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Performance of a generalist grasshopper on a C₃ and a C₄ grass: compensation for the effects of elevated CO₂ on plant nutritional quality

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Abstract The increasing CO₂ concentration in Earth's atmosphere is expected to cause a greater decline in the nutritional quality of C₃ than C₄ plants. As a compensatory response, herbivorous insects may increase their feeding disproportionately on C₃ plants. These hypotheses were tested by growing the grasses *Lolium multiflorum* (C₃) and *Bouteloua curtipendula* (C₄) at ambient (370 ppm) and elevated (740 ppm) CO₂ levels in open top chambers in the field, and comparing the growth and digestive efficiencies of the generalist grasshopper *Melanoplus sanguinipes* on each of the four plant × CO₂ treatment combinations. As expected, the nutritional quality of the C₃ grass declined to a greater extent than did that of the C₄ grass at elevated CO₂; protein levels declined in the C₃ grass, while levels of carbohydrates (sugar, fructan and starch) increased. However, *M. sanguinipes* did not significantly increase its consumption rate to compensate for the lower nutritional quality of the C₃ grass grown under elevated CO₂. Instead, these grasshoppers appear to use post-ingestive mechanisms to maintain their growth rates on the C₃ grass under elevated CO₂. Consumption rates of the C₃ and C₄ grasses were also similar, demonstrating a lack of compensatory feeding on the C₄ grass. We also examined the relative efficiencies of nutrient utilization from a C₃ and C₄ grass by *M. sanguinipes* to test the basis for the C₄ plant avoidance hypothesis. Contrary to this hypothesis, neither protein nor

sugar was digested with a lower efficiency from the C₄ grass than from the C₃ grass. A novel finding of this study is that fructan, a potentially large carbohydrate source in C₃ grasses, is utilized by grasshoppers. Based on the higher nutrient levels in the C₃ grass and the better growth performance of *M. sanguinipes* on this grass at both CO₂ levels, we conclude that C₃ grasses are likely to remain better host plants than C₄ grasses in future CO₂ conditions.

Keywords *Melanoplus sanguinipes* (grasshopper) · *Lolium multiflorum* (C₃ grass) · *Bouteloua curtipendula* (C₄ grass) · Nutrient · Digestion

Introduction

The CO₂ level in Earth's atmosphere is widely expected to double during this century (e.g., Falkowski et al. 2000; Houghton et al. 2001), substantially altering the nutritional quality of C₃ plants; levels of nonstructural carbohydrates will increase and protein (nitrogen) will decrease (Poorter 1993; Poorter et al. 1997; Wand et al. 1999). The effects of these changes on leaf-chewing insect herbivores have been examined primarily on caterpillars feeding on C₃ dicots (Bezemer and Jones 1998). The responses of caterpillars vary widely, ranging from effective compensation to reduced fitness. Little work has been done on the effects of elevated CO₂ on the nutritional ecology of grasshoppers (Johnson and Lincoln 1990, 1991). We are aware of one unpublished study examining the effects of elevated CO₂ on grasshoppers feeding on a C₄ grass (Montjoy 1992), and none examining the effects of elevated CO₂ on C₃ grasses and grasshoppers. C₃ grasses are more strongly affected by elevated CO₂ than are C₄ grasses (Wand et al. 1999), suggesting that elevated CO₂ will more strongly affect grasshoppers feeding on C₃ grasses than those feeding on C₄ grasses. Indeed, it has been hypothesized that herbivores will disproportionately increase their feeding damage on C₃ plants to compensate for the larger changes in C₃ plants in elevated CO₂ (Lincoln et al. 1984, 1986; Lambers 1993).

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Grasshoppers play important roles in rangeland ecology (Parker and Root 1981; Hewitt and Onsager 1983; Tschamtko and Greiler 1995; Belovsky and Slade 2000). Much of our knowledge about the nutritional ecology of grasshoppers on grasses has been generated by tests of the C₄ plant avoidance hypothesis, which states that C₄ plants should be avoided by herbivores because these plants have lower levels of nutrients and lower nutrient availability than C₃ plants (Caswell et al. 1973; Caswell and Read 1975, 1976). Field and laboratory studies on diet selection have produced mixed results, however (Capinera 1978; Boutton et al. 1978, 1980; Landa and Rabinowitz 1983; Heidorn and Joern 1984; Pinder and Kroh 1987; Pinder and Jackson 1988). Although Caswell and Reed (1975, 1976) present microscopical evidence that some fraction of the nutrient-rich bundle sheath cells in C₄ grasses remains intact following ingestion by grasshoppers, no analyses of nutrient assimilation efficiencies were made to determine whether intact bundle sheath cells necessarily result in lower nutrient availability from C₄ grasses. A single unpublished study comparing protein and soluble carbohydrate digestibility by a grass-specialist grasshopper (Boys 1981) found no significant reduction in nutrient digestibility from a C₄ grass. It was suggested, however, that generalist grasshoppers might not be equally efficient at processing C₄ grasses because they lack the specialized mandibles of graminivorous species (Isely 1944; Boys 1981; Patterson 1984). In this study, we compared the efficiency of nutrient utilization from a C₃ and a C₄ grass to determine whether nutrient utilization efficiencies are necessarily lower from C₄ grasses by generalist grasshoppers.

This study tested four hypotheses: (1) elevated atmospheric CO₂ reduces the nutritional quality of C₃ grasses but has little effect on C₄ grasses, (2) generalist grasshoppers compensate for changes in plant quality under elevated CO₂ by increasing their consumption rates on C₃ grasses, but elevated CO₂ causes no change in consumption rates on C₄ grasses, (3) grasshoppers compensate for the lower levels of nutrients in C₄ grasses by consuming greater amounts of these plants than C₃ grasses, and (4) nutrients from C₄ grasses are utilized with lower efficiencies by generalist grasshoppers than are those from C₃ grasses. To test these hypotheses, we compared the effects of elevated CO₂ (740 ppm) and ambient CO₂ (370 ppm) concentrations on the nutritional quality of two grasses (Poaceae): *Lolium multiflorum* Lam. (Italian ryegrass), a common introduced C₃ pasture grass, and *Bouteloua curtipendula* (Michx.) Torr. (sideoats grama), a native C₄ rangeland grass. The nutritional quality of these species was determined by measuring foliar protein, sugar, starch, fructan, water, and fiber content and leaf toughness. *Melanoplus sanguinipes* (Fabricius) (Orthoptera: Acrididae) was used to examine the effect of varying grass nutritional quality on the feeding, digestion and growth of a generalist (forb- and grass-feeding) grasshopper.

Materials and methods

Grasses

Lolium multiflorum and *B. curtipendula* were grown from seed at the University of Michigan Biological Station, Pellston, Mich., USA. Seeds were examined for endophytic fungi, which were absent, and seedlings were grown in a greenhouse for approximately 3 weeks (Barbehenn et al. 2004a). Seedlings were transplanted to tall 3-l pots containing an 80:20 (v/v) mixture of potting soil and sand. Pots contained two *L. multiflorum* or four *B. curtipendula*; these numbers resulted in approximately equal biomass among pots. Each chamber contained six pots of *L. multiflorum* and six pots of *B. curtipendula* arranged in a checkerboard array and recessed into the ground approximately 20 cm. Open top chambers (0.5 m³) were constructed of PVC tubing, covered on four sides and a frustum with clear polyester film (Drake et al. 1989; Karowe et al. 1997). Grasses were grown either in 370 ppm or 740 ppm CO₂ ($n=20$ /treatment) from early June to early August, 2001 (Barbehenn et al. 2004a).

Grasshopper performance

Melanoplus sanguinipes is a common, economically important species throughout most of the United States. Eggs of *M. sanguinipes* were obtained from a non-diapause colony (USDA, Bozeman, Mont., USA). Grasshopper nymphs were reared to the fourth instar on *Triticum aestivum* (wheat; C₃) seedlings, *Cynodon dactylon* (bermuda grass; C₄) and wheat bran. Grasshoppers were provided with all three food types together in a screen cage (ca. 30 × 30 × 50 cm), and maintained in an incubator at 30°C with a 16:8 h light: dark photoperiod. A 60-W lightbulb was placed near the cage to allow insects to thermoregulate. Insects appeared to eat both grasses equally, and pots of grasses were replaced as needed. Recently molted fourth-instar nymphs were starved for 4–5 h to ensure that their guts were empty, weighed and randomly assigned to feed on one of the grasses from the four species × CO₂ treatment combinations. Grasshoppers were placed individually in ventilated plastic containers (ca. 600 ml) and fed the second fully expanded leaves from the grasses. Cut leaves were weighed and placed with an end in water in microcentrifuge tubes. After 24 h, grasshoppers were given a new group of freshly cut leaves from uncut plants in the same chamber used to feed them originally. Thus, each insect fed on plants from a different chamber. The total feeding period was 48 h. Fecal pellets were collected daily, frozen at –80°C, and freeze-dried. All uneaten food was dried at 70°C for at least 72 h and weighed. Consumption was determined as the difference in the initial and final dry weights of the food. The percent dry weight of food and insects were determined from representative samples of each leaf type and grasshopper nymphs, respectively (dried at 70°C for 72 h). Leaves were sampled from each of the chambers used for food at the end of the experiment to collect samples for nutrient analysis (dried at 70°C). At the end of each feeding trial, grasshoppers were starved for 3 h to collect all remaining fecal pellets, and frozen (–80°C). Final dry weights of grasshoppers were taken after they were freeze-dried. Consumption rate (CR), growth rate (GR) and nutritional indices were calculated on a dry weight basis (Waldbauer 1968). Nutritional indices measuring the overall efficiency of food assimilation and insect growth included approximate digestibility [AD = (ingested mass – fecal mass)/ingested mass], efficiency of conversion of ingested matter (ECI = mass gained/ingested mass), and efficiency of conversion of digested matter [ECD = mass gained/(ingested mass – fecal mass)]. In addition, we measured the assimilation efficiencies of nutrients [AE = (mass of nutrient ingested – mass of nutrient egested)/mass of nutrient ingested], and nutrient assimilation rates [AR = (mass of a nutrient ingested – mass of nutrient egested)/day].

Nutrient analyses

Dried grass and fecal samples were ground to a homogeneous powder using a dental amalgamator, and stored in screw-cap centrifuge tubes in the dark at room temperature, or at 4°C under a nitrogen atmosphere for long-term storage. Protein was measured as total amino acids in 6 M HCl hydrolysates with ninhydrin reagent (Sigma) (Barbehenn 1995). Uric acid was measured with uricase in the frass of 6–14 insects in each species \times CO₂ treatment combination (Martin and Van't Hof 1988). The uric acid concentration was multiplied by 2.4 to account for its color factor in the ninhydrin assay, and mean uric acid concentrations were used to correct fecal protein measurements. Sugar (the sum of glucose, fructose and sucrose) and fructan (in the C₃ grass) were measured in ethanol extracts (Barbehenn et al. 2004a). Starch was hydrolyzed with α -amylase and amyloglucosidase in the residue remaining after ethanol extraction, and was measured as glucose (Barbehenn et al. 2004a). All reaction mixtures were scaled to fit in 96-well microtiter plates (200 μ l), and absorbance measurements were made with a Bio-Rad Benchmark microplate reader. Neutral detergent fiber (primarily cellulose, hemicellulose and lignin) was measured gravimetrically after non-fiber components were solubilized (Van Soest et al. 1991).

Leaf toughness

Leaf toughness was measured in freshly cut leaves with a penetrometer during the same time that samples were collected for chemical analysis. Toughness was expressed as the mass (g) necessary to puncture the leaf (Barbehenn et al. 2004a). Toughness was measured in the lamina of the second fully expanded leaf at a point midway along its length. Measurements were made alternately between the C₃ and C₄ grasses and CO₂ treatments.

Statistical analyses

Measures of C₃ and C₄ grass nutritional quality were analyzed with a split-plot, Type III ANOVA (PROC MIXED) (SAS 2000). Models for analyzing grass nutritional quality included CO₂ level and grass species as fixed effects, and block and CO₂ \times block as random effects. CO₂ \times grass species interactions were examined to determine whether there was a differential effect of CO₂ on the nutritional quality of the C₃ and C₄ grass. Rates of consumption, growth and nutrient assimilation and final weights were analyzed by analysis of covariance (ANCOVA), using grasshopper initial dry weight as the covariate (PROC MIXED) (Raubenheimer and Simpson 1992; Horton and Redak 1993). Models for analyzing grasshopper performance included grass species and CO₂ level as fixed effects, block and CO₂ \times block as random effects, and the CO₂ \times grass species interaction. No significant differences were observed between male and female growth and consumption rates, and sex was dropped from the models. ANCOVA models for AD used fecal mass as the dependent variable with amount ingested as the covariate, while the model for ECI used weight gained as the dependent variable with amount ingested as the covariate, and those for nutrient AEs used fecal nutrient mass as the dependent variable with nutrient mass ingested as the covariate. ECD could not be tested with ANCOVA because of a significant assimilation \times species interaction, indicating non-parallel slopes, and was analyzed with split-plot ANOVA as described above. Split-plot ANOVA and ANCOVA produced the same pattern of significant differences for most variables, suggesting that ANOVA provided an accurate analysis of these data. Substantial differences between the results of ANOVA and ANCOVA analyses were observed only for protein and sugar AEs, in which case ANOVA detected no significant main effects or interactions for protein or sugar AE. When residuals could not be transformed to fit a normal distribution (e.g., water) Kruskal–Wallis tests were used to determine the significance of differences between grass species and between CO₂ levels (Wilkinson 2000).

Pairwise differences between means were examined by differences of least squares means, generated by PROC MIXED (SAS 2000). These multiple comparisons tested a priori hypotheses, and consequently differences significant at $P=0.05$ are indicated in the tables.

Results

Grasses

Elevated CO₂ significantly decreased protein levels in the C₃ grass, but not in the C₄ grass, although the CO₂ \times species interaction was not significant (Table 1). When grown at ambient CO₂, protein levels in the C₃ grass were 37% higher than in the C₄ grass (Table 1). However, when grown under elevated CO₂, the protein level in *L. multiflorum* was no longer significantly greater than the level in *B. curtipendula*.

Elevated CO₂ markedly increased sugar levels in the C₃ grass, but had no significant effect on those in the C₄ grass, producing a significant CO₂ \times grass species interaction. Sugar levels were over twice as high in *L. multiflorum* C₃ as in *B. curtipendula* C₄ at ambient CO₂, and threefold higher at elevated CO₂ (Table 1). Starch levels in the C₄ grass were 245% higher than in the C₃ grass at ambient CO₂. The difference between starch levels in the grasses under elevated CO₂ was smaller, but still significant, because starch levels increased in the C₃ grass but not in the C₄ grass. Fructan, present only in the C₃ grass, doubled at elevated CO₂. Overall, levels of total nonstructural carbohydrates (TNC; sugar, starch and fructan) were not significantly different in the C₃ grass and the C₄ grass at ambient CO₂, but were 78% higher in the C₃ grass than the C₄ grass at elevated CO₂, producing a significant CO₂ \times grass species interaction (Table 1).

The water content in *L. multiflorum* was significantly higher than in *B. curtipendula*, but did not differ between CO₂ treatments (Table 1). As expected, the fiber content in the C₄ grass was substantially higher than in the C₃ grass, but was not significantly affected by elevated CO₂. Leaf toughness, like fiber, was significantly greater in the C₄ grass than in the C₃ grass, and also was not significantly affected by elevated CO₂ (Table 1).

Grasshopper performance

Melanoplus sanguinipes grew 71% more rapidly on the C₃ than on the C₄ grass (Table 2). Most notably, elevated CO₂ had no detrimental effect on the performance of *M. sanguinipes* on either grass species. Contrary to our hypotheses, changes in the nutritional quality of the C₃ grass at elevated CO₂ did not cause grasshoppers to feed at a compensatory rate, nor were consumption rates significantly higher on the C₄ grass than on the C₃ grass (Table 2).

As expected, the C₃ grass was more digestible than the C₄ grass (Table 2), and CO₂ treatment had no effect on the

Table 1 Effect of atmospheric CO₂ concentration on levels of nutrients, fiber and toughness in a C₃ grass (*L. multiflorum*) and a C₄ grass (*B. curtipendula*). Data are presented as mean ±SE. Fructan is not present in C₄ grasses. Non-overlapping letters within columns designate statistically significant differences between means ($P < 0.05$). (TNC total nonstructural carbohydrate, ND not determined)

Grass species	CO ₂ (ppm)	Protein (%DW) n=18–20	Sugar (%DW) n=18–20	Starch (%DW) n=19	Fructan (%DW) n=16–20	TNC (%DW) n=16–20	Water (%FW) n=20	Fiber (%DW) n=10	Toughness (g) n=20
<i>L. multiflorum</i>	370	27.9±1.6 ^b	11.2±0.8 ^b	2.2±0.3 ^a	1.0±0.2 ^a	14.5±1.2 ^a	79.4±2.5 ^b	29.1±0.7 ^a	306±21 ^a
<i>L. multiflorum</i>	740	22.8±1.5 ^a	16.8±1.1 ^c	4.3±0.5 ^b	2.2±0.4 ^b	23.1±0.4 ^b	78.5±1.2 ^b	32.1±1.4 ^a	369±28 ^a
<i>B. curtipendula</i>	370	20.4±1.5 ^a	4.9±0.4 ^a	7.6±0.8 ^c	ND	11.4±1.3 ^a	71.0±1.0 ^a	55.3±1.3 ^b	706±33 ^b
<i>B. curtipendula</i>	740	19.0±1.1 ^a	5.5±0.4 ^a	8.2±0.8 ^c	ND	13.0±1.2 ^a	70.7±1.2 ^a	57.0±1.8 ^b	718±28 ^b
Significance of effects ^d		<0.001	<0.001	<0.001	ND	<0.001	<0.001	<0.001	<0.001
Grass species		0.040	0.003	0.099	0.007	<0.001	NS	NS	NS
CO ₂		NS	0.031	NS	ND	0.006	ND	NS	NS
CO ₂ × grass species									

^dP-values <0.10 are listed, and NS indicates $P > 0.10$. The significance of differences in water and fructan levels were determined by Kruskal–Wallis tests where possible

AD of either species. Similarly, the efficiency of conversion of ingested mass to body mass (ECI) was significantly greater for grasshoppers that fed on the C₃ grass than on the C₄ grass at both CO₂ levels. The conversion of digested (and assimilated) mass to body mass (ECD) did not differ significantly between the grass species at either CO₂ level. Again, CO₂ treatment had no significant effect on any of the nutritional indices, nor were any significant species × CO₂ interactions observed. The final weights attained by *M. sanguinipes* on the C₃ grass were 38% higher than on the C₄ grass (Table 2).

Nutrient AEs are the equivalent of ADs for individual nutrients. Protein AE was similar from both grasses, averaging 73% from the C₃ grass and 74% from the C₄ grass (Table 3). A small, but significant, increase in protein AE was observed at elevated CO₂. *Melanoplus sanguinipes* assimilated sugar with a similar, high efficiency from both grasses. Unlike other variables analyzed in this study, ANCOVA and ANOVA analyses resulted in different patterns of pairwise differences in sugar AE; the pairwise differences determined using an ANCOVA model (Table 3) did not match the results expected from the untransformed means. Unlike sugar, starch was assimilated from the C₃ grass 35% more efficiently than from the C₄ grass. Fructan was, in general, efficiently assimilated from the C₃ grass, although low efficiencies were observed in some individuals that fed on *L. multiflorum* from the elevated CO₂ treatment, producing a significant CO₂ treatment effect (Table 3).

The combined effects of foliar nutrient level, consumption rate and nutrient assimilation efficiency were expressed as nutrient AR (Table 4). Grasshoppers had a 32% higher protein AR from the C₃ grass than from the C₄ grass, largely as a result of the higher levels of protein in the C₃ grass. The protein AR was not significantly affected by elevated CO₂ in either grass species. Carbohydrate ARs each increased significantly under elevated CO₂ from the C₃ grass, but not from the C₄ grass, resulting in a significant CO₂ × grass species interaction in each case. Under elevated CO₂, the sugar AR from the C₃ grass increased 63%, the starch AR increased 133% and the fructan AR increased 102%. The sugar AR was higher in grasshoppers that fed on the C₃ grass than on the C₄ grass at both CO₂ levels. In contrast, the starch AR was 167% higher from the C₄ grass than from the C₃ grass at ambient CO₂, but did not differ between insects feeding on the two grasses at elevated CO₂ because of increased starch levels in *L. multiflorum*. Although the fructan AR from the C₃ grass doubled under elevated CO₂, fructan remained a minor component (7–8%) of the total carbohydrates assimilated from *L. multiflorum*. Overall, the AR of total nonstructural carbohydrates from the C₃ grass increased 73% at elevated CO₂, but was unaffected in grasshoppers feeding on the C₄ grass. This pattern was largely the result of higher sugar levels in the C₃ grass at elevated CO₂, although marked increases in both starch and fructan AR also contributed (Table 4).

Starch comprised a significantly greater proportion of the total carbohydrates assimilated from the C₄ grass than

Table 2 Growth and consumption rates, nutritional indices and final weights of *M. sanguinipes* on a C₃ grass (*L. multiflorum*) and a C₄ grass (*B. curtipendula*) grown at ambient or elevated atmospheric CO₂ concentrations. Data are presented as mean±SE. Growth and

consumption rates=mg/day. (*AD* approximate digestibility, *ECI* efficiency of conversion of ingested material, *ECD* efficiency of conversion of digested material). Non-overlapping letters within columns designate significantly different means ($P<0.05$)

Grass species	CO ₂ (ppm)	Growth rate	Consumption rate	AD	ECI	ECD ^c	Final weight (mg)	<i>n</i>
<i>L. multiflorum</i>	370	7.3±0.5 ^b	46±3 ^a	52.8±3.0 ^{ab}	16.5±1.2 ^b	33.9±3.8 ^a	34±2 ^b	14
<i>L. multiflorum</i>	740	8.2±0.6 ^b	53±5 ^a	56.3±2.7 ^b	16.1±1.0 ^b	30.0±3.2 ^a	38±2 ^b	13
<i>B. curtipendula</i>	370	4.8±0.4 ^a	46±4 ^a	44.2±2.9 ^a	11.0±1.0 ^a	26.6±3.7 ^a	26±2 ^a	10
<i>B. curtipendula</i>	740	4.3±0.6 ^a	47±3 ^a	45.0±4.8 ^a	9.5±1.3 ^a	26.2±3.1 ^a	26±2 ^a	16
Significance of effects ^d								
Grass species		0.011	NS	0.014	<0.001	NS	<0.001	
CO ₂		NS	NS	NS	NS	NS	NS	
CO ₂ × Grass species		NS	NS	NS	NS	NS	NS	

^cECD was analyzed by ANOVA, rather than by ANCOVA

^d*P*-values <0.10 are listed, and *NS* indicates $P>0.10$

from the C₃ grass ($P<0.001$). While sugar and starch were assimilated in approximately a 1:1 ratio from the C₄ grass, sugar comprised approximately 75% of the carbohydrates assimilated from the C₃ grass at ambient and elevated CO₂ levels.

Discussion

As expected, elevated CO₂ had little effect on the nutritional quality of the C₄ grass (*B. curtipendula*), but significantly changed the nutritional quality of the C₃ grass (*L. multiflorum*). Under elevated CO₂, levels of nonstructural carbohydrates (sugar, starch and fructan) increased in the C₃ grass, while protein decreased. This finding supports the hypothesis that elevated CO₂ reduces the nutritional quality of C₃ grasses to a greater extent than that of C₄ grasses, and is consistent with many previous findings (Poorter 1993; Poorter et al. 1997; Wand et al. 1999). Nevertheless, even at future CO₂ levels *L. multi-*

florum would be expected to remain a superior host plant compared to *B. curtipendula*; the C₃ grass contained higher levels of carbohydrates and water than the C₄ grass in both CO₂ treatments and higher levels of protein at ambient CO₂, while the C₄ grass contained a higher level of fiber and was significantly tougher than the C₃ grass. Results of feeding trials with *M. sanguinipes* support this expectation; grasshoppers grew more rapidly and attained higher final weights when they fed on *L. multiflorum* from both CO₂ treatments.

Contrary to the hypothesis that insect herbivores will increase their feeding rates disproportionately on C₃ plants under elevated atmospheric CO₂ (Lincoln et al. 1984, 1986; Lambers 1993), *M. sanguinipes* did not significantly increase its consumption rate when feeding on the C₃ grass grown under elevated CO₂. Instead, *M. sanguinipes* maintained a high growth rate on the C₃ grass grown under elevated CO₂ despite significant changes in its foliar nutritional quality, suggesting that post-ingestive mechanisms enable these grasshoppers to compensate for variable

Table 3 Assimilation efficiencies (*AE*) of carbohydrates and protein by *M. sanguinipes* from a C₃ grass (*L. multiflorum*) and a C₄ grass (*B. curtipendula*) grown at ambient or elevated atmospheric CO₂ concentrations. *AE* are presented as mean±SE. Fructan is not present in C₄ grasses. *AE* was calculated as (ingested nutrient mass –

egested nutrient mass)/ingested nutrient mass × 100. Non-overlapping letters within columns designate significantly different means ($P<0.05$) as determined by ANCOVA on transformed data. Untransformed summary statistics are presented. (*ND* not determined)

Grass species	CO ₂ (ppm)	AE (%)				<i>n</i>
		Protein	Sugar	Starch	Fructan	
<i>L. multiflorum</i>	370	70.0±2.6 ^a	87.8±1.5 ^a	93.1±0.8 ^b	94.4±0.6 ^b	14
<i>L. multiflorum</i>	740	75.8 ±1.6 ^b	90.4±1.2 ^{ab}	93.5±1.1 ^b	73.2±8.5 ^a	13
<i>B. curtipendula</i>	370	73.1±1.6 ^{ab}	91.0±1.8 ^b	70.7±4.9 ^a	ND	10
<i>B. curtipendula</i>	740	75.5±1.7 ^b	88.5±2.1 ^b	67.3±5.2 ^a	ND	16
Significance of effects ^c						
Grass species		NS	0.066	<0.001	ND	
CO ₂		0.029	NS	NS	0.034	
CO ₂ × Grass species		NS	NS	NS	ND	

^c*P*-values <0.10 are listed, and *NS* indicates $P>0.10$. Data were analyzed with ANCOVA, using nutrient mass egested as the dependent variable and nutrient mass ingested as the covariate. The significance of differences in *AE* of fructan were determined by Kruskal–Wallis tests

Table 4 Assimilation rates (*AR*) of protein and carbohydrates by *M. sanguinipes* from a C₃ grass (*L. multiflorum*) and a C₄ grass (*B. curtipendula*) grown at ambient or elevated atmospheric CO₂ concentrations. *AR* (mg/day) are presented as mean±SE. *TNC* (total

nonstructural carbohydrate) *AR* is the sum of sugar, starch and fructan *AR*. Non-overlapping letters within columns designate significantly different means ($P < 0.05$)]

Grass species	CO ₂ (ppm)	AR (mg/day)					<i>n</i>
		Protein	Sugar	Starch	Fructan	TNC	
<i>L. multiflorum</i>	370	9.3±0.8 ^b	4.6±0.3 ^b	0.9±0.06 ^a	0.44±0.03 ^a	6.0±0.4 ^b	14
<i>L. multiflorum</i>	740	8.6±0.8 ^b	7.5±0.7 ^c	2.1±0.2 ^b	0.89±0.16 ^b	10.4±0.8 ^c	13
<i>B. curtipendula</i>	370	6.8±0.6 ^a	2.0±0.2 ^a	2.4±0.3 ^b	0	4.5±0.4 ^a	10
<i>B. curtipendula</i>	740	6.8±0.4 ^a	2.3±0.2 ^a	2.6±0.3 ^b	0	4.9±0.5 ^a	16
Significance of effects ^d							
Grass species		0.019	<0.001	<0.001	ND	<0.001	
CO ₂		NS	<0.001	0.028	0.030	0.001	
CO ₂ × grass species		NS	0.003	0.016	ND	0.004	

^d*P*-values <0.10 are listed, and *NS* indicates $P > 0.10$. Data were analyzed with ANCOVA, using insect initial dry weight as the covariate

nutritional quality in their host plants. Post-ingestive compensatory responses in grasshoppers include changes in gut size, food residence time, digestive enzyme levels, and nutrient metabolism (Simpson and Simpson 1990; Bernays and Simpson 1990; Hinks et al. 1991; Zanotto et al. 1993; Yang and Joern 1994a, b). Thus, the hypothesis that feeding would be increased on the C₃ grass at elevated CO₂ was not supported, but the prediction that elevated CO₂ would not influence consumption rates on the C₄ grass was supported. In addition, *M. sanguinipes* did not compensate for the lower nutritional quality of the C₄ grass by increasing consumption over that on the C₃ grass, leading us to reject our third hypothesis. While post-ingestive mechanisms were sufficient to deal with changes in the nutritional quality of the C₃ grass at elevated CO₂, *M. sanguinipes* was unable to compensate for the lower nutritional quality of the C₄ grass. However, further work is needed to examine the efficacy of compensatory mechanisms that may require a longer time to develop than was possible in a 2-day feeding trial.

Although compensatory feeding was commonly observed in early studies, the absence of compensatory feeding on C₃ plants grown under elevated CO₂ has since been observed frequently among herbivorous insects (Bezemer and Jones 1998). Generalist and specialist caterpillars also did not feed at compensatory rates on *L. multiflorum* grown under elevated CO₂ (Barbehenn et al. 2004a). Again, it is noteworthy that post-ingestive mechanisms, rather than increased consumption rates, appear to enable these caterpillars to compensate for decreased nutritional quality in a C₃ grass at elevated CO₂.

Higher protein *AR* in grasshoppers feeding on the C₃ grass is likely to have contributed strongly to their better performance on this grass. Although higher carbohydrate *AR* from the C₃ grass may also have increased the performance of *M. sanguinipes*, the substantially higher carbohydrate *AR* in grasshoppers that fed on the C₃ grass grown in elevated CO₂ did not significantly increase their growth any further. *Locusta migratoria* grasshoppers also grew no faster when fed a carbohydrate-rich artificial diet

(Zanotto et al. 1993). An additional explanation for the better performance of *M. sanguinipes* on the C₃ grass is the possibility that the C₃ grass requires lower costs for processing than the C₄ grass. Although our results did not demonstrate a statistically significant difference in ECD between insects that fed on the two grass species, the C₄ grass was almost certainly more energetically costly to chew (Bernays 1986; Bernays and Hamai 1987), and might have required greater costs for digestion as well. *M. sanguinipes* had higher digestion efficiencies (AD) and conversion efficiencies (ECI) on the C₃ grass than the C₄ grass, but these results were most likely caused by the higher content of indigestible fiber found in C₄ grasses (Van Soest 1994).

Our results show that different host plants provide insect herbivores with different mixtures of carbohydrates, e.g., sugar contributed a greater fraction of the total carbohydrates assimilated by *M. sanguinipes* from the C₃ grass, while starch was the most important carbohydrate source from the C₄ grass. Since fructan is not produced by C₄ grasses, its presence in C₃ grasses is potentially a major difference between the carbohydrates provided by C₃ and C₄ grasses. Previous work on fructan (inulin) digestion by grasshoppers, including *M. sanguinipes*, found that it was not utilized or was only weakly digested (Morgan 1976; Dadd 1977). Thus, contrary to our expectations that grass fructan would act as an indigestible diluent of essential nutrients, our results demonstrate that grass fructan is utilized by grasshoppers. The potential for C₃ grasses to synthesize unusually high levels of fructan (e.g., 30% dry weight) suggests that fructan could be an important energy source in some environmental conditions, such as in cold weather (Volenc and Nelson 1984; Chatterton et al. 1989; Pollack and Cairns 1991). However, our results with *L. multiflorum* showed that elevated CO₂ does not change fructan levels in this C₃ grass in a nutritionally significant way. Recent results suggest that the effects of elevated CO₂ on *L. multiflorum* and *B. curtipendula* represent a common pattern among grasses (Barbehenn et al. 2004b).

The results of this study do not support the C₄ plant avoidance hypothesis as originally stated; levels of nutrients are commonly lower in C₄ grasses than in C₃ grasses (Barbehenn et al. 2004b), but they are not necessarily less digestible. Protein, which is commonly regarded as the most limiting macronutrient for herbivores (Mattson 1980), was assimilated with equal efficiencies from the C₃ and C₄ grass by *M. sanguinipes*. Given that as much as 50% of the soluble protein in C₄ plants resides in their thick-walled bundle sheath cells (Ku et al. 1979), the high efficiencies of digestion of protein from *B. curtipendula* demonstrate that bundle sheath cells are not a significant barrier to protein digestion, as was hypothesized (Caswell et al. 1973). Similarly, the bundle sheath cell anatomy of C₄ grasses was not an impediment to sugar assimilation by *M. sanguinipes*; the efficiencies of sugar assimilation from a C₃ and C₄ grass by *M. sanguinipes* in this study were approximately 90%, similar to previous measurements in grasshoppers on C₃ plants (Simpson 1982; Ferreira et al. 1992). In contrast to protein and sugar, starch was significantly less digestible from the C₄ grass than from the C₃ grass. While sugars and proteins are small enough to permeate cell walls during digestion (Barbehenn 1992), starch resides in relatively massive grains. Lower starch assimilation from the C₄ grass is, therefore, consistent with a greater structural integrity of the ingested C₄ than C₃ grass tissues (Caswell et al. 1973; Caswell and Reed 1975, 1976). Overall, the results of this study lead us to reject the fourth hypothesis that nutrients are more efficiently digested from C₃ than C₄ grasses. This is consistent with previous findings in a grass-specialist grasshopper (Boys 1981). Although this study examined only a single species of grass from each photosynthetic pathway, the differences in leaf anatomy between these plants (i.e., the presence or absence of bundle sheath cells) are sufficiently distinct to test whether C₄ grass nutrients are necessarily less digestible than those from C₃ grasses.

In conclusion, our results suggest that nutritional differences between C₃ and C₄ grasses may be diminished, but not eliminated, under future CO₂ conditions. Based on foliar nutrient composition and the performance of *M. sanguinipes*, the C₃ grass (*L. multiflorum*) would be expected to remain a superior host plant to the C₄ grass (*B. curtipendula*) at elevated CO₂ levels. However, even at ambient CO₂, grasshoppers make extensive use of *B. curtipendula* and other C₄ grasses in the field (e.g., Boutton et al. 1978; Heidorn and Joern 1984; Pinder and Jackson 1988). Although one would expect grasshoppers that fed primarily on C₄ grasses to have lower fitness than those that fed primarily on C₃ grasses, it is not surprising that these mobile herbivores commonly include C₄ grasses in their diets. The results of this study suggest that the nutritional quality of C₄ grasses is not as poor as has been hypothesized previously for grasshoppers (Caswell et al. 1973; Caswell and Reed 1975, 1976), and that the relative extent of feeding on C₄ grasses will likely depend on such factors as seasonal variation in the quality of the available host plants and the relative abundance of C₄ grasses in

different geographical regions (Teeri and Stowe 1976; Heidorn and Joern 1984).

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