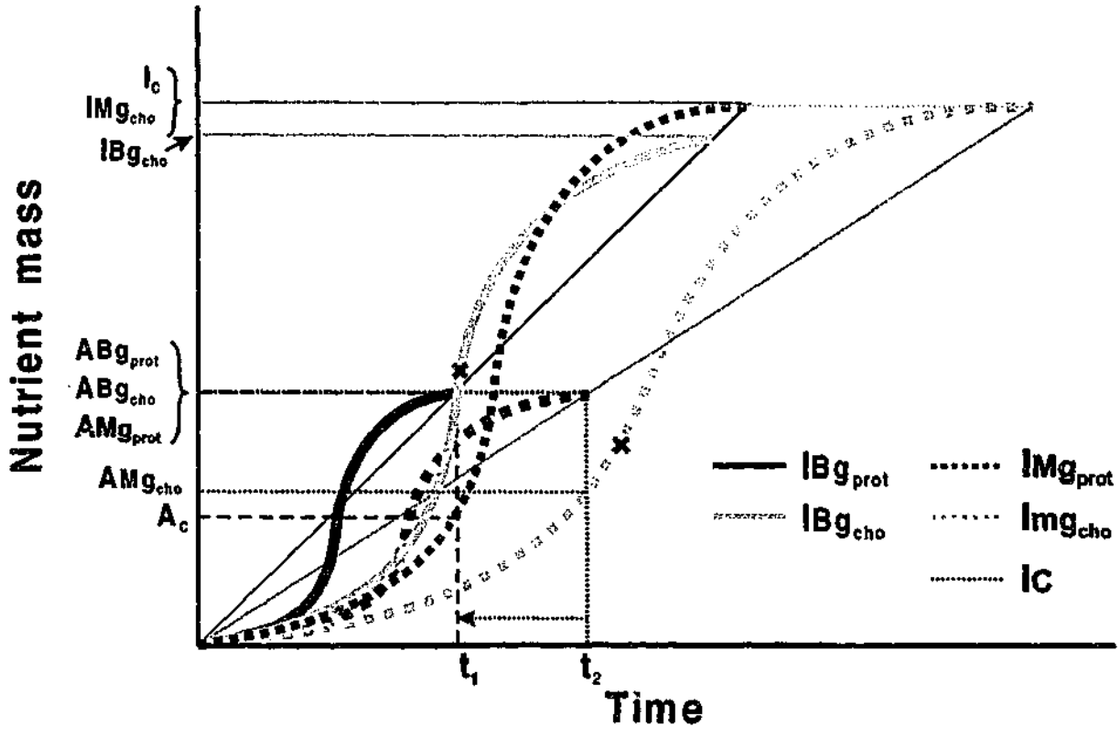


Fig. 8.16 The relationship between the proportion of protein and non-structural carbohydrates in Button grass and Mitchell grass and food retention time for Instar V nymphs. Instar V nymphs consumed equivalent sized meals of whole blades of both diets (Chapter 5) with nymphs feeding on Mitchell grass retaining food for *c.* 58% longer. This resulted in equal amounts of protein being assimilated (ABg_{prot} and AMg_{prot}) (same protein intake and digestibility (Chapter 4)). Per meal, nymphs consuming Mitchell grass ingested more non-structural carbohydrates (IMg_{cho}) than nymphs feeding on Button grass (IBg_{cho}) (Chapter 4). The model shows that the longer intermeal duration for nymphs feeding on Mitchell grass (t_2) will result in less non-structural carbohydrates being *assimilated* (AMg_{cho}) than for nymphs feeding on Button grass (ABg_{cho}), even though Mitchell grass has a higher concentration of carbohydrate (IMg_{cho}). Nymphs feeding on Mitchell grass would have assimilated even less if the intermeal duration was the same as for Button grass (t_1) (AMg_{cho} compared to A_C). I_C is what would have occurred if Mitchell grass was assimilated at the same rate as Button grass. The X marks the actual amount of non-structural carbohydrates digested by the nymphs feeding on either diet.

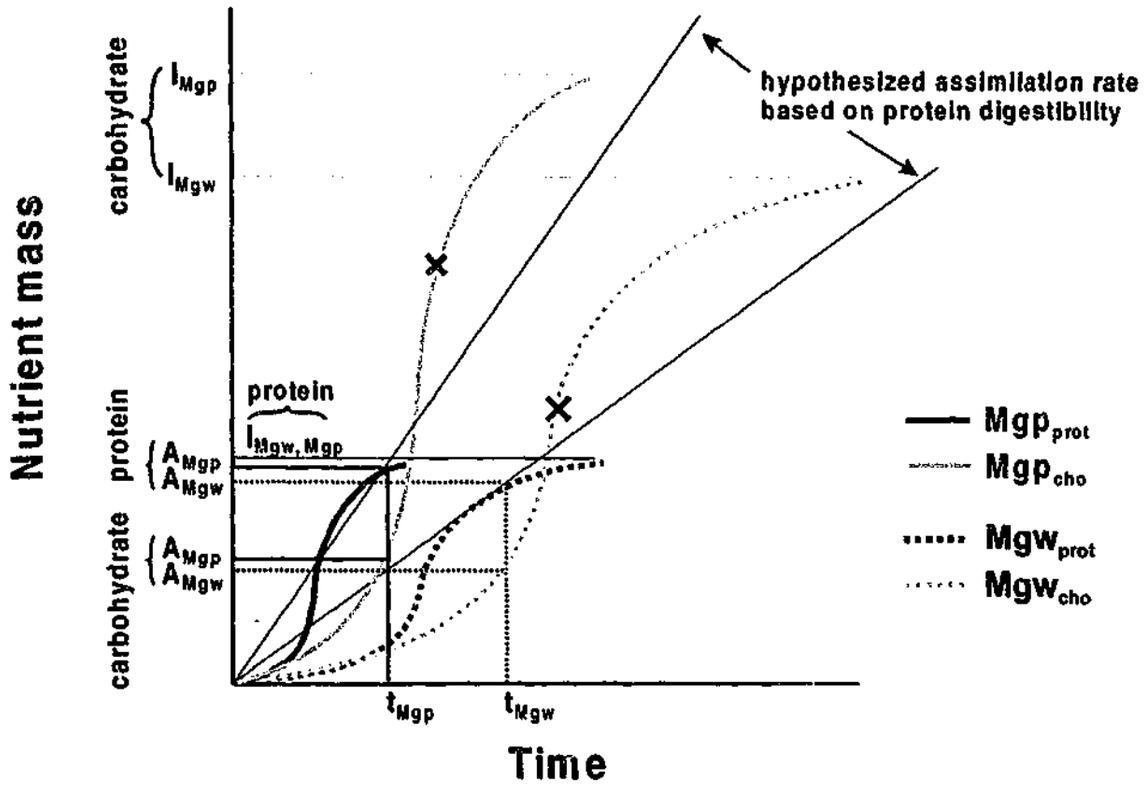


Fig. 8.17 The hypothesized relationship between the transfer functions of protein and carbohydrate from whole Mitchell grass blades (Mgw) and powdered Mitchell grass blades (Mgp). Both forms of Mitchell grass had the same amounts of protein ($I_{Mgw, Mgp}$), but powdered Mitchell grass had more carbohydrate (I_{Mgp}). The digestibility of protein was higher for the powdered diet (A_{Mgp}, protein) than the intact diet (A_{Mgw}, protein) even though the intermeal duration was shorter for nymphs feeding on powdered Mitchell grass (t_{Mgp}) compared to intact blades (t_{Mgw}). Thus nymphs consuming powdered Mitchell grass assimilated more carbohydrate (A_{Mgp}, carbohydrate) but maybe not proportionally more than nymphs feeding on intact grass blades (A_{Mgw}, carbohydrate). The X marks the actual amount of non-structural carbohydrates digested by the nymphs feeding on both diets.

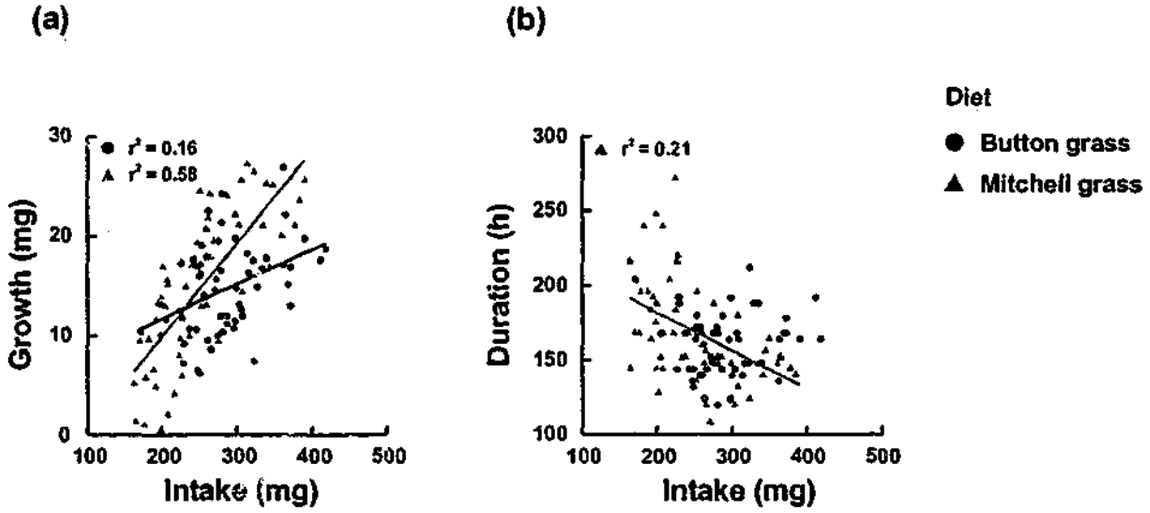


Fig. 8.18 Relationship between intake and (a) growth, and (b) instar duration for nymphs consuming both diets. Lines signify a significant ($P < 0.05$) relationship between the variables being compared.

APPENDIX I EFFECT OF PARTICLE SIZE ON DETERMINATION OF PLANT CELL WALL MATERIAL BY THE VAN SOEST METHOD

INTRODUCTION

The neutral detergent fibre method allows quick and repeatable quantification of cell wall from the more easily digestible cell contents (Van Soest *et al.* 1991). Typically, dried plant material is milled into small particles prior to chemical analysis that fractures the plant material. The homogeneity of particle sizes produced depends on the type of mill used (e.g. cutting or hammer), the type of plant, its age and previous history (i.e. has it already been 'milled' by mastication). Previous experiments have shown that different particle sizes resulting from the milling process, when separated into different size classes yield different values of Neutral Detergent Fibre (NDF) (Ehle 1982; McArthur 1988). It was also found in those studies that the larger particles had higher NDF values. Foley and Cork (1992) hypothesized that researchers who work on mammals that grind their diet very finely may be overestimating the amount of NDF digested. The amount of NDF in faeces may be underestimated compared to that in the diet when the proportion of very fine particles is much larger in the faecal sample than the milled plant sample either due to loses of NDF during the filtration process used to recover the residue or due to the correlation of particle size and NDF. However, the interpretation by both Ehle (1982) and McArthur (1988) were confounded because the difference in NDF in each particle size class could have been because (1) the different fractions represented different parts of the plant, i.e. more fibrous veins might not shatter as easily and be larger in size than that of the lamina, or (2) the size of the particle itself might influence how the detergent solution acts on it. This can be resolved by separating milled plant

material into fractions based on size and then further grinding each fraction to the same size, thus removing the effect of particle size. The aim of the following experiments was to determine if particle size affected the amount of NDF recovered from ground plant material.

Materials and Methods

Wheat was grown in a glasshouse for two weeks (Stage 12: two leaves unfolded; Tottman and Makepeace 1979). Leaf blades were harvested and freeze-dried to a constant weight, then milled, using a Tecator Cyclotec 1093 Sample Mill, to pass through a 1 mm sieve (the size suggested by Van Soest *et al.* (1991)). Sample division was performed using a Fritsch rotary fraction splitter with vibratory feeder that divides the original sample into identical sub-samples. A portion of the original sample was kept while the remainder was divided into four fractions based on particle size, < 0.2 mm, 0.2 - <0.5 mm, 0.5 - <1 mm, and > 1 mm (Fig. I.1). A dry sieve method was used, which tends to separate particles on a cross-sectional basis (Van Soest 1994b). All samples were then halved and one half was haphazardly selected and milled again using a Spex® freezer/mill (hammer-type mill) until the particles were able to pass through a 0.2 mm sieve (Fig. I.2). Cell wall was determined from four replicate *c.* 50 mg samples using the Van Soest method omitting sodium sulphite (Van Soest *et al.* 1991).

To separate the cell wall fraction from the solubilized cell, a sintered glass crucible (porosity 2) was used to collect the cell wall. To test if smaller particles were being lost through the sintered glass filter in the crucible, a trial using cellulose (Sigma C6413) was performed. This cellulose had particles ranging in size from 0.025-0.075 mm with an average of approximately 0.06mm (Sigma-Aldrich *pers. comm.*). Six replicates were used. It could be argued that the plant material was fragmented into smaller particles in the Spex® freezer/mill than occurred in the cellulose powder, so in an attempt to mimic the plant material, a trial using ashless cellulose filter paper was milled with the Spex® freezer/mill, using six replicates.

Calculated percentages of cell wall for the eight grass fractions were compared using two-factor ANOVA (two milling levels x four particle sizes) after box plots were used to check for normality and homogeneity of variances across the treatments. As there was a significant interaction between the two factors, one-way ANOVA with post-hoc Tukey's tests were performed between all eight groups. All analyses were undertaken with SYSTAT® 10.

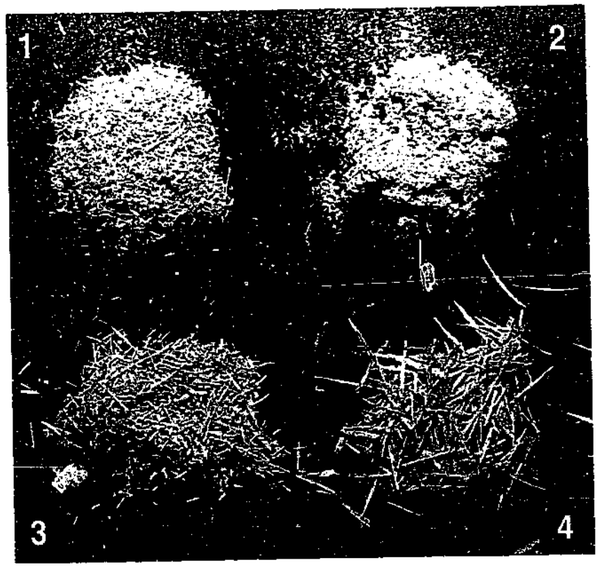
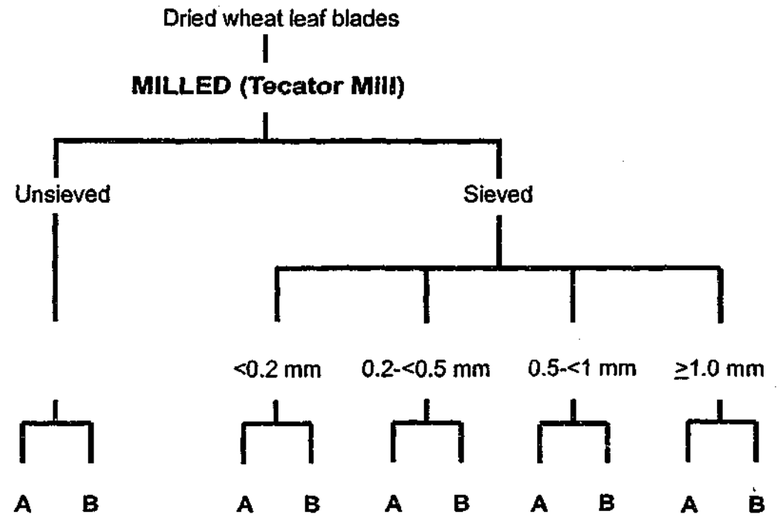


Fig. I.1 Image depicting the four different particle sizes, (1) < 0.2 mm fraction, (2) 0.2 - < 0.5 mm fraction, (3) 0.5 - < 1 mm fraction and (4) ≥ 1.0 mm fraction.



A Milled - Tecator Mill

B Re-milled to < 0.2 mm - Spex® freezer/mill

Fig. I.2 Schematic diagram outlining the method used to analyze the effect of particle size on the amount of cell wall recovered; A, milled using the Tecator mill only, B, re-milled with the Spex® freezer/mill to pass through a sieve < 0.2 mm.

RESULTS AND DISCUSSION

Analysis of the four fractions obtained after initial milling and dry sieving gave significantly different values of NDF ($F_{3,12} = 234.5$, $P < 0.001$). Tukey's post-hoc test showed that all fractions were significantly different with the biggest particles having the most cell wall (Table I.1). However, with the fractions that were reground to less than 0.2 mm, only the fraction that originally had the smallest particle size was significantly different from the other three size classes ($F_{3,12} = 15.4$, $P < 0.001$). Both fractions less than 0.5 mm were not significantly different from their re-ground counterparts. The larger the particles the greater the difference in cell wall recovered compared to their more finely ground counterpart. The amount of cell wall obtained from the original sample when analyzed after initial milling (Tecator mill) was significantly greater than when it was re-ground to pass through a 0.2 mm sieve. This was to be expected as approximately 50% of the sample was made up of particles > 0.5 mm.

It was concluded that the reduced amount of cell wall obtained with the smaller particles was not likely to be due to losses of the smaller particles through the crucibles. The particle size distribution of the Spex® freezer/milled plant samples is not known and the particles could have been smaller than the cellulose powder. Further, sieving of the Spex® freezer/milled plant material suggested the majority of particles were between 0.1-0.2 mm in size. All the powdered cellulose and Spex® freezer/milled ashless filter paper was retained 99.5 ± 0.9% (mean ± s.e.) with a 95% confidence interval of 97.6–101.4% and 100.6 ± 0.7% with a 95% confidence interval of 98.7–102.4% respectively.

These results suggest that particle size affects the amount of cell wall measured but also that when the plant is milled it fragments into different sizes related to the amount of cell wall that it contains. From the Tecator milled material, the larger particles had a higher ratio of cell wall to cell contents suggesting they are from different parts of the plant than the smaller fragments. However, the size of these particles also affects the results obtained. Larger particles gave a much higher value of cell wall than their finely ground counterparts probably because larger particles have a small surface area to volume ratio, which may prevent the neutral detergent solution from accessing the interior of fragments to remove non-cell wall material.

These results suggest that particles must be ground to be able to pass through a 0.5 mm sieve.

This pattern was observed with different aged wheat with the Tecator ground fraction giving significantly larger values for cell wall than its more finely ground counterpart; *c.* 6 week old Wheat 52.3 ± 1.6% versus 46.1 ± 1.0% and *c.* 2 week old wheat 40.6 ± 0.4% versus 37.0 ± 0.2%. Not surprisingly there was a greater difference the higher the percentage of cell wall in the dry matter. Some small mammals are known to have over 50% of the faecal particles less than 0.075 mm (Foley & Cork 1992), our results suggest analysis of diet cell wall intake would have been overestimated compared to that from the faeces.

To prevent differences in particle size affecting digestibility results, all plant and frass material was ground to less than 0.2 mm prior to cell wall analysis. It is also important to grind the plant material to a uniform small size so that (1) sub-sampling does not bias the selection of particles of a particular size due to the settling of the sample in a vial or by retention on the spatula; and (2) differences in cell wall material between different plants are not enhanced or lost by their fracture properties if a cutting mill is used.

Table I.1 Percentage of Neutral Detergent Fibre in different fractions based on particle size analyzed in its raw state and ground to a particle size of less than 0.2 mm. Results are for four replicates and those superscripted by the same letter within a column and # within a row are not significantly different (P>0.05).

Fraction	% of original (w/w)	raw state % dry matter	milled to < 0.2 mm % dry matter
< 0.2 mm	4.9	43.8 ± 0.3[#]	43.5 ± 0.1[b,#]
0.2-< 0.5 mm	49.0	45.2 ± 0.1[#]	45.9 ± 1.0[b#]
0.5-< 1 mm	43.9	50.2 ± 0.3	47.4 ± 0.7[a,]
> 1 mm	2.3	55.8 ± 0.7	47.8 ± 0.3[a,]
unsieved	100.0	48.2 ± 0.3	44.5 ± 0.6

APPENDIX II PILOT STUDY TO DETERMINE GERMINATION CONDITIONS AND GROWTH RATES OF BUTTON GRASS AND MITCHELL GRASS

INTRODUCTION:

For this experiment I required the grass blades within each species to be of a similar condition, e.g. nutrient status. As I was growing Mitchell grass from seed it was not known how long it would take for the plants to develop. I wanted to feed Button grass once it had matured seeds and Mitchell grass as it was developing seeds, the stage the grasses would be in when the locusts were in *c.* Instar III. Both species occur naturally in soils of low N and P concentration, and I was concerned that commercially available soil mixes may be too nutrient-rich. A trial was performed to grow the grasses under glasshouse conditions to determine (1) if the grasses grew 'better' when fertilized with a particular nutrient regime, and (2) how quickly the grasses grew and matured.

Both grasses were grown from seed. While Mitchell grass germinates readily when sown into the soil, Button grass germination is extremely low, less than 2% (Silcock *et al.* 1990). To be able to propagate sufficient plants from seed on demand, a mechanism to increase the germination rate of Button Grass seeds was required. Silcock and Williams (1975b) significantly increased (*c.* 27%) Button Grass seed germination and extended the viability from 2.5–8 to years, by soaking the caryopses in 70% sulphuric acid for 4minutes. Button grass seed germination is maximized 3-5 yrs post collection from fresh material (Silcock *et al.* 1990). However, germination of field collected Button grass seed was increased by soaking in 70% sulphuric acid for 4 min, the germination rate was still negligible. It was not possible to obtain seed aged between 3–5 years post-collection. The gibberellins are known to break seed dormancy and promote seed germination.

A pilot study was performed to ascertain if gibberellic acid would enhance the germination rate of Button grass and, to investigate the effect of different nutrient treatments on plant growth rates.

MATERIALS AND METHODS:

Germination trials of Button grass seed

Twenty-seven month old Button grass seeds collected from plants transplanted from Fowlers Gap to Monash University (grown in soil from Fowlers Gap) were subjected to five different treatments to determine whether percentage germination could be increased. Treatments consisted of (1) none (control), soaking seeds in 70% sulphuric acid for either (2) 4 min. or (3) 5 min., or germinating the seeds in either (4) 1 mM or (5) 5 mM gibberellic acid (Sigma G 7645) solution. Seeds for treatments 1, 4 and 5 were soaked in sterile distilled water for 5 mins while treatment 2 and 3 seeds were soaked in 70 % sulphuric acid for 4 and 5 mins respectively. All seeds were then rinsed in sterile distilled water before 20 caryopses of each treatment were evenly distributed on filter paper discs (Whatmans No 1, 44 mm diameter) which were placed on filter paper risers *c.* 4 mm high in 5 cm petri dishes. To the petri dishes containing treatments 1–3, 1 ml of distilled H_2O was added, while to treatment 4 and 5 seeds 1 ml of 1 mM and 5 mM gibberellic acid solution respectively was added. Lids were placed on the petri dishes and they were sealed with parafilm. Each treatment was replicated five times. The treatments were randomly placed in a germination cabinet set at 30°C with constant light ('gro-lux'). Petri dishes were checked daily for five days (germination complete by then) and the number of seeds germinated/dish recorded. The results were evaluated using ANOVA performed after arcsine transformation of the data, with a Tukey's post-hoc test.

Pilot study of growth of both grasses

Individual seedlings of both grasses were grown individually in plastic bag pots (750 ml) in a glasshouse under nine different treatments. In one treatment the grasses were grown directly in 'organic mix' soil, and the other eight were grown in sand with one of eight Hoaglands' II nutrient mix, modified with respect to strength and phosphorus concentration (Fig. II.1). Approximately 200 ml (1 cup full) of the appropriate nutrient treatment was applied to each pot every second day except for treatment one where 100% 3 $\mu g\ g^{-1}$ P Hoaglands was applied daily and treatment 9 where no nutrients were applied. All pots were flushed with water every fourth day

to prevent the build-up of salts. All plants were watered as required. The glasshouses were maintained between 10 – 30°C.

Fig. II.1 Description of the nine nutrient treatments used to test growth responses of both grasses and the recipe of Hoaglands' II nutrient solution.

Treatments:
c. 200 ml applied to each pot every second day, except treatment 1 and 9

1. 100% Hoaglands 3 $\mu g\ g^{-1}$ P, applied daily,
2. 100% Hoaglands 3$\mu g\ g^{-1}$ P;
3. 50% Hoaglands 3 $\mu g\ g^{-1}$ P;
4. 25% Hoaglands 3 $\mu g\ g^{-1}$ P;
5. 10% Hoaglands 3 $\mu g\ g^{-1}$ P;
6. 100% Hoaglands 30 $\mu g\ g^{-1}$ P ($NH_4H_2PO_4$ adjusted to 1 mM no $(NH_4)_2SO_4$ added);
7. 100% Hoaglands 15 $\mu g\ g^{-1}$ P ($NH_4H_2PO_4$ adjusted to 0.5 mM, + 7 $\mu g\ g^{1}$ N as $(NH_4)_2SO_4$);
8. 100% Hoaglands 0.003 $\mu g\ g^{-1}$ P ($NH_4H_2PO_4$ adjusted to 0.0001 mM + 14 $\mu g\ g^{-1}$ N as $(NH_4)_2SO_4$);
9. 'organic mix' soil, no added nutrients.

Hoaglands II (modified to 3 μg g^{-1} phosphorus)

Chemical	mM	Trace elements:	μg g^{-1}
KNO_3	6.0	H_3BO_3	0.5
$Ca(NO_s)_2.4H_2)$	4.0	$MnCl_2.4H_2O$	0.5
$NH_4H_2PO_4$	0.1	$CuSO_4.5H_2O$	0.02
$(NH_4)_2SO_4$	0.9	$ZnSO_4.7H_2O$	0.05
$MgSO_4.7H_2O$	2.0	MoO_3	0.01
$FeSO_4.7H_2O$	0.1		
EDTA-Disodium salt	0.05		

RESULTS AND DISCUSSION:

The results for the control (group 1) were consistent with those found previously (Silcock and Williams 1975a; Silcock *et al.* 1990). Treatment with 70% sulphuric acid increased seed germination, with 4 min being better than 5 min (Fig. II.2). Silcock and Williams (1975b) found the same pattern but with a larger increase in germination following treatment with 70% sulphuric acid for 4 min, (70%) and 5 min, (36%). However, their seed was 5 years old and untreated (control) seed had a germination rate of 18%. Gibberellic acid had the strongest effect on germination rate, with the seeds incubated in 5 mM gibberellic acid treatment having the highest germination rate, 27 ± 4% with 16 ± 3% germination found for the seeds incubated in 1 mM gibberellic acid (Fig. II.2).

The growth experiments showed that both grasses grew fastest and maximized above-ground biomass when planted in 'organic mix' soil. When growing in 'organic mix', Button grass plants produced spikelets after four weeks and Mitchell grass after 11–12 weeks.

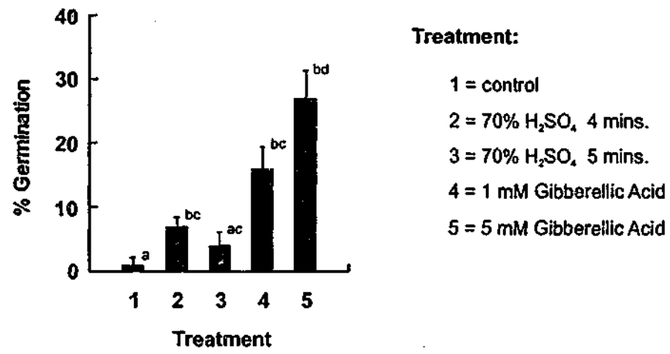


Fig. II.2 Effect of five treatments on the germination of *Dactyloctenium radulan* seeds after 5 days. Bars with the same letters are not significantly different ($P > 0.05$). Results are from an ANOVA following arcsin transformation with post-hoc Tukey's test.

CONCLUSION:

Given these results, for all experiments, Button grass seeds were germinated in 5 mM gibberllic acid before being transplanted into tubs with 'organic' mix. Mitchell grass seeds were planted 12 weeks and Button grass six weeks prior to being harvested to feed to the locusts. Both grasses were planted at two weekly intervals.

APPENDIX III ANOVA AND ANCOVA RESULTS FOR THE LOCUSTS FEEDING ON BUTTON GRASS AND MITCHELL GRASS

Table III.1 Results of ANOVA and ANCOVA of performance measures of the different aged locusts feeding on Button grass and Mitchell grass. Where the test for differences in regression slopes was significant (diet x covariate; age x covariate, diet x age x covariate) the effects of diet and age were analyzed separately. *Post-hoc* effect of age was only investigated where results are given.

*Significant interaction of the treatment x covariate term, with results given for Dunnett's test ($P < 0.05$) according to the Johnson-Neyman Technique. Post hoc testing of adjusted means was performed using pairwise comparisons with *P*-values corrected for multiple testing with the sequential Holm method.

Type of analysis	Source of variation	df	MS	F	P
ANOVA					
Log initial weight	Age	3	3998.219	1184.372	< 0.001
	Diet	1	0.756	0.224	0.637
	Age*Diet	3	0.567	0.168	0.918
	Residual	150	3.376		
Instar duration	Age	3	40911.385	61.220	< 0.001
	Diet	1	17573.700	26.298	< 0.001
	Age*Diet	3	2412.107	3.610	0.015
	Residual	150	668.263		
Simple main effects:					
Age = 2	Diet	1	0.4658	0.001	0.979
Age = 3	Diet	1	3132.900	4.688	0.032
Age = 4	Diet	1	10112.400	15.132	< 0.001
Age = 5	Diet	1	12040.900	18.018	< 0.001

Type of analysis	Source of variation	df	MS	F	P
ANCOVA					
Final weight: Wet	Age	4	0.191	58.580	< 0.001
Interaction	Diet	1	0.022	6.678	0.011
P =0.059	Age*Diet	4	0.010	2.964	0.021
	Log initial weight	1	0.281	86.214	< 0.001
	Residual	187	0.003		
Simple main effects:					
Age = 2	Diet	1	0.000	0.008	0.930
Interaction	Log initial weight	1	0.253	7.810	0.008
P=0.064	Residual	35	0.032		
Age = 3	Diet	1	0.013	0.782	0.382
Interaction	Log initial weight	1	0.233	14.351	0.001
P = 0.755	Residual	37	0.016		
Age = 4	Diet	1	0.151	7.988	0.008
Interaction	Log initial weight	1	0.671	35.578	< 0.001
P = 0.656	Residual	37	0.019		
Age = 5	Diet	1	0.182	15.101	< 0.001
Interaction	Log initial weight	1	0.151	12.489	0.001
P = 0.078	Residual	37	0.012		
Age = 6	Diet	1	0.007	1.557	0.220
Interaction	Log initial weight	1	0.360	79.482	< 0.001
P = 0.240	Residual	37	0.005		
Diet = Button grass	Age	4	0.129	43.548	< 0.001
*Interaction	Log initial weight	1	0.116	39.213	< 0.001
P=0.002	Residual	192	0.003		
Results Wilcox Procedure:					
Lines significantly different between stated values					
	III vs IV	1.251	- 1.576		
	IV vs Adult	1.495	- 2.195		
	V vs Adult	1.743	- 2.290		
Lines not significantly different for the remaining comparisons					
Diet = Mitchell grass	Age	4	0.069	19.322	< 0.001
Interaction	Log initial weight	1	0.164	45.825	< 0.001
P=0.295	Residual	192	0.004		

Type of analysis	Source of variation	df	MS	F	P
Final weight: Dry	Age	4	0.082	17.957	< 0.001
*Interaction	Diet	1	0.024	5.369	0.022
P =0.008	Age*Diet	4	0.011	2.356	0.055
	Log initial weight	1	0.226	49.565	< 0.001
	Residual	187	0.005		
Analyzed each age separately due to significant interaction (age x log initial dry weight *P* = 0.014) diets combined as there was not a significant interaction (diet x log initial dry weight) *P* = 0.754					
Simple main effects:					
Age = 2	Diet	1	0.070	2.021	0.165
Interaction	Log initial weight	1	0.425	12.274	0.001
P=0.138	Residual	33	0.035		
Age = 3	Diet	1	0.014	0.947	0.337
Interaction	Log initial weight	1	0.170	11.283	0.002
P = 0.844	Residual	37	0.015		
Age = 4	Diet	1	0.086	3.571	0.067
Interaction	Log initial weight	1	0.302	12.473	0.001
P = 0.452	Residual	37	0.024		
Age = 5	Diet	1	0.186	14.754	< 0.001
Interaction	Log initial weight	1	0.139	11.051	0.002
P = 0.069	Residual	37	0.013		
Age = 6	Diet	1	0.058	4.302	0.045
Interaction	Log initial weight	1	0.505	37.280	< 0.001
P = 0.279	Residual	37	0.014		
Diets combined	Age	4	0.082	17.111	< 0.001
Interaction	Log initial weight	1	0.228	47.697	< 0.001
P=0.152	Residual	192	0.005		

Type of analysis	Source of variation	df	MS	F	P
Growth rate	Age	3	3.223	41.134	< 0.001
Interaction P =0.240	Diet	1	0.175	2.233	0.137
	Age*Diet	3	0.343	4.379	0.006
	Instar duration	1	0.015	0.197	0.658
	Residual	148	0.078		
Simple main effects:					
Diet = Button grass	Age	3	2.619	44.116	< 0.001
Interaction P=0.174	Instar duration	1	0.020	0.339	0.562
	Residual	75	0.059		
Post-hoc tests significant differences between all ages					
Diet = Mitchell	Age	3	0.950	9.586	< 0.001
Interaction P=0.222	Instar duration	1	0.003	0.034	0.854
	Residual	72	0.099		
Post-hoc tests significant differences between all ages except Instar IV and Instar V					

Type of analysis	Source of variation	df	MS	F	P
Consumption: Total fresh matter					
Interaction P =0.875	Age	4	0.450	74.093	< 0.001
	Diet	1	4.833	795.950	< 0.001
	Age*Diet	4	0.011	1.865	0.118
	Log initial weight	1	0.0003	0.051	0.822
	Residual	187	0.006		
Simple Main Effects:					
Age = 2	Diet	1	4.855	62.203	< 0.001
Interaction P=0.383	Log initial weight	1	0.031	0.398	0.532
	Residual	35	0.078		
Age = 3	Diet	1	3.531	156.188	< 0.001
Interaction P = 0.345	Log initial weight	1	0.004	0.177	0.676
	Residual	37	0.023		
Age = 4	Diet	1	5.186	172.021	< 0.001
Interaction P = 0.250	Log initial weight	1	0.066	2.182	0.148
	Residual	37	0.030		
Age = 5	Diet	1	6.216	521.260	< 0.001
Interaction P = 0.740	Log initial weight	1	0.004	0.345	0.560
	Residual	37	0.012		
Age = 6	Diet	1	6.066	289.609	< 0.001
Interaction P = 0.136	Log initial weight	1	0.017	0.810	0.374
	Residual	37	0.021		
Diets combined	Age	4	0.469	14.949	< 0.001
Interaction P=0.927	Log initial weight	1	0.007	0.223	0.637
	Residual	192	0.031		

Type of analysis	Source of variation	df	MS	F	P
Consumption: Total dry matter					
Interaction *P* =0.546	Age	4	0.368	44.482	< 0.001
	Diet	1	0.682	82.304	< 0.001
	Age*Diet	4	0.009	1.095	0.360
	Log initial weight	1	0.0280	3.376	0.068
	Residual	187	0.008		
Simple main effects:					
Age = 2	Diet	1	0.744	6.718	0.014
Interaction *P*=0.354	Log initial weight	1	0.040	0.362	0.551
	Residual	35	0.111		
Age = 3	Diet	1	0.248	6.563	0.015
Interaction *P* = 0.416	Log initial weight	1	0.032	0.854	0.362
	Residual	37	0.038		
Age = 4	Diet	1	0.777	28.526	< 0.001
Interaction *P* = 0.433	Log initial weight	1	0.394	14.469	0.001
	Residual	37	0.027		
Age = 5	Diet	1	1.147	70.317	< 0.001
Interaction *P* = 0.139	Log initial weight	1	0.016	1.006	0.322
	Residual	37	0.016		
Age = 6	Diet	1	0.896	36.587	< 0.001
Interaction *P* = 0.682	Log initial weight	1	0.088	3.612	0.065
	Residual	37	0.024		
Diets combined	Age	4	0.373	31.574	< 0.001
Interaction *P*=0.155	Log initial weight	1	0.021	1.808	0.180
	Residual	192	0.012		

Type of analysis	Source of variation	df	MS	F	P
Consumption: Water					
Interaction *P* =0.941	Age	4	0.506	52.101	< 0.001
	Diet	1	8.030	826.412	< 0.001
	Age*Diet	4	0.013	1.382	0.242
	Log initial weight	1	0.008	0.848	0.358
	Residual	187	0.010		
Simple main effects:					
Age = 2	Diet	1	1.638	71.772	< 0.001
Interaction *P*=0.490	Log initial weight	1	0.004	0.172	0.681
	Residual	35	0.023		
Age = 3	Diet	1	1.159	134.886	< 0.001
Interaction *P* = 0.597	Log initial weight	1	0.005	0.549	0.463
	Residual	37	0.009		
Age = 4	Diet	1	1.593	194.469	< 0.001
Interaction *P* = 0.404	Log initial weight	1	0.001	0.177	0.677
	Residual	37	0.008		
Age = 5	Diet	1	1.819	481.427	< 0.001
Interaction *P* = 0.861	Log initial weight	1	0.0002	0.060	0.807
	Residual	37	0.004		
Age = 6	Diet	1	1.859	289.596	< 0.001
Interaction *P* = 0.121	Log initial weight	1	0.018	2.789	0.103
	Residual	37	0.006		

Type of analysis	Source of variation	df	MS	F	P
Consumption Non-cell wall material					
Interaction P =0.290	Age	4	0.369	41.419	< 0.001
	Diet	1	1.034	116.147	< 0.001
	Age*Diet	4	0.020	2.213	0.069
	Log initial weight	1	0.046	5.120	0.025
	Residual	187	0.009		
Simple main effects:					
Age = 2	Diet	1	0.797	7.232	0.011
Interaction P=0.253	Log initial weight	1	0.032	0.287	0.596
	Residual	35	0.110		
Age = 3	Diet	1	0.293	7.686	0.009
Interaction P = 0.260	Log initial weight	1	0.053	1.379	0.248
	Residual	37	0.038		
Age = 4	Diet	1	1.449	44.377	0.000
Interaction P = 0.845	Log initial weight	1	0.490	15.008	0.000
	Residual	37	0.033		
Age = 5	Diet	1	1.826	82.719	0.000
Interaction P = 0.105	Log initial weight	1	0.023	1.061	0.310
	Residual	37	0.022		
Age = 6	Diet	1	1.562	56.294	0.000
Interaction P = 0.467	Log initial weight	1	0.149	5.380	0.026
	Residual	37	0.028		
Diets combined	Age	4	0.373	25.783	< 0.001
Interaction P=0.168	Log initial weight	1	0.36	2.461	0.118
	Residual	192	0.014		

Type of analysis	Source of variation	df	MS	F	P
Consumption: Protein					
Interaction P =0.523	Age	4	0.357	40.819	< 0.001
	Diet	1	0.051	5.851	0.017
	Age*Diet	4	0.017	2.023	0.093
	Log initial weight	1	0.021	2.445	0.120
	Residual	187	0.009		
Simple main effects:					
Age = 2	Diet	1	0.199	1.722	0.198
Interaction P=0.436	Log initial weight	1	0.044	0.382	0.540
	Residual	35	0.116		
Age = 3	Diet	1	0.071	2.028	0.163
Interaction P = 0.581	Log initial weight	1	0.017	0.495	0.486
	Residual	37	0.035		
Age = 4	Diet	1	0.061	1.815	0.186
Interaction P = 0.443	Log initial weight	1	0.319	9.498	0.004
	Residual	37	0.034		
Age = 5	Diet	1	0.224	12.121	0.001
Interaction P = 0.052	Log initial weight	1	0.022	1.73	0.286
	Residual	37	0.019		
Age = 6	Diet	1	0.072	2.616	0.114
Interaction P = 0.864	Log initial weight	1	0.087	3.166	0.083
	Residual	37	0.028		

Type of analysis	Source of variation	df	MS	F	P
Consumption: Carbohydrates					
Interaction P=0.686	Age	4	0.394	40.527	< 0.001
	Diet	1	0.304	31.212	< 0.001
	Age*Diet	4	0.008	0.821	0.513
	Log initial weight	1	0.017	1.731	0.190
	Residual	187	0.010		
Simple main effects:					
Age = 2	Diet	1	0.432	3.639	0.072
Interaction P=0.657	Log initial weight	1	0.101	0.804	0.376
	Residual	35	0.125		
Age = 3	Diet	1	0.100	2.119	0.154
Interaction P = 0.374	Log initial weight	1	0.011	0.233	0.632
	Residual	37	0.047		
Age = 4	Diet	1	0.263	7.565	0.009
Interaction P = 0.334	Log initial weight	1	0.526	15.106	< 0.001
	Residual	37	0.035		
Age = 5	Diet	1	0.684	36.990	0.000
Interaction P = 0.266	Log initial weight	1	0.008	0.408	0.527
	Residual	37	0.018		
Age = 6	Diet	1	0.304	12.483	0.001
Interaction P = 0.381	Log initial weight	1	0.072	2.978	0.093
	Residual	37	0.024		

Type of analysis	Source of variation	df	MS	F	P
Consumption rate	Age	3	3.014	354.284	< 0.001
Interaction P =0.365	Diet	1	0.615	72.316	< 0.001
	Age*Diet	3	0.017	1.960	0.123
	Instar duration	1	0.122	14.304	< 0.001
	Residual	149	0.009		
Simple main effects:					
Age = 2	Diet	1	0.147	8.315	0.007
Interaction P=0.532	Instar duration	1	0.121	6.827	0.013
	Residual	35	0.017		
Age = 3	Diet	1	0.072	11.285	0.002
Interaction P = 0.996	Instar duration	1	0.034	5.436	0.025
	Residual	37			
Age = 4	Diet	1	0.129	19.282	<0.001
*Interaction P = 0.041	Instar duration t	1	0.016	2.400	0.130
	Residual	37	0.007		
Results Wilcox Procedure: lines significantly different over instar duration (-500 – 500 h)					
Age = 5	Diet	1	0.165	52.819	< 0.001
Interaction P = 0.410	Instar duration	1	0.001	0.365	0.549
	Residual	37	0.003		
Diets combined	Age	3	3.817	304.569	< 0.001
*Interaction P=0.001	Instar duration	1	0.001	0.095	0.758
	Residual	153	0.012		
Results Wilcox Procedure: all lines significantly different					

Type of analysis	Source of variation	df	MS	F	P
Dry matter assimilated					
Interaction *P* =0.431	Age	4	0.066	16.733	< 0.001
	Diet	1	3.300	83.675	< 0.001
	Age*Diet	4	0.088	2.230	0.067
	Log initial weight	1	0.037	0.927	0.337
	Residual	187	0.039		
Simple main effects:					
Age = 2	Diet	1	0.707	1.446	0.237
Interaction *P*=0.706	Log initial weight	1	0.016	0.034	0.856
	Residual		0.489		
Age = 3	Diet	1	2.183	7.410	0.010
Interaction *P* = 0.217	Log initial weight	1	0.003	0.011	0.918
	Residual	37	0.295		
Age = 4	Diet	1	5.617	44.325	< 0.001
Interaction *P* = 0.110	Log initial weight	1	0.967	7.630	0.009
	Residual	37	0.127		
Age = 5	Diet	1	4.503	75.623	< 0.001
Interaction *P* = 0.439	Log initial weight	1	0.000	0.000	0.986
	Residual	37	0.060		
Age = 6	Diet	1	6.877	75.588	< 0.001
Interaction *P* = 0.163	Log initial weight	1	0.039	0.430	0.516
	Residual	37	0.091		
Diets combined	Age	4	0.314	5.586	< 0.001
Interaction *P*=0.419	Log initial weight	1	0.052	0.921	0.340
	Residual	92	0.056		

Type of analysis	Source of variation	df	MS	F	P
Protein assimilated					
Interaction *P* =0.139	Age	4	0.181	20.770	< 0.001
	Diet	1	0.059	6.774	0.011
	Age*Diet	4	0.025	2.876	0.027
	Log initial weight	1	0.081	9.234	0.003
	Residual	77	0.009		
Simple Main Effects:					
Age = 2	Diet	1	0.004	0.050	0.826
*Interaction *P*=0.026	Log initial weight	1	0.110	1.318	0.267
	Residual	17	0.083		
Results Wilcox Procedure: lines not significantly different					
Age = 3	Diet	1	0.010	0.152	0.702
Interaction *P* = 0.859	Log initial weight	1	0.003	0.052	0.823
	Residual	16	0.067		
Age = 4	Diet	1	0.049	1.383	0.258
Interaction *P* = 0.182	Log initial weight	1	0.330	9.323	0.008
	Residual	15	0.035		
Age = 5	Diet	1	0.101	8.093	0.012
*Interaction *P* = 0.035	Log initial weight	1	0.134	10.689	0.005
	Residual	16	0.013		
Results Wilcox Proccdure: lines significantly different when protein assimilated > 0.9 mg					
Age = 6	Diet	1	0.638	21.097	< 0.001
Interaction *P* = 0.624	Log initial weight	1	0.063	2.098	0.165
	Residual	18	0.030		

Type of analysis	Source of variation	df	MS	F	P
Assimilation rate	Age	3	3.136	69.265	< 0.001
Interaction	Diet	1	1.946	42.980	< 0.001
P =0.361	Age*Diet	3	0.081	1.787	0.152
	Instar duration	1	0.023	0.501	0.480
	Residual	148	0.045		
Simple main effects:					
Age = 2	Diet	1	0.138	1.517	0.226
Interaction	Instar duration	1	0.054	0.596	0.445
P=0.258	Residual	35	0.091		
Age = 3	Diet	1	0.451	8.295	0.007
Interaction	Instar duration	1	0.043	0.787	0.381
P = 0.995	Residual	37	0.054		
Age = 4	Diet	1	0.669	23.210	< 0.001
Interaction	Instar duration t	1	0.001	0.014	0.904
P = 0.090	Residual	37	0.029		
Age = 5	Diet	1	0.498	45.495	< 0.001
Interaction	Instar duration	1	0.011	0.986	0.327
P = 0.205	Residual	37	0.011		
Diets combined	Age	3	4.526	78.365	< 0.001
Interaction	Instar duration	1	0.281	4.859	0.029
P=0.062	Residual	153	0.058		

Type of analysis	Source of variation	df	MS	F	P
Frass					
Interaction	Age	4	0.352	55.355	< 0.001
P =0.320	Diet	1	0.287	45.067	< 0.001
	Age*Diet	4	0.029	4.544	0.002
	Log initial weight	1	0.045	7.016	0.009
	Residual	187	0.006		
Simple main effects:					
Age = 2	Diet	1	1.325	17.134	< 0.001
Interaction	Log initial weight	1	0.004	0.056	0.815
P=0.170	Residual	35	0.077		
Age = 3	Diet	1	0.054	1.890	0.177
Interaction	Log initial weight	1	0.072	2.532	0.120
P = 0.784	Residual	37	0.029		
Age = 4	Diet	1	0.098	4.085	0.051
Interaction	Log initial weight	1	0.303	12.605	0.001
P = 0.832	Residual	37	0.024		
Age = 5	Diet	1	0.379	24.449	< 0.001
Interaction	Log initial weight	1	0.030	1.960	0.170
P = 0.107	Residual	37	0.016		
Age = 6	Diet	1	0.191	8.555	0.006
Interaction	Log initial weight	1	0.083	3.745	0.061
P = 0.853	Residual	37	0.022		

Type of analysis	Source of variation	df	MS	F	P
Frass (AD)					
Interaction P=0.231	Age	4	1746.322	20.346	< 0.001
	Diet	1	2795.058	32.564	< 0.001
	Age*Diet	4	649.357	7.565	< 0.001
	Consumption	1	53948.4	628.530	< 0.001
	Residual	187	85.8326		
Simple main effects:					
Age = 2	Diet	1	87.960	9.395	0.004
Interaction P=0.443	Consumption	1	307.972	32.894	< 0.001
	Residual	35	9.362		
Age = 3	Diet	1	5.170	0.341	0.563
Interaction P = 0.504	Consumption	1	676.2	44.589	< 0.001
	Residual	37	15.166		
Age = 4	Diet	1	588.765	15.681	< 0.001
Interaction P = 0.938	Consumption	1	5204.865	138.627	< 0.001
	Residual	37	37.546		
Age = 5	Diet	1	355.681	2.0426	0.161
*Interaction P = 0.019	Consumption	1	10585.7	60.791	< 0.001
	Residual	37	174.132		
Results Wilcox Procedure: lines significantly different between intake of 261.6 – 355.8 mg. Lines not significantly different where Button grass and Mitchell grass consumption overlaps					
Age = 6	Diet	1	3475.379	19.340	< 0.001
Interaction P = 0.220	Consumption	1	37864.99	211.043	< 0.001
	Residual	37	179.418		

Diet = Button grass	Age	4	965.716	7.828	< 0.001
Interaction P=0.174	Consumption	1	32544.5	263.797	< 0.001
	Residual	94	123.369		
Post-hoc Pair wise Holm adjusted means comparison Non- significant ($P > 0.05$) differences:					
	II vs III	P =	0.506		
	II vs IV	P =	0.444		
	III vs IV	P =	0.588		
	III vs V	P =	0.097		
Post-hoc Pair wise Holm adjusted means comparison Significant ($P < 0.05$) differences:					
	II vs V	P =	0.045		
	II vs Adult	P <	0.001		
	III vs Adult	P =	0.001		
	IV vs V	P =	0.047		
	IV vs Adult	P <	0.001		
	V vs Adult	P <	0.001		
Diet = Mitchell	Age	4	638.239	15.273	< 0.001
*Interaction P = 0.012	Consumption	1	22013.3	526.766	< 0.001
	Residual	92	41.789		
Results Wilcox Procedure:					
Lines significantly different between stated values * not significantly different for consumption means					
	II vs III	28.6	- 38.4*		
	II vs IV	39.1	- 44.9*		
	IV vs Adult	145.4	- 231.1*		
	V vs Adult	177.9	- 268.8		
Lines not significantly different for the remaining comparisons					

Type of analysis	Source of variation	df	MS	F	P
Non-Cell Wall Frass (AD)					
Interaction P=0.941	Age	4	1636.273	25.872	< 0.001
	Diet	1	113.528	1.795	0.182
	Age*Diet	4	109.278	1.728	0.146
	Non-CW consumption	1	2104.999	33.283	< 0.001
	Residual	187	63.245		
Simple main effects					
Diets combined	Age	4	2954.637	46.177	< 0.001
Interaction P=0.168	Non-C.W. consumption	1	2709.474	42.345	< 0.001
	Residual	192	63.986		

Post-hoc Pair wise Holm adjusted means comparison
Non- significant ($P > 0.05$) differences:

III vs IV	P = 0.061

Post-hoc Pair wise Bonferonni adjusted means comparison
Significant ($P < 0.05$) differences:

II vs III	$P <$ 0.001
II vs IV	$P <$ 0.001
II vs V	$P <$ 0.001
II vs Adult	$P <$ 0.001
III vs V	$P <$ 0.001
III vs Adult	$P <$ 0.001
IV vs V	$P <$ 0.001
IV vs Adult	$P <$ 0.001
V vs Adult	$P <$ 0.001

Type of analysis	Source of variation	df	MS	F	P
Protein frass (AD)					
Interaction P=0.319	Age	4	6.095	7.304	< 0.001
	Diet	1	8.463	10.141	0.002
	Age*Diet	4	5.557	6.659	< 0.001
	Protein consumption	1	10.414	12.480	< 0.001
	Residual	86	0.834		
Simple main effects:					
Age = 2	Diet	1	0.031	3.370	0.084
Interaction P=0.600	Protein consumption	1	< 0.0001	0.0001	0.992
	Residual	16	0.009		
Age = 3	Diet	1	0.174	1.221	0.286
Interaction P = 0.180	Protein consumption	1	0.046	0.326	0.576
	Residual		0.142	1.678	0.215
Age = 4	Diet	1	0.149	3.884	0.067
Interaction P = 0.424	Protein consumption	1	0.345		
	Residual	15	0.089		
Age = 5	Diet	1	0.507	0.455	0.510
Interaction P = 0.159	Protein consumption	1	0.674	0.605	0.448
	Residual	16	1.114		
Age = 6	Diet	1	28.199	10.542	0.004
Interaction P = 0.468	Protein consumption	1	11.369	4.250	0.054
	Residual	18	2.675		
Diet = Button grass	Age	4	1.024	1.576	0.199
Interaction P=0.080	Prot3ein onsumption	1	4.582	7.054	0.011
	Residual	40	0.650		
Diet = Mitchell grass	Age	4	7.764	7.632	< 0.001
Interaction	Protein consumption	1	5.834	5.735	0.021
	Residual	45	1.017		

Continued over

Post-hoc Pair wise Holm adjusted means comparison.
Non- significant ($P > 0.05$) differences only given:

II vs III	$P =$ 1.000
II vs IV	$P =$ 1.000
II vs V	$P =$ 1.000
II vs Ad	$P =$ 0.274
III vs IV	$P =$ 1.000
III vs V	$P =$ 1.000
III vs AD	$P =$ 0.257
IV vs V	$P =$ 1.000

Post-hoc Pair wise Holm adjusted means comparison
Significant ($P < 0.05$) differences:

IV vs Adult	$P =$ 0.016
V vs Adult	$P <$ 0.001

Type of analysis	Source of variation	df	MS	F	P
Fresh weight gain (ECI)					
Interaction P =0.985	Age	4	17438.2	130.211	< 0.001
	Diet	1	264.979	1.979	0.161
	Age*Diet	4	534.245	3.990	0.004
	Consumption	1	1548.041	11.560	< 0.001
	Residual	187	133.922		
Simple main effects:					
Diet = Button grass	Age	4	11734.753	99.752	< 0.001
Interaction P=0.368	Consumption	1	462.794	3.934	0.050
	Residual	94	117.639		

Post-hoc Pair wise Bonferonni adjusted means comparison.
Non- significant ($P > 0.05$) differences:

II vs III	$P =$ 0.379
II vs V	$P =$ 1.000
II vs Adult	$P =$ 1.000
III vs IV	$P =$ 0.109
III vs Adult	$P =$ 1.000
IV vs V	$P =$ 0.120
IV vs Adult	$P =$ 1.000

Post-hoc Pair wise Bonferonni adjusted means comparison
Significant ($P < 0.05$) differences:

II vs IV	$P =$ 0.042
V vs Adult	$P <$ 0.001

Diet = Mitchell grass	Age	4	6369.108	44.142	< 0.001
Interaction P = 0.796	Consumption	1	1796.416	12.450	0.001
	Residual	92	144.285		

Post-hoc Pair wise Bonferonni adjusted means comparison.
Non- significant ($P > 0.05$) differences only given:

II vs III	$P =$ 0.968
II vs IV	$P =$ 0.534
III vs IV	$P =$ 0.532

Continued over

Post-hoc Pair wise Bonferonni adjusted means comparison
Significant ($P < 0.05$) differences:

II vs V	$P =$ 0.038
IIIvs V	$P =$ 0.018
IV vs V	$P =$ 0.014
II vs Adult	$P <$ 0.001
III vs Adult	$P <$ 0.001
IV vs Adult	$P <$ 0.001
V vs Adult	$P <$ 0.001

Type of analysis	Source of variation	df	MS	F	P
Dry weight gain (ECI)					
Interaction P=0.702	Age	4	397.959	42.031	< 0.001
	Diet	1	32.344	3.416	0.066
	Age*Diet	4	3.624	0.383	0.821
	Consumption	1	862.456	91.090	< 0.001
	Residual	187	9.468		
Simple main effects:					
Diets combined	Age	4	437.891	46.458	< 0.001
Interaction P=0.841	Consumption	1	1329.216	141.024	< 0.001
	Residual	192	9.425		

Post-hoc Pair wise Holm adjusted means comparison.
Non- significant ($P > 0.05$) differences only given:

II vs III	$P =$ 0.864
II vs IV	$P =$ 0.657
III vs IV	$P =$ 0.657

Post-hoc Pair wise Holm adjusted means comparison
Significant ($P < 0.05$) differences:

II vs V	$P <$ 0.001
IIIvs V	$P <$ 0.001
IV vs V	$P <$ 0.001
II vs Adult	$P <$ 0.001
III vs Adult	$P <$ 0.001
IV vs Adult	$P <$ 0.001
V vs Adult	$P <$ 0.001

Type of analysis	Source of variation	df	MS	F	P
Dry weight gain (ECD)					
Interaction P=0.053	Age	4	239.910	23.160	< 0.001
	Diet	1	44.177	4.265	0.040
	Age*Diet	4	5.888	0.568	0.686
	Dry matter assimilated	1	695.921	67.182	< 0.001
	Residual	187	10.359		
Simple main effects:					
Age = 2	Diet	1	0.106	0.087	0.769
Interaction P=0.376	Dry matter assimilated	1	3.962	3.275	0.079
	Residual	35	1.210		
Age = 3	Diet	1	2.036	1.641	0.208
*Interaction P = 0.028	Dry matter assimilated	1	1.410	1.136	0.293
	Residual	37	1.241		
Results Wilcox Procedure:	lines not significantly different				
Age = 4	Diet	1	2.310	0.348	0.559
Interaction P = 0.616	Dry matter assimilated	1	61.112	9.208	0.004
	Residual	37	6.637		
Age = 5	Diet	1	9.910	0.648	0.426
*Interaction P = 0.002	Dry matter assimilated	1	67.925	4.439	0.042
	Residual	37	15.300		
Results Wilcox Procedure:	lines not significantly different				
Age = 6	Diet	1	170.755	7.411	0.010
Interaction P = 0.215	Dry matter assimilated	1	746.170	32.386	< 0.001
	Residual	37	23.040		

Diets combined	Age	4	229.359	22.131	< 0.001
Interaction P=0.284	Dry matter assimilated	1	1149.079	110.875	< 0.001
	Residual	192	10.364		

Post-hoc Pair wise Holm adjusted means comparison.
Non- significant ($P > 0.05$) differences only given:

II vs III	P =	0.327
III vs IV	P =	0.702
III vs IV	P =	0.327
III vs V	P =	1.000
IV vs V	P =	1.000

Post-hoc Pair wise Holm adjusted means comparison
Significant ($P < 0.05$) differences:

II vs IV	P =	0.023
II vs V	P <	0.001
II vs Adult	P <	0.001
III vs Adult	P <	0.001
IV vs Adult	P <	0.001
V vs Adult	P <	0.001

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