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# Relative nutritional quality of $C_3$ and $C_4$ grasses for a graminivorous lepidopteran, *Paratrytone melane* (Hesperiidae)

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**Summary.** We tested the hypothesis that $C_4$ grasses are inferior to $C_3$ grasses as host plants for herbivorous insects by measuring the relative performance of larvae of a graminivorous lepidopteran, *Paratrytone melane* (Hesperiidae), fed $C_3$ and $C_4$ grasses. Relative growth rates and final weights were higher in larvae fed a $C_3$ grass in Experiment I. However, in two additional experiments, relative growth rates and final weights were not significantly different in larvae fed $C_3$ and $C_4$ grasses. We examined two factors which are believed to cause $C_4$ grasses to be of lower nutritional value than $C_3$ grasses: foliar nutrient levels and nutrient digestibility. In general, foliar nutrient levels were higher in $C_3$ grasses. In Experiment I, protein and soluble carbohydrates were digested from a $C_3$ and a $C_4$ grass with equivalent efficiencies. Therefore, differences in larval performance are best explained by higher nutrient levels in the $C_3$ grass in this experiment. In Experiment II, soluble carbohydrates were digested with similar efficiencies from $C_3$ and $C_4$ grasses but protein was digested with greater efficiency from the $C_3$ grasses. We conclude (1) that the bundle sheath anatomy of $C_4$ grasses is not a barrier to soluble carbohydrate digestion and does not have a nutritionally significant effect on protein digestion and (2) that *P. melane* may consume $C_4$ grasses at compensatory rates.

**Key words:** $C_3$ and $C_4$ grasses – Lepidoptera – Hesperiidae – *Paratrytone melane* – Nutrients

Caswell et al. (1973) hypothesized that $C_4$ plants may be poorer host plants for herbivores than $C_3$ plants and, therefore, should be avoided relative to $C_3$ plants. This hypothesis was based on the anatomical and nutritional differences between $C_3$ and $C_4$ plant photosynthetic tissues. Plants with the $C_4$ photosynthetic pathway have a characteristic Kranz anatomy: vascular bundles are surrounded by an inner cylinder of thick-walled bundle sheath cells (BSC), which itself is surrounded by an outer layer of mesophyll cells (Laetsch 1974). Photosynthesis occurs solely in these adjoining layers in $C_4$ plants. Therefore, these are the most nutrient-rich tissues in $C_4$ grass leaves. $C_3$ grasses have a diffusely arranged photosynthetic mesophyll and no thick-walled, photosynthetic BSC. Thus, $C_4$ grasses are believed to be poorer hosts than $C_3$ grasses for phytophagous insects for two reasons: (1) the nutrients found in the BSC of $C_4$ grasses are believed to be indigestible because of the inability of insects to crush BSC (Caswell and Reed 1975, 1976) and (2) concentrations of nutrients, such as protein and soluble carbohydrates, are generally lower in $C_4$ plants than in $C_3$ plants (Caswell et al. 1973; Boutton et al. 1978; Van Soest 1982).

Research on the relative nutritional value of $C_3$ and $C_4$ grasses for insects has been based largely on grasshoppers (Orthoptera: Acrididae) and has generally concluded that $C_4$ grasses are poorer host plants than $C_3$ grasses because of the lower digestibility of $C_4$ grasses (Caswell and Reed 1975, 1976; Heidorn and Joern 1984). However, the digestibility of nutrients from a $C_3$ and a $C_4$ grass has been measured in only one study (Boys 1981) and no grass-specialist Lepidoptera have been used to test this hypothesis mechanistically.

Three lines of evidence suggest that generalizations about the relative nutritional value of $C_3$ and $C_4$ grasses need reevaluation: (1) no significant differences were found between protein and soluble carbohydrate digestibilities for *Locusta migratoria* (Orthoptera: Acrididae) fed a $C_3$ and a $C_4$ grass (Boys 1981); (2) *Hemileuca oliviae* (Lepidoptera: Saturniidae) preferred $C_4$ grasses and had higher growth rates on these grasses (Capinera 1978); and (3) tests of the hypothesis that $C_4$ plants tend to be avoided by phytophagous insects have produced variable results (Pinder and Kroh 1987; Pinder and Jackson 1988).

*Paratrytone melane* Edwards (Lepidoptera: Hesperiidae) feeds exclusively on a variety of $C_3$ and $C_4$ grasses and some sedges (Scott 1986). Its range extends from northern California, where $C_3$ species are dominant, to northern Baja California, where $C_4$ species are dominant (Heppner

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1972; Teeri and Stowe 1976). In order to test whether $C_4$ grasses are nutritionally poorer host plants than $C_3$ grasses for a graminivorous insect, we measured both the relative effect of $C_4$ grass herbivory on the performance of *P. melane* and the relative digestibility of nutrients from $C_3$ and $C_4$ grasses. If the availability of nutrients from $C_4$ grasses is reduced because of the inability of insects to crush BSC, then nutrient digestion efficiencies should be lower from $C_4$ grasses than from $C_3$ grasses. We measured the digestibilities of specific nutrients, rather than total dry mass digestibility, because a greater percentage of $C_4$ grass dry mass is composed of indigestible structural materials, such as lignin, cellulose and hemicellulose (Van Soest 1982). Therefore, a lower percentage of $C_4$ grass dry mass may be digested regardless of whether BSC impede nutrient digestion.

## Materials and methods

### *Grasses*

Grasses were grown in 15-cm pots in a standard soil mixture ("UC mix"). Grasses were watered daily and were fertilized weekly with approximately 150 ml of Hoagland's solution. *Cynodon dactylon* (L.) Pers. ($C_4$) and *Lolium multiflorum* Lam. ($C_3$) were grown from seed in a greenhouse in Expt I. In Expt II, *Pennisetum clandestinum* Hochst. ex Chiov. ($C_4$), *Dactylis glomerata* L. ($C_3$), and *Ehrharta erecta* Lam. ($C_3$) were dug from field sites and grown in standard soil in a lathhouse. *C. dactylon*, *L. multiflorum*, and *Paspalum dilatatum* Poir. ($C_4$) were grown from seed. Each species was grown in a lathhouse except *C. dactylon*, which was grown in a greenhouse. In Expt III, *Agrostis palustris* Huds. ($C_3$), *Festuca myuros* L. ($C_3$), *Festuca rubra* L. ($C_3$), *Sorghum sudanense* (Piper) Stapf. ($C_4$), *Agropyron cristatum* (L.) ($C_3$), *P. dilatatum*, and *E. erecta* were grown from seed in a lathhouse and *P. clandestinum* was dug from a field site. *C. dactylon* and *Muhlenbergia rigens* (Benth) Hitch. ($C_4$) were grown from seed in a greenhouse. All grasses were over three months old before their leaves were fed to larvae, with the exception of *L. multiflorum* in Expt II, which was one month old.

### *Insects*

*P. melane* adults were caught in Berkeley, CA and a colony was maintained in an outdoor screen cage (approx. $13 \times 7 \times 3$ m). Eggs were collected from the undersurfaces of leaves of *C. dactylon*, *E. erecta*, *P. clandestinum*, and *S. sudanense* growing in a mixed stand in the cage. *C. dactylon* and *P. dilatatum* are known hosts. In addition, we used several newly-observed hosts: *Bromus carinatus* (Hook. and Arn.) ($C_3$), *E. erecta*, *L. multiflorum*, *P. clandestinum*, and *S. sudanense* (Barbehenn, unpub.). *P. melane* has not been observed to feed on *A. cristatum*, *A. palustris*, *F. myuros*, *F. rubra* or *M. rigens*.

### *Consumption, digestion and growth of larvae fed $C_3$ and $C_4$ grasses*

*Experiment I.* Eggs from a summer generation of *P. melane* were placed on *C. dactylon* or *L. multiflorum*. Colonies on each host plant were reared indoors in natural light at an average temperature of 28° C (range = 24–32° C) and 50% relative humidity. Pharate fifth-instar larvae were taken from each colony and weighed the day on which they completed molting. The initial dry weights (DW) of larvae were estimated from the percentage DW of newly-molted larvae of the same age sacrificed from each colony. Larval gut contents were removed before drying (Barbehenn and Keddie 1992). Larvae were kept individually in 275-ml plastic cups with gauze tops in an incubator (Percival) maintained at 30° C:20° C with a 12 h light: 12 h dark photoperiod and 62% average relative humidity. We observed that *P. melane* larvae generally feed from the tips of leaves. Therefore, entire, fully-expanded leaves (*C. dactylon*) or the distal ends of fully-expanded leaves (*L. multiflorum*) were fed to larvae in each treatment daily. Cut leaves were weighed and placed immediately in a vial of water in each larval container. The fresh weight (FW) of food and DW of frass and uneaten food were weighed on a Sartorius electronic balance to the nearest 0.1 mg. Relative growth rate (RGR), relative consumption rate (RCR), and relative nutrient consumption rates were measured gravimetrically on a DW basis (Waldbauer 1968) and were calculated using exponential mean weights of larvae (Montgomery 1983). FW of experimental pupae were converted to DW using the average percentage DW of pupae from each host plant. Nutrient digestibilities were calculated as (mg nutrient ingested-mg nutrient egested)/mg nutrient ingested $\times$ 100. Relative nutrient consumption rates were calculated as (mg nutrient ingested/mg mean larval DW/day) $\times$ 100.

*Experiment II.* This experiment expanded the number of species of $C_3$ and $C_4$ grasses fed to *P. melane*. Larvae from a fall generation were reared on *C. dactylon* in a greenhouse until the fourth instar. Pharate fourth instar larvae were assigned at random to each of six grass species and a group used for determining larval percentage DW. Experimental conditions were repeated from Expt I. Entire, fully-expanded leaves (*C. dactylon*) or the distal ends of fully-expanded leaves were fed to larvae. The final DW of newly-molted, fifth-instar larvae were measured after their gut contents were removed. One month old *L. multiflorum* was commonly rejected by larvae and/or did not support growth and therefore was discontinued as a treatment.

*Experiment III.* Expt II was repeated to measure the performance of fourth-instar *P. melane* larvae fed a similar group of $C_3$ and $C_4$ grasses. Larvae from a summer generation were reared on *C. dactylon* in conditions described for Expt II through the third instar. When these larvae molted to the fourth-instar they were assigned at random to one of six grass species or a group sacrificed to determine percent DW (Expt IIIa). Fourth-instar larvae from the same cohort which molted approximately one week later were assigned at random to feed on *A. cristatum* ($C_3$) or *S. sudanense* ($C_4$) (Expt IIIb). The experimental conditions of Expt I were repeated with the exceptions of a 15 h light: 9 h dark photoperiod and 70% average relative humidity. Entire, fully-expanded leaves or the distal ends of fully-expanded leaves (*E. erecta*, *M. rigens*, *P. dilatatum*, and *S. sudanense*) were fed to larvae. *M. rigens* and *F. myuros* were commonly rejected by larvae and/or did not support growth and therefore were discontinued as treatments.

### *Host preference*

Twelve fourth-instar larvae of similar age reared on *C. dactylon* were maintained as described for Expt I, with the exception of temperatures of 20° C:14° C. Each pair of nine combinations of $C_3$ and $C_4$ grasses (*B. carinatus*, *D. glomerata*, *E. erecta* ($C_3$ species) and *C. dactylon*, *P. dilatatum*, *P. clandestinum* ($C_4$ species)) was offered to each larva over a nine-day period. The order in which each grass pair was fed to each larva was randomized in order to eliminate any biases due to changes in plant quality during the experiment. Similar amounts of each grass were weighed and placed together in vials of water in each larval container. Larvae were allowed to feed overnight on each pair of grasses. Consumption was measured gravimetrically on a DW basis. Preference was defined as the consumption of a greater amount of either grass species in each pair.

*Nutrient analyses*

Representative samples of grass leaves (n = 3 replicates) were cut daily from each grass species and weighed immediately (500–800 mg FW). Samples were dried at 70° C to a constant weight and ground in a Wiley mill through 60-mesh (Expt I) or 40-mesh screens (Expt II).

Crude protein (%N × 5.3) was measured by Kjeldahl analysis (Tecator) in Expt I. It was necessary to pool frass from larvae in each treatment on each date analyzed ($n = 6$–9 dates per treatment). Uric acid was measured in 0.6% $LiCO_3$ extracts of frass with a uric acid test kit (Sigma) to correct for excreted N. Average protein levels in grasses were calculated from samples taken at 11–14 dates during the course of the experiment. In Expt II, protein was measured as total amino acids in duplicate frass samples from each larva. Grass samples from the same four dates were analyzed from each species. Samples were hydrolyzed in 6M HCl at 110° C for 24 h in screw-cap vials (Pierce) flushed with nitrogen. Amino acid levels were quantified using ninhydrin (Sigma) (Moore and Stein 1954). Hydrolyzed bovine serum albumin was used as a standard. The common forms of excreted nitrogen in Lepidoptera do not interfere with the ninhydrin method (Barbehenn unpub.).

Soluble carbohydrates were measured using the anthrone method (Yemm and Willis 1954). Grass and frass samples (10 mg) were extracted in 5.0 ml water at 25° C for 1.5 h. Extracts (0.5 ml) were mixed with 5.0 ml anthrone reagent and heated at 80° C for 15 min. The absorbance of each solution was measured with a spectrophotometer at a wavelength of 620 nm.

Water content was measured as (1-DW/FW) × 100 for samples from 34–39 dates, 43–47 dates, and 10–15 dates in Expts I-III, respectively.

*Statistical analyses*

Consumption and growth rates in Expt I were analyzed with ANCOVA using initial DW as a covariate in order to take into account differences in initial DW between treatments (Wilkinson 1986). Initial larval DW did not differ significantly in Expts II and III. Comparisons of consumption, digestion, larval growth, and grass nutrient levels in Expts II and III were made between $C_3$ and $C_4$ grasses with nested ANOVA (Wilkinson 1986). Although not all data sets met the assumptions of the nested ANOVA, none of the resulting probabilities concerning *P. melane* were close to significance ($0.7 > P > 0.2$). Tests of differences in grass nutrient levels in Expts II and III which were close to significance were also made with Mann-Whitney U-tests on pooled $C_3$ and $C_4$ grass data to corroborate the results of nested ANOVAs. In cases in which data were not normally distributed and/or had nonhomogeneous variances and could not be transformed, unplanned pairwise comparisons were made with Mann-Whitney U-tests followed by sequential Bonferroni tests ($\alpha = 0.05$/number of pairwise tests remaining) (Rice 1989). In all other cases, unplanned pairwise comparisons of means were made with Tukey's honestly significant difference test (Wilkinson 1986). Data from all treatments in Expt II which were analyzed for correlations were pooled following ANCOVA results showing that all treatments fit a common regression line (Wilcoxon 1986). Fisher's method for combining probabilities from independent experiments testing the same hypothesis was used as indicated (Sokal and Rohlf 1981). Host preferences were analyzed with Wilcoxon signed ranks tests. Survivorship was compared with G-tests of independence on 2 × 2 tables (Sokal and Rohlf 1981).

## Results

*Grass nutrient levels*

Some $C_4$ grasses contained comparable or higher levels of certain nutrients than some $C_3$ grasses. However, no $C_4$ grass was as nutritious in all aspects measured as any of the $C_3$ grasses. Protein levels were significantly higher in *L. multiflorum* ($C_3$) than in *C. dactylon* ($C_4$) in Expt I ($P < 0.0001$; Table 1). $C_3$ grasses also had higher protein levels than $C_4$ grasses in Expt II ($P = 0.058$; Table 1). Soluble carbohydrate levels in *L. multiflorum* were higher than in *C. dactylon* in Expt I ($P < 0.0001$; Table 1), but no significant difference between soluble carbohydrate levels in $C_3$ and $C_4$ grasses was found in Expt II ($P = 0.330$). Overall, water concentrations in $C_3$ grasses were higher than in $C_4$ grasses, averaging 81.2% ($n = 7$ species) and 75.4% ($n = 5$ species), respectively (combined $P < 0.001$; Table 1). All probabilities from nested ANOVA which were near significance were found to be of greater significance using the Mann-Whitney *U*-test to corroborate these results ($P < 0.001$). Therefore, the probabilities presented using nested ANOVA on data which could not be transformed appear to be conservative.

*Consumption of grasses and nutrients*

RCR of *P. melane* larvae fed $C_3$ and $C_4$ grasses in Expt I were not significantly different ($P = 0.10$; Table 4). In Expt II, RCR of larvae fed $C_4$ grasses were close to being significantly higher than those of larvae fed $C_3$ grasses ($P = 0.057$; Table 4). When probabilities from both experiments were combined, larvae fed $C_4$ grasses were found to have a significantly greater RCR than those fed $C_3$ grasses ($P < 0.05$). Relative nutrient consumption rates of larvae fed *C. dactylon* in Expt I were lower for each nutrient measured (Table 2). In Expt II, by comparison, no significant differences were found between relative nutrient consumption rates of larvae fed $C_3$ and $C_4$ grasses (Table 2). This suggests that larvae in Expt II were able to compensate for the generally lower nutrient levels in the $C_4$ grasses.

*Nutrient digestibility*

Average soluble carbohydrate digestibilities in *P. melane* fed *C. dactylon* and *L. multiflorum* in Expt I were similar (Table 3). Soluble carbohydrate digestibilities also did not differ significantly between larvae fed $C_3$ and $C_4$ grasses in Expt II (Table 3), suggesting that the BSC anatomy of $C_4$ grasses did not impede the digestion of these nutrients.

Average protein digestibilities for larvae reared on *C. dactylon* ($C_4$) and *L. multiflorum* ($C_3$) were similar in Expt I (Table 3), suggesting that $C_4$ grass BSC anatomy also was not a barrier to protein digestion. No uric acid was measured in the frass of fifth-instar *P. melane* and no further attempt was made to correct for other forms of excreted N (Bursell 1967). In Expt II larvae had significantly higher protein digestion efficiencies when fed $C_3$ grasses (58.6% vs 47.6% on $C_4$ grasses) ($P < 0.004$; Table 3).

*Larval growth and survivorship*

RGR and final weights of larvae fed *L. multiflorum* ($C_3$) in Expt I were significantly higher than RGR of larvae fed

**Table 1.** Protein, soluble carbohydrate, and water contents of $C_3$ and $C_4$ grasses[1]

| Grass species | Protein (%)[2] | Soluble carbohydrate (%) | Water (%) |
|---|---|---|---|
| *Experiment I* | | | |
| *L. multiflorum* ($C_3$) | 11.9±0.6 | 31.8±4.2 | 80.2±0.4 |
| *C. dactylon* ($C_4$) | 8.0±0.5 | 7.9±0.4 | 67.4±0.4 |
| Probability[3] | <0.0001 | <0.0001 | <0.0001 |
| *Experiment II* | | | |
| *D. glomerata* ($C_3$) | 29.8±0.9$^a$ | 3.5±0.3$^a$ | 80.7±0.2$^c$ |
| *E. erecta* ($C_3$) | 18.8±0.5$^a$ | 11.3±0.8$^b$ | 79.2±0.3$^d$ |
| *L. multiflorum* ($C_3$) | 26.5±2.0$^a$ | 3.8±0.3$^a$ | 89.4±0.2$^e$ |
| *C. dactylon* ($C_4$) | 17.9±1.0$^a$ | 3.0±0.3$^a$ | 74.3±0.2$^a$ |
| *P. dilatatum* ($C_4$) | 16.7±0.7$^a$ | 4.0±0.2$^a$ | 75.8±0.3$^b$ |
| *P. clandestinum* ($C_4$) | 14.7±0.3$^a$ | 3.1±0.2$^a$ | 76.4±0.2$^b$ |
| F (df) | 6.97 (1,4) | 1.21 (1,4) | 5.65 (1,4) |
| Probability[4] | 0.058 | 0.330 | 0.076 |
| *Experiment III* | | | |
| *F. myuros* ($C_3$) | ---- | ---- | 83.5±0.6$^f$ |
| *E. erecta* ($C_3$) | ---- | ---- | 81.8±0.3$^{ef}$ |
| *A. palustris* ($C_3$) | ---- | ---- | 81.1±0.9$^{def}$ |
| *A. cristatum* ($C_3$) | ---- | ---- | 79.4±1.2$^{cdef}$ |
| *F. rubra* ($C_3$) | ---- | ---- | 78.7±0.5$^{bcd}$ |
| *P. dilatatum* ($C_4$) | ---- | ---- | 80.5±0.6$^{def}$ |
| *P. clandestinum* ($C_4$) | ---- | ---- | 77.9±0.4$^{bcd}$ |
| *S. sudanense* ($C_4$) | ---- | ---- | 76.4±1.5$^{bcd}$ |
| *M. rigens* ($C_4$) | ---- | ---- | 75.5±0.7$^{bc}$ |
| *C. dactylon* ($C_4$) | ---- | ---- | 67.7±0.6$^a$ |
| F (df) | | | 5.14 (1,8) |
| Probability[4] | | | 0.053 |

[1] Data are presented as mean±standard error. Means in columns followed by different letters are significantly different ($P<0.05$)
[2] Protein was measured with the Kjeldahl method in Expt I and with ninhydrin on hydrolyzed samples in Expt II
[3] Mann-Whitney *U*-test
[4] Nested ANOVA comparing $C_3$ and $C_4$ grasses

**Table 2.** Relative nutrient consumption rates of *P. melane* larvae fed $C_3$ and $C_4$ grasses[1]

| Grass species | RPCR[2] (mg/mg/d) | RCCR (mg/mg/d) | RWCR (mg/mg/d) |
|---|---|---|---|
| *Experiment I* | | | |
| *L. multiflorum* ($C_3$) | 0.11±0.004 | 0.26±0.01 | 3.38±0.13 |
| *C. dactylon* ($C_4$) | 0.08±0.004 | 0.08±0.004 | 1.96±0.10 |
| F (df) | 15.57 (1,26) | 278.8 (1,26) | 63.9 (1,26) |
| Probability[3] | <0.001 | <0.001 | <0.001 |
| *Experiment II* | | | |
| *D. glomerata* ($C_3$) | 0.17±0.01$^b$ | 0.020±0.001$^a$ | 2.36±0.18$^a$ |
| *E. erecta* ($C_3$) | 0.12±0.007$^a$ | 0.075±0.004$^c$ | 2.44±0.14$^a$ |
| *C. dactylon* ($C_4$) | 0.13±0.007$^{ab}$ | 0.021±0.001$^a$ | 2.14±0.12$^a$ |
| *P. dilatatum* ($C_4$) | 0.12±0.005$^a$ | 0.029±0.001$^b$ | 2.19±0.09$^a$ |
| *P. clandestinum* ($C_4$) | 0.12±0.02$^{ab}$ | 0.025±0.004$^{ab}$ | 2.65±0.40$^a$ |
| F (df) | 1.08 (1,3) | 0.96 (1,3) | 0.16 (1,3) |
| Probability[4] | 0.37 | 0.40 | 0.71 |

[1] Data are presented as mean±standard error. Means in columns followed by different letters are significantly different ($P<0.05$)
[2] RPCR = relative protein consumption rate, RCCR = relative carbohydrate consumption rate, RWCR = relative water consumption rate
[3] ANCOVA using initial larval dry weight as a covariate
[4] Nested ANOVA comparing larvae fed $C_3$ or $C_4$ grasses

**Table 3.** Digestibility of nutrients in $C_3$ and $C_4$ grasses by *P. melane* larvae[1]

| Grass species | Soluble carbohydrate digestibility (%) | Protein digestibility (%) |
|---|---|---|
| *Experiment I* | | |
| *L. multiflorum* ($C_3$) | 70.9 | 77.9 |
| *C. dactylon* ($C_4$)[2] | 78.0 | 79.7 |
| *Experiment II* | | |
| *D. glomerata* ($C_3$) | $78.9 \pm 3.5^{ab}$ | $59.4 \pm 3.1^{a}$ |
| *E. erecta* ($C_3$) | $89.5 \pm 0.9^{c}$ | $57.7 \pm 5.0^{a}$ |
| *C. dactylon* ($C_4$)[2] | $67.8 \pm 4.2^{a}$ | $46.8 \pm 3.9^{a}$ |
| *P. dilatatum* ($C_4$) | $81.5 \pm 2.0^{b}$ | $46.3 \pm 2.8^{a}$ |
| *P. clandestinum* ($C_4$) | $71.7 \pm 5.7^{ab}$ | $49.8 \pm 5.4^{a}$ |
| F (df) | 2.32 (1,3) | 63.91 (1,3) |
| Probability[3] | 0.22 | 0.004 |

[1] Data are presented as mean±standard error. Means in columns followed by different letters are significantly different ($P < 0.05$)
[2] *C. dactylon* data previously published (Barbehenn 1992)
[3] Nested ANOVA comparing larvae fed $C_3$ or $C_4$ grasses

*C. dactylon* ($C_4$) (Table 4). Since nutrient digestibilities were similar for larvae fed both grass species, higher RGR of larvae fed the $C_3$ grass are most simply interpreted as resulting from higher nutrient levels in this grass. In Expts II and IIIa,b no significant differences in RGR or final weights were found between larvae fed $C_3$ and $C_4$ grasses (Table 4). Development times did not differ between larvae fed $C_3$ or $C_4$ grasses in any of the experiments.

RGR were significantly correlated with grass protein levels in Expt II ($r = 0.35$, $P = 0.011$) and highly correlated with protein digestibility ($r = 0.69$, $P < 0.0001$). RGR was also significantly correlated with grass water contents in Expt II ($r = 0.34$, $P = 0.016$). RGR was not significantly correlated with soluble carbohydrate digestibility ($r = 0.11$, $P = 0.46$), however, and RGRs were not correlated with grass soluble carbohydrate levels ($r = 0.071$, $P = 0.64$), suggesting that soluble carbohydrate levels did not limit growth.

Survivorship of *P. melane* fed $C_3$ and $C_4$ grasses was not significantly different ($P > 0.1$ in each experiment) (Table 5). In Expt I, II and III survivorship on $C_3$ grasses

**Table 4.** Consumption and growth of *P. melane* larvae fed $C_3$ and $C_4$ grasses[1]

| Grass species | RCR (mg/mg/d) | RGR (mg/mg/d) | Final weight (mg) | Development time (d) |
|---|---|---|---|---|
| *Experiment I* | | | | |
| *L. multiflorum* ($C_3$) | $0.83 \pm 0.03$ | $0.110 \pm 0.005$ | $74.8 \pm 2.0$ | $16.9 \pm 0.6$ |
| *C. dactylon* ($C_4$) | $0.95 \pm 0.05$ | $0.090 \pm 0.003$ | $56.9 \pm 1.3$ | $18.9 \pm 0.9$ |
| F (df) | 2.88 (1,26) | 10.98 (1,26) | ---- | ---- |
| Probability | 0.10[2] | 0.003[2] | <0.0001[3] | 0.19[3] |
| *Experiment II* | | | | |
| *D. glomerata* ($C_3$) | $0.56 \pm 0.04^{a}$ | $0.054 \pm 0.005^{b}$ | $12.0 \pm 0.8^{a}$ | $20.3 \pm 1.3^{a}$ |
| *E. erecta* ($C_3$) | $0.64 \pm 0.04^{ab}$ | $0.047 \pm 0.006^{ab}$ | $10.4 \pm 0.7^{a}$ | $22.0 \pm 2.3^{a}$ |
| *C. dactylon* ($C_4$) | $0.74 \pm 0.04^{ab}$ | $0.038 \pm 0.005^{ab}$ | $9.1 \pm 0.7^{a}$ | $23.0 \pm 1.9^{a}$ |
| *P. dilatatum* ($C_4$) | $0.70 \pm 0.03^{ab}$ | $0.044 \pm 0.004^{ab}$ | $10.3 \pm 0.6^{a}$ | $23.2 \pm 1.3^{a}$ |
| *P. clandestinum* ($C_4$) | $0.82 \pm 0.12^{b}$ | $0.032 \pm 0.006^{a}$ | $10.2 \pm 1.4^{a}$ | $28.0 \pm 3.5^{b}$ |
| F (df) | 9.10 (1,3) | 5.86 (1,3) | 1.52 (1,3) | 3.85 (1,3) |
| Probability[4] | 0.057 | 0.094 | 0.30 | 0.14 |
| *Experiment IIIa* | | | | |
| *A. palustris* ($C_3$) | ---- | $0.150 \pm 0.007^{ab}$ | $9.4 \pm 0.3^{a}$ | $11.3 \pm 0.3^{ab}$ |
| *E. erecta* ($C_3$) | ---- | $0.144 \pm 0.006^{ab}$ | $9.6 \pm 0.4^{a}$ | $11.1 \pm 0.3^{ab}$ |
| *F. rubra* ($C_3$) | ---- | $0.161 \pm 0.004^{b}$ | $9.9 \pm 0.2^{ab}$ | $10.2 \pm 0.2^{a}$ |
| *C. dactylon* ($C_4$) | ---- | $0.137 \pm 0.006^{a}$ | $8.9 \pm 0.2^{a}$ | $11.5 \pm 0.5^{ab}$ |
| *P. dilatatum* ($C_4$) | ---- | $0.138 \pm 0.006^{a}$ | $9.7 \pm 0.2^{a}$ | $11.6 \pm 0.5^{ab}$ |
| *P. clandestinum* ($C_4$) | ---- | $0.143 \pm 0.005^{ab}$ | $10.8 \pm 0.2^{b}$ | $12.0 \pm 0.3^{b}$ |
| F (df) | ---- | 5.10 (1,4) | 0.090 (1,4) | 5.71 (1,4) |
| Probability[4] | ---- | 0.087 | 0.78 | 0.075 |
| *Experiment IIIb* | | | | |
| *A. cristatum* ($C_3$) | ---- | $0.175 \pm 0.010$ | $10.2 \pm 0.5$ | $10.6 \pm 0.5$ |
| *S. sudanense* ($C_4$) | ---- | $0.185 \pm 0.008$ | $9.9 \pm 0.4$ | $10.3 \pm 0.4$ |
| Probability[3] | | 0.49 | 0.79 | 0.61 |

[1] Data are presented as mean±standard error. Means within columns followed by different letters are significantly different ($P < 0.05$). RCR = relative consumption rate, RGR = relative growth rate
[2] ANCOVA using initial larval dry weight as a covariate
[3] Mann-Whitney U-test
[4] Nested ANOVA comparing larvae fed $C_3$ or $C_4$ grasses

**Table 5.** Survivorship of *P. melane* larvae fed $C_3$ and $C_4$ grasses

| Grass species | Final *n* | Percent survival |
|---|---|---|
| *Experiment I* | | |
| *L. multiflorum* ($C_3$) | 14 | 93 |
| *C. dactylon* ($C_4$) | 15 | 94 |
| *Experiment II* | | |
| *D. glomerata* ($C_3$) | 13 | 93 |
| *E. erecta* ($C_3$) | 10 | 71 |
| *C. dactylon* ($C_4$) | 8 | 57 |
| *P. dilatatum* ($C_4$) | 15 | 100 |
| *P. clandestinum* ($C_4$) | 5 | 42 |
| *Experiment IIIa* | | |
| *A. palustris* ($C_3$) | 11 | 100 |
| *E. erecta* ($C_3$) | 11 | 100 |
| *F. rubra* ($C_3$) | 11 | 100 |
| *C. dactylon* ($C_4$) | 12 | 100 |
| *P. dilatatum* ($C_4$) | 13 | 93 |
| *P. clandestinum* ($C_4$) | 9 | 100 |
| *Experiment IIIb* | | |
| *A. cristatum* ($C_3$) | 9 | 100 |
| *S. sudanense* ($C_4$) | 7 | 88 |

averaged 93%, 78%, and 100%, respectively, and on $C_4$ grasses 94%, 66% and 95%.

*Host preference*

*P. melane* showed no significant preference for $C_3$ grasses (combined probabilities for individuals $> 0.1$). RCR of $C_3$ and $C_4$ grasses averaged $2.94 \pm 0.083$ (SE) and $2.18 \pm 0.062$ mg/mg/day, respectively.

## Discussion

The hypothesis that $C_4$ grasses are nutritionally inferior to $C_3$ grasses as a result of both lower nutrient levels and the indigestibility of BSC was not supported by Expt I. The results of Expt I, in which the digestive efficiencies and growth of *P. melane* fed *L. multiflorum* ($C_3$) and *C. dactylon* ($C_4$) were compared, show that nutrient digestibilities were similar from each grass. This evidence suggests that the BSC anatomy of at least some $C_4$ grasses is not a barrier to digestion, as has been proposed (Caswell et al. 1973; Caswell and Reed 1975, 1976). Higher RGR and final weights of larvae fed *L. multiflorum* are most simply explained by the higher nutrient levels in this grass (Mattson 1980; Dadd 1985; Martin and Van't Hoff 1989).

The results of Expt II, in which digestive efficiencies and growth of *P. melane* were compared on two $C_3$ grasses and three $C_4$ grasses, also show that the BSC anatomy of $C_4$ grasses does not reduce soluble carbohydrate digestibilities. However, reduced protein digestibility from $C_4$ grasses suggests that their BSC anatomy may have impeded protein digestion in this experiment. The results of Expt II and of Expt III, in which the growth of *P. melane* was compared on eight $C_3$ and $C_4$ grasses, do not support the hypothesis that $C_3$ grasses are better host plants than $C_4$ grasses; neither RGR nor final weights of larvae fed $C_3$ and $C_4$ grasses were significantly different. In general, therefore, there appears to be a relatively small cost to feeding on $C_4$ grasses in terms of RGR and final weight for *P. melane*, and no cost in terms of survival.

An absolute difference of 11.0% in protein digestibilities between $C_3$ and $C_4$ grasses in Expt II, though statistically significant, had no apparent effect on performance and is less than would be expected if BSC protein in $C_4$ grasses was completely indigestible. BSC may contain on the order of 50% of the soluble protein in the leaves of some grass species (Ku et al. 1979). Protein digestibilities of less than 30% would be expected if BSC protein was indigestible (assuming the remaining 50% of the protein was digested with an efficiency of 58.6%, the average $C_3$ grass protein digestibility in Expt II).

*P. melane* appears to have a digestive strategy based on leaching nutrients from uncrushed leaf tissues (Barbehenn 1992). In the snipped leaf tissues of *C. dactylon*, for example, only 15–30% of leaf cells are crushed during the digestive process. Therefore, nutrient digestibility would not be expected to differ between $C_3$ and $C_4$ grasses because of the inability of *P. melane* to crush BSC. Effects of the BSC anatomy of $C_4$ grasses on digestive efficiencies in *P. melane* could result from the centralized location of nutrients in $C_4$ grass tissues, creating longer diffusion paths for nutrients and/or digestive enzymes.

The hypothesis that the BSC anatomy of $C_4$ plants directly limits nutrient digestibility is still in need of quantitative testing in insect species in which this factor is believed to be important, such as orthopterans (Caswell and Reed 1975; 1976). In neither of the species tested to date (*L. migratoria* and *P. melane*) has the BSC anatomy of $C_4$ grasses been found to be associated with consistently lower nutrient digestibilities. In *P. melane*, the ability to digest uncrushed leaf tissues and consume some $C_4$ grasses at compensatory rates appear to allow this grass-specialist to successfully utilize $C_4$ grasses. Therefore, a lack of preference for $C_3$ grasses in *P. melane* is not surprising and may help explain some of the variation which has been found between studies on the relative utilization of $C_3$ and $C_4$ grasses by other insects (Pinder and Kroh 1987). Further work is needed on the relative effects of $C_4$ grass consumption on the fitness of other insects, along with measurements of nutrient digestibility, in order for the mechanisms underlying the $C_4$ plant avoidance hypothesis of Caswell et al. (1973) to be properly tested.

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