Oecologia (Berl.) 45, 299–306 (1980)

Oecologia

Carbon Isotope Ratios and Crop Analyses of *Arphia* (Orthoptera: Acrididae) Species in Southeastern Wyoming Grassland

Thomas W. Boutton[1, 3], Bruce N. Smith[1, 4], and A. Tyrone Harrison[2]

[1] Department of Botany and Range Science, Brigham Young University, Provo, Utah 84602, U.S.A.
[2] School of Life Sciences, University of Nebraska, Lincoln, Nebraska 68588, U.S.A.
[3] Present address: Department of Biology, Augustana College, Sioux Falls, South Dakota 57197, U.S.A., and Department of Botany, University of Nairobi, P.O. Box 30197, Nairobi, Kenya

Summary. Food habits of *Arphia conspersa* Scudder and *Arphia pseudonietana* (Thomas) were studied along an altitudinal transect in southeastern Wyoming shortgrass mixed prairie. Stable carbon isotope ratios indicated that diets were significantly different between study sites, between species, and between sexes. These differences were found to be primarily related to the availability of different food plants along the transect, although species with the C_3 pathway of photosynthesis were consumed in greater proportion than their availability in the habitat. The preference for C_3 species is presumably related to their higher nutritional value and digestibility, in spite of the fact that more time and energy must be spent to locate these food plants in some of the habitats studied. This study demonstrates the utility of the carbon isotope method in studying plant-animal interactions in habitats containing both C_3 and C_4 plants.

Introduction

The distribution and relative abundances of the stable isotopes of carbon in the biotic and abiotic components of nature has been a topic of considerable interest and importance for many years (Craig, 1953; Degens, 1969; Smith, 1972). Recent studies have shown two distinct groups of carbon isotope ratios in higher plants (Smith, in Laetsch, 1968; Bender, 1968), and that these differences are related to the C_3 and C_4 pathways of photosynthesis. Plants with C_3 photosynthesis have $\delta^{13}C$ values ranging from -21 to $-33^0/_{00}$ vs. PDB, with a mean value of $-26^0/_{00}$. The depletion of ^{13}C in C_3 plants relative to its abundance in atmospheric CO_2 has been shown to be due chiefly to discrimination by the primary carbon fixing enzyme, RuBP carboxylase, during photosynthesis (Wong et al., 1979). By contrast, C_4 plants have $\delta^{13}C$ values ranging from -9 to $-17^0/_{00}$ vs. PDB with an average value of $-13^0/_{00}$, and are only slightly depleted in ^{13}C relative to atmospheric CO_2. This is due to the lesser extent of ^{13}C discrimination by PEP carboxylase, the primary carbon fixing enzyme of C_4 plants (Whelan et al., 1973).

Unlike plants, animals do not appreciably change the carbon isotope ratios of their carbon sources (Parker, 1964; Smith and Epstein, 1970; Minson et al., 1975; DeNiro and Epstein, 1978a), resulting in the isotope ratio of an animal being the same as its food source. The differences in $\delta^{13}C$ values between C_3 and C_4 plants and the fact that animals reflect the $\delta^{13}C$ value of their diets have enabled the use of carbon isotope ratios as tracers of carbon flow between trophic levels (e.g. producer→consumer) and between ecosystems (e.g. terrestrial→marine). Carbon isotope ratios have been successfully employed in the study of food habits of ungulates (Tieszen et al., 1979; Vogel, 1978), domestic rangeland herbivores (Minson et al., 1975; Ludlow et al., 1976), small mammals (De Niro and Epstein, 1978b; Fry et al., 1978a), grasshoppers (Boutton et al., 1978; Fry et al., 1978b), and humans (Vogel, 1977; DeNiro and Epstein, 1978c). Sources of organic carbon in sediments of marine (Hedges and Parker, 1976) and freshwater (Rau, 1978) systems, as well as sources of soil organic matter (Dzurec, 1979) have also been determined by $\delta^{13}C$ values. Carbon isotope ratios clearly have great utility and potential for the study of ecosystem interactions, particularly in areas where C_3 and C_4 plants coexist. However, some entirely C_3 communities possess carbon pools with sufficiently different $\delta^{13}C$ values to allow use of this method to trace carbon transfers (Rau, 1979).

C_3 and C_4 plants coexist over most of the Great Plains of North America, making this region ideal for studying biotic processes with stable carbon isotope methodology. In a previous paper, we have described a temperature-precipitation gradient along an elevational transect in southeast Wyoming. Along the gradient, plant community composition changed from nearly all C_3 biomass at the coolest site to nearly all C_4 biomass at the warmest site (Boutton et al., in press). Simultaneously, extensive collections of grasshoppers (Orthoptera: Acrididae) were made at the seven study sites where plant community composition was analyzed. This study reports the food habits of two *Arphia* species (Oedipodinae) along the transect using $\delta^{13}C$ values.

Arphia species have been reported to consume both C_3 and C_4 plants (Mulkern et al., 1969; Ueckert and Hansen, 1971), and their reported food plants change in abundance along the elevational gradient. We were thus able to assess food preference under conditions of variable food plant abundance, a type of study that has previously been confined to laboratories.

The hypotheses tested were: 1) that diets of both *Arphia* species would be different between sites, 2) that the differences in diets between sites would be due to changes in food plant abundance, 3) that because of the temporal displacement of these two *Arphia* species, their diets would differ from each other at the same sites, and 4) that C_3 plants would be preferred over C_4 plants (Caswell et al., 1973; Caswell and Reed, 1975, 1976).

---

[4] To whom reprint requests should be sent

0029-8549/80/0045/0299/$01.60

Study Area

This study was conducted along an altitudinal transect from 2652 m to 1650 m in the grasslands of southeastern Wyoming, U.S.A. Vegetation at the high elevations represents a transition between the subalpine and shortgrass mixed prairie zones, while the lower elevations are representative of the shortgrass mixed prairie (Sims et al., 1978). Mean annual temperature ranges from 8.2° C at 1,650 m to 3.2° C at 2,533 m, with July the hottest and January the coldest months at all sites. Annual precipitation is 515 mm at 2,704 m, decreasing to 311 mm at 1,650 m. Approximately 70% of the annual precipitation falls as rain from the beginning of April through the end of September, with May being the wettest month at all sites. A detailed description of the location, climate, and vegetation of all sites can be found in Boutton et al. (in press).

Biology of Arphia Species

Arphia conspersa Scudder and *Arphia pseudonietana* (Thomas) are members of the subfamily Oedipodinae and are sympatric over most of their ranges. *A. conspersa* is found in Alaska and northern Canada south to Mexico, while *A. pseudonietana* occupies a similar area but extends north only to southern Canada (Alexander and Hilliard, 1969; Barnum, 1964). This difference in latitudinal distribution is reflected altitudinally in the Rocky Mts., with *A. conspersa* extending above 3,050 m, while *A. pseudonietana* seldom is found above 2,335 m (Alexander and Hilliard, 1969).

These two congeners seem to be reproductively isolated by temporal displacement of life cycles. *Arphia conspersa* is usually the first Oedipodine to appear as adult in the spring. Eggs usually hatch during late July or early August, and fifth (final) instar is usually reached in November. Individuals overwinter in the fifth instar, and adults appear in April at 1,830 m, and in mid-June at 3,050 m (Alexander and Hilliard, 1969). Adult females average 2.5 cm in length, with males considerably shorter at 1.8 cm.

Eggs of *Arphia pseudonietana* are laid from mid-September through mid-October, and overwintering occurs in the egg stage. Juveniles hatch from mid-June through July, adults first appearing during the first week of August at about 1,830 m, somewhat later at higher elevations. *A pseudonietana* is typically larger than *A. conspersa* with adult females averaging 3.3 cm in length, and males 2.3 cm. The sexual dimorphism in body size exhibited by both species is very common among Acrididae.

On the basis of mandibular morphology, Isely (1944) classified these species as mixed feeders, feeding on a mixture of forbs and grasses. Gangwere (1961), however, classified them as having graminivorous mandibles, indicating a diet consisting entirely of grasses. Crop analyses by several investigators (Mulkern et al., 1964; Ueckert and Hansen, 1971; Ueckert et al., 1972) indicate that grasses and sedges usually constitute 100% of both of their diets, with *Bouteloua gracilis*, *Stipa comata*, *Carex* sp., and *Agropyron smithii* usually among the most preferred species. Forbs usually compose less than 2% of the diets of both species.

Methods

In order to determine the resources available to the herbivores, plant biomass was determined at each of seven study areas by clip samples in June and August of 1978. In June, ten circular 0.5 m^2 plots were placed in two parallel rows of five, each plot 10 m from the two nearest plots. August plots were adjacent to plots clipped in June. All photosynthetically active green biomass rooted within a plot was clipped at ground level and sorted by species. Although above-ground biomass is not simply equated with food for herbivores (Sinclair, 1975), we feel this inequality is minimized by separating live biomass from dead biomass. Wet weights of all species were determined in the field. Clipped plants were then transported back to the laboratory where they were oven-dried at 70° C for two weeks and then weighed to the nearest 0.1 g. Wet weights and dry weights were used to compute water content of each species. Photosynthetic pathways of plant species were determined from the literature (Downton, 1975; Brown, 1977; Szarek and Ting, 1977; Raghavendra and Das, 1978).

Grasshoppers were hand-collected at each of the study areas. Individuals of *Arphia conspersa* were killed immediately in cyanide. After a hind leg was removed for isotopic analysis, individuals of *Arphia pseudonietana* were killed and preserved in 95% ethanol so that crop analyses could be performed later.

Sixty-five individuals of *A. pseudonietana* (thirteen from each of five sites) were analyzed for crop contents by the Composition Analysis Laboratory at Colorado State University. Crop contents were analyzed as explained in Ueckert and Hansen (1971). Twenty microscope fields/slide were examined and % relative density determined for each food item present. % relative density is equivalent to the relative amount of biomass of each plant species consumed.

$\delta^{13}C$ values were determined on the same sixty-five individuals of *A. pseudonietana* that were analyzed for crop contents, and on thirty-five individuals of *A. conspersa*. Two factors need to be considered in the selection of body parts of grasshoppers for $\delta^{13}C$ analysis. First, there is considerable variation in the composition of each grasshopper meal, and the amount of food in the crop of a grasshopper usually constitutes a large proportion of an individual's total weight. Thus, food in the gut is a major source of contamination when determining grasshopper $\delta^{13}C$ values (DeNiro and Epstein, 1978a). Second, grasshoppers have fat bodies which may constitute 30% or more of their dry weight at different times during their adult lives (Uvarov, 1966). Because lipid synthesis discriminates against ^{13}C incorporation (DeNiro and Epstein, 1977), inclusion of the fat body in isotopic analyses could produce a $\delta^{13}C$ value considerably more negative than the value which would be a true reflection of the animal's diet (Teeri and Schoeller, 1979).

In order to avoid the influence of the most recent meal and the fat body on grasshopper isotope ratios, only legs were used in analyses. Legs contain about 0.4% lipid (Uvarov, 1966), and are constructed from carbon obtained throughout the lifetime of the organism. Therefore, we feel that $\delta^{13}C$ values of grasshopper legs provide a lifetime integration of dietary $\delta^{13}C$ and provide the most reliable information about food sources.

5–20 mg of legs from each individual were combusted in a stream of oxygen at 800° C over copper turnings. Combustion products were trapped in liquid nitrogen and the excess oxygen pumped away. The trap was then warmed to dry ice temperature, which retained water but liberated the CO_2 for collection in a sample tube. CO_2 samples were analyzed for $^{13}C/C^{12}$ on an isotope ratio mass spectrometer. Results are expressed as:

$$\delta^{13}C‰ = \left[\frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}}\right] \times 1{,}000$$

where R standard is the mass 45 to mass 44 ratio in CO_2 of fossil carbonate of *Belemnitella americana* from the Peedee formation of South Carolina (Craig, 1953; Craig, 1957). Values were corrected for errors from ^{17}O contribution to mass 45 abundance, switching valve leakage, and background. Precision of combustion was determined to be 0.2$^{0}/_{00}$, and machine precision 0.09$^{0}/_{00}$, for an overall precision of 0.3$^{0}/_{00}$ on each determination.

Results

109 plant species from 31 families were found in plots from all seven study areas during both sample periods. A detailed account of the distribution and abundance of these species along the transect is presented in Boutton et al. (in press).

Arphia conspersa was found at five sites from 2,652 m to 1,936 m during June. No members of this species were found in August, although nymphs were probably present at that time. $\delta^{13}C$ values were different at all sites, and became increasingly more positive as elevation decreased (Table 1). A $\delta^{13}C$ value of $-25^{0}/_{00}$ would indicate a diet composed entirely of C_3 plants, while a $\delta^{13}C$ value of $-12^{0}/_{00}$ would indicate a diet of 100% C_4 material. Diets of both C_3 and C_4 plant material would give intermediate values. Single classification analysis of variance (Sokal and Rohlf, 1969) showed that the differences in $\delta^{13}C$ values between sites were highly significant (Table 1), indicating that some correlate of elevation has an effect on the diet of *A. conspersa.*

In order to determine which $\delta^{13}C$ values were different from each other, an a posteriori Student-Newman-Keuls test (Sokal and Rohlf, 1969) was performed. Nine of the ten possible dietary comparisons were significantly different for *A. conspersa* (Table 1). Only $\delta^{13}C$ values of grasshoppers from 2,652 m and 2,421 m were not significantly different from each other.

Previous grasshopper studies have shown that the relative amounts of the different food plants consumed are positively correlated with the availability of those food plants (Bernays and Chapman, 1970; Ueckert and Hansen, 1971; Ueckert et al., 1972; Sheldon and Rogers, 1978) although this might not always be the rule (Otte and Joern, 1977; Joern, 1979). Because $\delta^{13}C$ values cannot discriminate individual food plants, we cannot determine the role of availability of individual food plants. However, $\delta^{13}C$ values of grasshoppers are determined by the relative amounts of C_3 and C_4 biomass consumed. Therefore, a regression analysis was performed to determine if $\delta^{13}C$ values of *A. conspersa* at different sites were a function of the percentage of plant community biomass with C_4 photosynthesis. Figure 1 shows that the percentage of C_4 biomass available in the community accounts for 96% of the variability in $\delta^{13}C$ values observed at the five study areas.

Single classification analysis of variance of isotope ratios of *Arphia pseudonietana* was highly significant (Table 1), again suggesting some correlate of elevation is influencing diet. Student-Newman-Keuls analysis showed that six of the ten possible site comparisons were significantly different. Mean $\delta^{13}C$ values from 2,335 m and 2,165 m were not significantly different, and no two pairs of means from 2,335 m, 2,052 m, or 1,936 m were significantly different from each other (Table 1). The relative abundance of C_4 biomass along the transect was not able to account for a significant proportion of the variability of *A. pseudonietana* $\delta^{13}C$ values ($R^2=53\%$), although isotope ratios generally show that more C_4 material is consumed as more becomes available in the environment.

Table 1. Results of $\delta^{13}C$ determinations (means ± std. deviations), analysis of variance, Student-Newman-Keuls tests, and Mann-Whitney U-tests. Dashes indicate that no individuals were found at that site. Data for 2,335 m and 2,165 m are out of order to improve presentation of Student-Newman-Keuls results. $N=13$ for all *A. pseudonietana* values, while N is listed for each *A. conspersa* value. Results of Student-Newman-Keuls tests are indicated by vertical brackets. Any pair of means enclosed by the range of any one bracket is not significantly different. All other pairs are significantly different at least to $P<0.05$. U-statistics are listed next to the pairs tested. ** indicates $P \ll 0.001$, and * indicates $P<0.05$

Elevation (M)	*A. pseudonietana*	*A. conspersa*	U-statistic
2652	–	-24.8 ± 0.3 ($N=8$)	–
2421	–	-24.5 ± 0.6 ($N=9$)	–
2165	-22.2 ± 1.4	-23.3 ± 0.6 ($N=6$)	58*
2335	-21.2 ± 1.3	-23.9 ± 0.4 ($N=6$)	77*
2052	-20.8 ± 1.1	–	–
1936	-20.0 ± 1.5	-22.1 ± 1.2 ($N=6$)	68*
1650	-17.6 ± 1.8	–	–
df	4, 60	4, 30	
F_s	17.4**	14.7**	

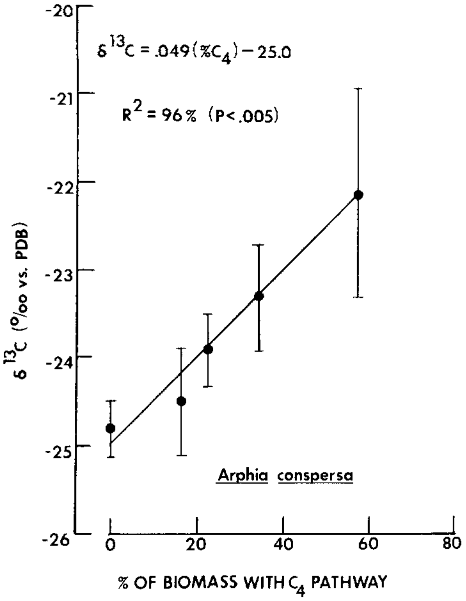

Fig. 1. Regression analysis of $\delta^{13}C$ values of *Arphia conspersa* on % of plant biomass with C_4 pathway at each site. Solid circles are means, and vertical bars are standard deviations

Dietary differences between sexes of the same species of grasshopper have been shown by Ueckert and Hansen (1971). To test this possibility, a nonparametric Mann-Whitney U-test (Sokal and Rohlf, 1969) was used to test for male-female differences in *A. pseudonietana*. A nonparametric test was used because variances were significantly different between males and females of this species. The results showed that males had significantly more positive $\delta^{13}C$ values than females at three of the five study areas (Table 2). The same trend was apparent at the other two sites, but the differences were not as great. Average difference between males and females was $1.4 \pm 0.5^{0}/_{00}$. This difference indicates that males are consuming more C_4 plant material than are females. There

Table 2. Results of Mann-Whitney U-test for sexual differences in $\delta^{13}C$ values of *Arphia pseudonietana*. Values shown are means ± std. deviations. Numbers in parentheses are sample sizes. * indicates $P < 0.05$

Elevation (M)	$\delta^{13}C$ Values		
	Females	Males	U-statistic
2335	−22.1 ± 1.0 (7)	−20.1 ± 0.5 (6)	40*
2165	−23.0 ± 0.8 (6)	−21.6 ± 1.4 (7)	33*
2052	−21.4 ± 0.6 (6)	−20.3 ± 1.2 (7)	31
1936	−21.1 ± 0.3 (5)	−19.3 ± 1.5 (8)	34*
1650	−18.1 ± 1.7 (5)	−17.4 ± 1.8 (8)	22

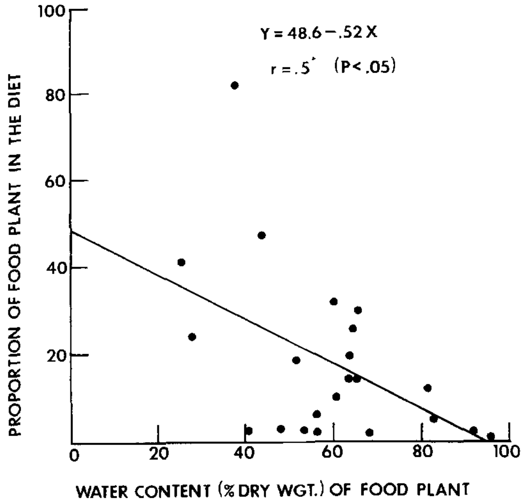

Fig. 2. Correlation analysis between moisture content (expressed as a percent of dry weight) of food plants and their % relative density in the crops of *Arphia pseudonietana*

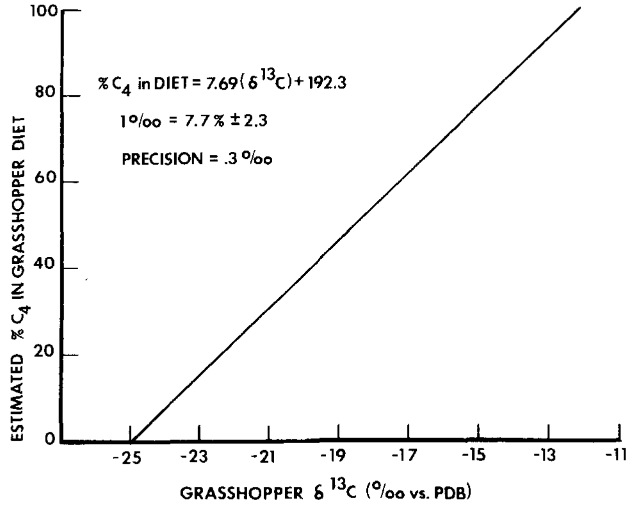

Fig. 3. Model for prediction of C_3-C_4 composition of grasshopper diets based on their $\delta^{13}C$ values

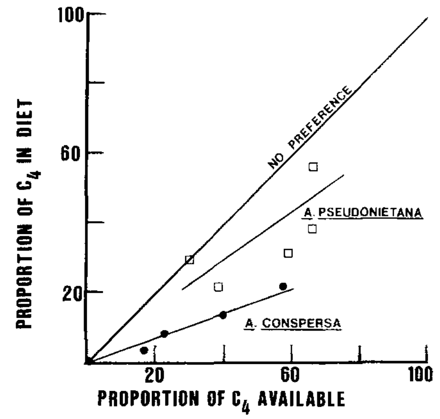

Fig. 4. Assessment of C_3-C_4 preference in *Arphia conspersa* and *Arphia pseudonietana*

was not enough data to test for sexual differences in *Arphia conspersa*.

Because variances were unequal for *A. conspersa* and *A. pseudonietana*, a nonparametric Mann-Whitney U-test was run to see if these two species had different $\delta^{13}C$ values at sites where they occurred together. Both species were collected at 2,335 m, 2,165 m, and 1,936 m. Results showed that *A. pseudonietana* had significantly more positive $\delta^{13}C$ values than *A. conspersa* (Table 1), indicating that *A. pseudonietana* consumes more C_4 material than *A. conspersa* at these sites. This could be due to the significantly greater proportion of C_4 biomass at all sites in August when *A. pseudonietana* is present than in June when *A. conspersa* is present (Boutton et al., in press).

Table 3 shows the results of the crop analyses for *Arphia pseudonietana*. In agreement with previous reports by Mulkern et al. (1969), Ueckert and Hansen (1971), and Ueckert et al. (1972), the most important food plants were *Carex eleocharis*, *Bouteloua gracilis*, *Koeleria nitida*, and *Stipa comata*. These four species consistently composed 80% of the diet of *A. pseudonietana*. No forb or shrub material was found in the crops except for small amounts of *Yucca glauca* at 2,335 m and *Artemisia* sp. at 1,650 m. This supports the contention of Gangwere (1961) that this species has graminivorous mandibles rather than mixed feeder mandibles, as proposed by Isely (1944).

The number of food plants utilized at each site ranged from four species at 2,165 m to ten species at 2,335 m, with an average of seven plant species utilized/site. Females had more total food plants/site and more plant species/crop than did males at the same sites (Table 3). No regular differences in diet between males and females were revealed by the crop analysis. However, *Agropyron smithii* was an important dietary component of females at 2,335 m, 2,052 m, and 1,936 m, but no males consumed this plant at any site along the transect. Females also consumed small quantities of *Vulpia octoflora* and *Oryzopsis hymenoides* while males did not. Thus, females are consuming a larger number of plant species/site and more plant species/meal than males, indicating that females have more generalized diets than males of *Arphia pseudonietana*.

A correlation analysis (Sokal and Rohlf, 1969) showed that there was no relationship between the abundance of each species in the crops and the abundance of those species in the environment. Many plant species were consumed in far greater proportion than they were available in the environment. For example, *Carex eleo-*

Table 3. Results of crop analyses for *Arphia pseudonietana*. Values shown in the table are % relative density ± std. deviation. 13 individuals were analyzed at each site

Food plants	Elevation (M)				
	2335	2165	2052	1936	1650
C-4 Grasses					
Bouteloua gracilis	28.79 ± 28.47	3.15 ± 4.97	13.80 ± 26.91	14.65 ± 28.65	17.04 ± 21.19
Buchloe dactyloides				4.34 ± 9.02	13.43 ± 15.01
Muhlenbergia filiculmis	18.72 ± 19.28			0.64 ± 2.21	
Muhlenbergia torreyi					19.02 ± 24.94
Totals	47.69	3.15	13.80	19.63	49.49
C-3 Grasses					
Agropyron smithii	3.33 ± 11.54		9.23 ± 26.74	5.35 ± 18.53	
Danthonia parryi	2.67 ± 6.60				
Koeleria nitida	1.14 ± 2.68	12.82 ± 29.38	31.49 ± 43.91	25.01 ± 39.08	
Oryzopsis hymenoides			0.59 ± 2.05		
Poa sp.	23.03 ± 34.59		1.09 ± 3.00		
Stipa comata	19.34 ± 30.00	2.71 ± 7.68	2.86 ± 9.91	2.70 ± 9.34	
Vulpia octoflora	0.26 ± 0.90				0.51 ± 1.76
Totals	49.77	15.53	45.26	33.06	0.51
C-3 Forbs/Shrubs					
Artemisia sp.					8.33 ± 28.87
Carex eleocharis	2.55 ± 8.84	81.82 ± 29.06	40.94 ± 44.88	47.32 ± 49.07	41.67 ± 51.49
Yucca glauca	trace				
Totals	2.55	81.82	40.94	47.32	50.00
% C-4 in Diet	47.69 ± 39.73	3.15 ± 4.97	13.80 ± 28.01	19.63 ± 38.17	49.49 ± 51.72
% C-3 in Diet	52.32 ± 39.73	97.35 ± 4.97	86.20 ± 28.22	80.38 ± 38.17	50.51 ± 51.72
# Species in Diet (all)	10	4	7	7	6
# Species in Diet (♀)	9	4	7	7	5
# Species in Diet (♂)	7	3	4	4	5
Mean # Sp./Crop (all)	3.2 ± 1.2	1.5 ± 0.9	1.8 ± 1.2	1.8 ± 1.1	1.9 ± 1.3
Mean #Sp./Crop (♂)	2.5 ± 0.6	1.3 ± 0.9	1.4 ± 0.8	1.3 ± 0.9	1.4 ± 1.1
Mean # Sp./Crop (♀)	3.7 ± 1.4	1.8 ± 0.8	2.3 ± 1.5	2.8 ± 0.8	2.8 ± 1.1

charis, the most preferred food plant at all sites except 2,335 m, averaged 43% relative density in the crops across all sites, but its average abundance in the environment was only 3.3% of the total plant biomass. Thus, it appears that factors other than abundance of food plants are playing a role in determining the choice of food plants by *A. pseudonietana*.

The succulence (i.e. water content) of food plants has often been regarded as an important factor in food selection by grasshoppers (Gangwere, 1961; Dadd, 1963; Sheldon and Rogers, 1978), since grasshoppers derive most of their water from food. To test this possibility for *A. pseudonietana*, correlation analysis was conducted between the % relative density of each food plant in the diet at a given site and the water content of those same food plants. Results showed that plants with lower water contents were found in larger quantities in the crops than were plants with higher water contents. Although the correlation was weak, it was statistically significant (Fig. 2). Other workers have also found that grasshoppers prefer dried or wilted vegetation over more succulent individuals of the same species (Isely, 1946; Kaufmann, 1965; Ueckert and Hansen, 1971).

In order to test the hypothesis of Caswell et al. (1973) that C_4 plants are avoided as a food source by herbivores, a model was constructed to determine the relationship betweeen the $\delta^{13}C$ value of a grasshopper and the proportion of C_4 material in its diet. Based on the $\delta^{13}C$ values of C_3 and C_4 plants reported in the literature (Smith and Brown, 1973; Troughton et al., 1974; Smith and Turner, 1975), the average value for a C_3 plant is $-26^0/_{00}$, and the average C_4 plant value is $-13^0/_{00}$. Because animals are usually about $1^0/_{00}$ more enriched in ^{13}C than their food source, a herbivore with a 100% C_3 diet would have a $\delta^{13}C$ value of $-25^0/_{00}$, and a herbivore with a 100% C_4 diet would have a $\delta^{13}C$ value of $-12^0/_{00}$. Other investigators (DeNiro and Epstein, 1978a; Teeri and Schoeller, 1979) have shown that $\delta^{13}C$ values of animals are linear over the $\delta^{13}C$ values of their diets, so we have constructed a linear model to predict the proportion of C_4 material in a diet (Fig. 3). According to the model, a change of $1^0/_{00}$ in the grasshopper $\delta^{13}C$ value is due to a 7.7% difference in C_4 intake. Because our analytical precision is $0.3^0/_{00}$, we impose limits of ±2.3% on all estimates of the proportion of C_4 material in a diet. This model shows good agreement with the model proposed by Fry et al. (1978b).

Preference for food items can be assessed graphically by plotting the proportion of a food type in the diet against the proportion of that food type available in the environment (Murdoch, 1969; Cock, 1978). If an animal consumes that food type in the proportions that it is available, no preference is shown (Fig. 4). Points lying above the no preference line would indicate a preference for C_4 material, while points lying below the no preference line would indicate a preference for the alternate food source, in this case C_3 material. When diets for both species are converted from $\delta^{13}C$ values to % C_4 in the diet using Fig. 3 and then plotted in Fig. 4, the results show that both *Arphia* species prefer C_3 over C_4 material as a food source, supporting the hypothesis of Caswell et al. (1973). Lines fitted to the data for both species in Fig. 4 deviate most from the no preference line where C_3 abundance is lowest, indicating that preference for C_3 food items is greatest where C_3 availability is lowest.

Comparing the two methods of dietary analysis that were used in this study, we found that the observed abundances of C_3 and C_4 plants in the crops of *Arphia pseudonietana* showed no relationship with the C_3-C_4 composition of the diet as revealed by carbon isotope ratios. Grasshopper meals are highly variable, and a very large number of crops need to be examined in order to develop a realistic picture of the diet. Isotopic analysis does not give an instantaneous analysis of diet, but gives a long term integrated average diet and is not responsive to the variability between individual meals. Thus, the instantaneous measure of diet given by a few crop analyses may be expected to differ from the long term average diet indicated by carbon isotope ratios.

The disadvantage of the isotope method is that it gives no information about the plant species composition of the diet. $\delta^{13}C$ values alone do not reveal the species eaten, the number of species per meal, and the importance of each food plant in the diet. It can only determine the relative amounts of C_3 and C_4 vegetation consumed during the lifetime of the organism. The use of $\delta^{13}C$ values coupled with stomach analyses on the same individuals would be a very powerful method of dietary analysis.

Discussion

Natural selection should favor organisms which ingest food in such a manner that the genetic contribution to future generations is maximized (Emlen, 1968; Pulliam, 1974; Estabrook and Dunham, 1976; Stenseth and Hansson, 1979). This involves maximizing the nutritional and caloric intake per unit of time spent feeding. Availability of different food types in the environment may often be of major importance in food selection (Ueckert et al., 1972; Pulliam, 1974), since with increasing availability, the metabolic costs of food acquisition decline exponentially (Stenseth and Hansson, 1979). However, the value of the food (nutritional, caloric, etc.) also influences food habits (Dadd, 1963; Chapman and Bernays, 1977). A model of optimal diet devised by Estabrook and Dunham (1976) showed that small changes in the relative value of food items had a greater effect on the optimal diet than did changes in the relative abundance of potential food items. Diets thus seem to be constrained by both availability of food types and their quality. Therefore, we will try to interpret the food habits of *Arphia conspersa* and *Arphia pseudonietana* based on the relative availability and the nutritive quality of food items.

Diets of both *Arphia* species, as revealed by $\delta^{13}C$ values, showed significant differences between sites. In *Arphia conspersa*, the site variation in $\delta^{13}C$ values was strongly related to the abundance of C_4 biomass along the transect, suggesting that abundance is playing a role in the food habits of this species. Differences between sites in $\delta^{13}C$ values of *A. pseudonietana* could not be explained adequately on the basis of C_4 abundance, although there was a trend towards increasing consumption of C_4 biomass as C_4 biomass increased in the environment.

Where both species could be collected from the same sites, *A. pseudonietana* was shown to consume significantly more C_4 plant material than *A. conspersa*. Again food plant abundance may be involved, since in June, when *A. conspersa* is adult, there is a significantly lower proportion of C_4 biomass present than in August, when *A. pseudonietana* is adult. The ability to feed on food items that are abundant should be strongly selected for, since time and energy spent searching for less abundant food items could be costly in terms of reproductive potential.

However, these two species are not merely feeding in such a manner that they are consuming their food plants in the proportion they encounter them while foraging. If this were the case, all the data points in Fig. 4 would lie along the no preference line. In reality, both species lie below this line, indicating a preference for C_3 food plants, and supporting the hypothesis of Caswell et al. (1973) that C_3 plants are preferred over C_4 plants. Preference for C_3 food items also increases as availability of C_3 food decreases (Fig. 4), strongly suggesting that qualitative aspects of the host plants are playing some role in the determination of diet.

Differences in the nutritional value of C_3 and C_4 plants are well known. C_4 grasses contain more structural material (Akin and Burdick, 1975; Wilson and Ford, 1971) and less nitrogen (Wilson and Haydock, 1971; Lyttleton, 1973) than C_3 grasses. Caswell and Reed (1975, 1976) have shown that the bundle sheath cells, which compose a high proportion of all the cells present in a C_4 grass plant (Akin and Burdick, 1975), are difficult for some grasshoppers, including *Arphia pseudonietana*, to digest due to their thick cell walls. These cells often pass through the digestive systems of grasshoppers intact. The potential nutritional value of these cells thus may not be available to grasshoppers, although some of the contents of unbroken cells might be utilized (Richards and Davies, 1977).

In spite of the physical resistance of the bundle sheath cells to degradation and high structural carbohydrate content of C_4 grasses, animals in several taxa have diets composed entirely or largely of C_4 species (Tieszen et al., 1979; Vogel, 1978; Fry et al., 1978b). Several species of grasshoppers on the shortgrass mixed plains, such as *Amphitornus coloradus, Eritettix simplex, Opeia obscura*, and *Trachyrhachys kiowa* exist on diets approaching 100% C_4 material (Mulkern et al., 1969; Ueckert and Hansen, 1971; Ueckert et al., 1972), so some adaptation must be present that allows these species to utilize the nutrients in C_4 bundle sheath cells. Mandibular morphology, retention time of food in the digestive system, efficiency of nutrient absorption, or other factors related to the digestive process may be different in species that utilize large quantities of C_4 material in their diets.

The need for nitrogen by grasshoppers and other animals is well known (Smith and Northcott, 1951; White, 1978). C_4 grasses usually contain about 50% less nitrogen than C_3 grasses (Lyttleton, 1973). That insects may be limited by the nitrogen levels in C_4 plants is demonstrated by an increase in herbivores on C_4 plants treated with nitrogen fertilizer compared with herbivore levels on unfertilized plants (Vince, in Valiela and Teal, 1979). The negative correlation between water content of food plants and their abundance in the crops of *A. pseudonietana* lends further support to the importance of nitrogen, since water stressed plants contain more nitrogen than unstressed plants (White, 1976, 1978).

The greater consumption of C_4 material by males of *Arphia pseudonietana* compared with females (they consume 45% more C_3 biomass than do males) could also be related to the nutritional quality of the food. Grasshopper eggs are about 50% protein (Uvarov, 1966), representing a substantial drain on the nitrogen budget of a female. Furthermore, nitrogen and phosphorous levels have been shown to influence reproduction in grasshoppers, with fewer eggs laid when nitrogen or phosphorous are present at low levels in the food plants (Smith and Northcott, 1951; Smith, 1960). Both nitrogen and phosphorous are significantly lower in C_4 plants (Wilson and Haydock, 1971; Christie, 1979), so reproduction might be improved if females fed on the more nutritious C_3 species. Adaptations improving reproductive output should be favored by natural selection.

In conclusion, food selection by these two species of *Arphia* seems to be greatly influenced by the abundance of their food plants at the different study areas. However, the proportion of food in the diet was not directly proportional to the availability of the food plants in the environment, indicating that food was not consumed in the proportion that it was encountered while searching. Preference was shown for the C_3 species, probably due to the lower digestibility and nutrient levels of C_4 species. Females of *Arphia pseudonietana* showed a greater preference for C_3 food than did males, presumably due to the greater metabolic demands placed on them during reproduction. These two species show that diet is determined by resource abundance and the nutritive quality of those resources.

Acknowledgements. Dr. Robert E. Pfadt of the University of Wyoming helped make this study possible by sharing his knowledge of the taxonomy and ecology of Wyoming grasshoppers. Jerran T. Flinders, Kimball T. Harper, and Stephen L. Wood of Brigham Young University read and provided comments on the manuscript. R.M. Hansen, T. Foppe, and Van Baker of Colorado State University performed the crop analyses and provided vegetation data for the 1,650 m site. Janet Boutton, Ron Dzurec, and Glen Martin assisted in the lab. Financial support was provided by Sigma Xi, Provo City Rotary Club, and Brigham Young University Student Council.

References

Akin, D., Burdick, D.: Percentage of tissue types in tropical and temperate grass leaf blades and degradation of tissues by rumen microorganisms. Crop Sci. **15**, 661–668 (1975)

Alexander, G., Hilliard, J.: Altitudinal and seasonal distribution of Orthoptera in the Rocky Mountains of northern Colorado. Ecol. Monogr. **39**, 385–431 (1969)

Barnum, A.: Orthoptera of the Nevada Test Site. Brigham Young Univ. Sci. Bull., Biol. Series **4(3)**, 1–134 (1964)

Bender, M.: Mass spectrometric studies of carbon 13 variations in corn and other grasses. Radiocarbon **10**, 468–472 (1968)

Bernays, E., Chapman, R.: Food selection by *Chorthippus parallelus* (Zetterstedt) (Orthoptera: Acrididae) in the field. J. Anim. Ecol. **39**, 383–394 (1970)

Boutton, T., Cameron, G., Smith, B.: Insect herbivory on C_3 and C_4 grasses. Oecologia (Berl.) **36**, 21–32 (1978)

Boutton, T., Harrison, A., Smith, B.: Distribution of biomass of species differing in photosynthetic pathway along an altitudinal transect in southeastern Wyoming grassland. Oecologia (Berl.) (In press)

Brown, W.: The Kranz syndrome and its subtypes in grass systematics. Memoirs Torr. Bot. Club **23(3)**, 1–97 (1977)

Caswell, H., Reed, F.: Indigestibility of C_4 bundle sheath cells by the grasshopper, *Melanoplus confusus*. Ann. Ent. Soc. Amer. **68**, 686–688 (1975)

Caswell, H., Reed, F.: Plant-herbivore interactions: the indigestibility of C_4 bundle sheath cells by grasshoppers. Oecologia (Berl.) **26**, 151–156 (1976)

Caswell, H., Reed, F., Stephenson, S., Werner, P.: Photosynthetic pathways and selective herbivory: a hypothesis. Amer. Nat. **107**, 465–480 (1973)

Chapman, R., Bernays, E.: The chemical resistance of plants to insect attack. Pontif. Acad. Sci. Scripta Varia **41**, 603–643 (1977)

Christie, E.: Ecosystem processes in semiarid grasslands. II. Litter production, decomposition, and nutrient dynamics. Aust. J. Agric. Res. **30**, 29–42 (1979)

Cock, M.: The assessment of preference. J. Anim. Ecol. **47**, 805–816 (1978)

Craig, H.: The geochemistry of the stable carbon isotopes. Geochim. Cosmochim. Acta **3**, 53–92 (1953)

Craig, H.: Isotopic standards for carbon and oxygen and correction factors for mass spectrometric analysis of carbon dioxide. Geochim. Cosmochim. Acta **12**, 133–149 (1957)

Dadd, R.: Feeding behaviour and nutrition in grasshoppers and locusts. Advanc. Insect Physiol. **1**, 47–109 (1963)

Degens, E.: Biogeochemistry of stable carbon isotopes. In: Organic Geochemistry (G. Eglinton, M. Murphy, eds.), pp. 304–329. Berlin Heidelberg New York: Springer-Verlag 1969

DeNiro, M., Epstein, S.: Influence of diet on the distribution of carbon isotopes in animals. Geochim. Cosmochim. Acta **42**, 495–506 (1978a)

DeNiro, M., Epstein, S.: Carbon isotopic evidence for different feeding patterns in two hyrax species occupying the same habitat. Science **201**, 906–907 (1978b)

DeNiro, M., Epstein, S.: Dietary analysis from $^{13}C/^{12}C$ ratios of carbonate and collagen fractions of bone. Geol. Surv. Open-File Report **78-701**, 90–91 (1978c)

DeNiro, M., Epstein, S.: Mechanism of carbon isotope fractionation associated with lipid synthesis. Science **197**, 261–263 (1977)

Downton, W.: The occurrence of C_4 photosynthesis among plants. Photosynthetica **9**, 96–105 (1975)

Dzurec, R.: Carbon isotope ratios of soil organic matter and their use in assessing community composition changes in Curlew Valley, Utah. M.S thesis, Utah State University, Logan, Utah (1979)

Emlen, J.: Optimal choice in animals. Amer. Nat. **102**, 385–389 (1968)

Estabrook, G., Dunham, A.: Optimal diet as a function of absolute abundance, relative abundance, and relative value of available prey. Amer. Nat. **110**, 401–413 (1976)

Fry, B., Jeng, W., Scalan, R., Parker, P., Baccus, J.: $\delta^{13}C$ food web analysis of a Texas sand dune community. Geochim. Cosmochim. Acta **42**, 1299–1302 (1978a)

Fry, B., Joern, A., Parker, P.: Grasshopper food web analysis: use of carbon isotope ratios to examine feeding relationships among terrestrial herbivores. Ecology **59**, 498–506 (1978b)

Gangwere, S.: A monograph on food selection in Orthoptera. Trans. Am. Entomol. Soc. **87**, 67–230 (1961)

Hedges, J., Parker, P.: Land-derived organic matter in surface sediments from the Gulf of Mexico. Geochim. Cosmochim. Acta **40**, 1019–1029 (1976)

Isely, F.: Correlation between mandibular morphology and food specificity in grasshoppers. Ann. Ent. Soc. Amer. **37**, 47–67 (1944)

Isely, F.: Differential feeding in relation to local distribution of grasshoppers. Ecology **27**, 128–138 (1946)

Joern, A.: Feeding patterns in grasshoppers (Orthoptera: Acrididae): factors influencing diet specialization. Oecologia (Berl.) **38**, 325–347 (1979)

Kaufmann, T.: Biological studies of some Bavarian Acridoidea (Orthoptera), with special reference to their feeding habits. Ann. Ent. Soc. Am. **58**, 791–800 (1965)

Laetsch, W.: Chloroplast specialization in dicotyledons possessing the C_4 dicarboxylic acid pathway of photosynthetic CO_2 fixation. Amer. J. Bot. **55**, 875–883 (1968)

Ludlow, M., Troughton, J., Jones, R.: A technique for determining the proportion of C_3 and C_4 species in plant samples using stable natural isotopes of carbon. J. Agric. Sci. **87**, 625–632 (1976)

Lyttleton, J.: Proteins and Nucleic acids. In: Chemistry and biochemistry of herbage, Vol. I (G. Butler, R., Bailey, eds.), pp. 63-103. New York: Academic Press 1973

Minson, D., Ludlow, M., Troughton, J.: Differences in natural carbon isotope ratios of milk and hair from cattle grazing tropical and temperate pastures. Nature **256**, 602 (1975)

Mulkern, G., Pruess, K., Knutson, H., Hagen, A., Campbell, J., Lambley, J.: Food habits and preferences of grassland grasshoppers of the north central Great Plains. N. Dakota State Univ. Agric. Exp. Sta. Bull. **481**, 1–32 (1969)

Mulkern, G., Toczek, D., Brusven, M.: Biology and ecology of North Dakota grasshoppers. II. Food habits and preferences of grasshoppers associated with the Sand Hills Prairie. N. Dakota State Univ. Agric. Exp. Sta. Res. Rept. **11**, 1–59 (1964)

Murdoch, W.: Switching in general predators: experiments on predator specificity and stability of prey populations. Ecol. Monogr. **39**, 335–354 (1969)

Otte, D., Joern, A.: On feeding patterns in desert grasshoppers and the evolution of specialized diets. Proc. Acad. Nat. Sci. Philadelphia **128**, 89–126 (1977)

Parker, P.: The biogeochemistry of the stable isotopes of carbon in a marine bay. Geochim. Cosmochim. Acta **28**, 1155–1164 (1964)

Pulliam, H.: On the theory of optimal diets. Amer. Nat. **108**, 59–74 (1974)

Raghavendra, A., Das, V.: The occurrence of C_4 photosynthesis: a supplementary list of C_4 plants reported during late 1974–mid 1977. Photosynthetica **12**, 200–208 (1978)

Rau, G.: Carbon-13 depletion in a subalpine lake: carbon flow implications. Science **201**, 901–902 (1978)

Rau, G.: Carbon sources for aquatic insect production in a subalpine lake. Ph.D. thesis, Univ. of Washington, Seattle (1979)

Richards, O., Davies, R.: Imm's general textbook of entomology. Vol. I. Structure, physiology, and development. London: Chapman and Hall 1977

Sheldon, J., Rogers, L.: Grasshopper food habits within a shrub-steppe community. Oecologia (Berl.) **32**, 85–92 (1978)

Sims, P., Singh, J., Lauenroth, W.: The structure and function of ten western North American grasslands. I. Abiotic and vegetational characteristics. J. Ecol. **66**, 251–285 (1978)

Sinclair, A.: The resource limitation of trophic levels in tropical grassland ecosystems. J. Anim. Ecol. **44**, 497–520 (1975)

Smith, B.: Natural abundance of the stable isotopes of carbon in biological systems. BioScience **22**, 226–231 (1972)

Smith, B., Brown, W.: The Kranz syndrome in the Gramineae as indicated by carbon isotope ratios. Amer. J. Bot. **60**, 505–513 (1973)

Smith, B., Epstein, S.: Biogeochemistry of the stable isotopes of hydrogen and carbon in salt marsh biota. Plant Physiol. **46**, 738–742 (1970)

Smith, B., Turner, B.: Distribution of Kranz syndrome among Asteraceae. Amer. J. Bot. **62**, 541–545 (1975)

Smith, D.: Effects of changing the phosphorus content of the food plant on the migratory grasshopper, *Melanoplus bilituratus* (Walker) (Orthoptera: Acrididae). Can. Ent. **92**, 103–107 (1960)

Smith, D., Northcott, F.: The effects on the grasshopper, *Melanoplus mexicanus mexicanus* (Sauss.) (Orthoptera: Acrididae), of varying the nitrogen content in its food plant. Can. J. Zool. **29**, 297–304 (1951)

Sokal, R., Rohlf, F.: Biometry. San Francisco: Freeman 1969

Stenseth, N., Hansson, L.: Optimal food selection: a graphic model. Amer. Nat. **113**, 373–389 (1979)

Szarek, S., Ting, I.: The occurrence of crassulacean acid metabolism among plants. Photosynthetica **11**, 330–342 (1977)

Teeri, J., Schoeller, D.: $\delta^{13}C$ values of an herbivore and the ratio of C_3 to C_4 plant carbon in its diet. Oecologia (Berl.) **39**, 197–200 (1979)

Tieszen, L., Hein, D., Qvortrup, S., Troughton, J., Imbamba, S.: Use of $\delta^{13}C$ values to determine vegetation selectivity in East African herbivores. Oecologia (Berl.) **37**, 351–359 (1979)

Troughton, J., Card, K., Hendy, C.: Photosynthetic pathways and carbon isotope discrimination by plants. Carnegie Inst. Wash. Yearb. **73**, 768–780 (1974)

Ueckert, D., Hansen, R.: Dietary overlap of grasshoppers on sandhill rangeland in northeastern Colorado. Oecologia (Berl.) **8**, 276–295 (1971)

Ueckert, D., Hansen, R., Terwilliger, C.: Influence of plant frequency and certain morphological variations on diets of rangeland grasshoppers. J. Range Manage. **25**, 61–65 (1972)

Uvarov, B.: Grasshoppers and locusts: a handbook of general acridology. Vol. I. Anatomy, physiology, development, phase polymorphism, introduction to taxonomy. Cambridge: Cambridge University Press 1966

Valiela, I., Teal, J.: The nitrogen budget of a salt marsh ecosystem. Nature **280**, 652–656 (1979)

Vogel, J.: Isotopic evidence for early maize cultivation in New York State. Amer. Antiquity **42**, 238–242 (1977)

Vogel, J.: Isotopic assessment of the dietary habits of ungulates. S. Afr. J. Sci. **74**, 298–301 (1978)

Whelan, T., Sackett, W., Benedict, C.: Enzymatic fractionation of carbon isotopes by phosphoenolpyruvate carboxylase from C_4 plants. Plant Physiol. **51**, 1051–1054 (1973)

White, T.: Weather, food, and plagues of locusts. Oecologia (Berl.) **22**, 119–134 (1976)

White, T.: The importance of a relative shortage of food in animal ecology. Oecologia (Berl.) **33**, 71–86 (1978)

Wilson, J., Ford, C.: Temperature influences on the growth, digestibility, and carbohydrate composition of two tropical grasses, *Panicum maximum* var. *trichoglume* and *Setaria sphacelata,* and two cultivars of the temperate grass *Lolium perenne*. Aust. J. Agric Res. **22**, 563–571 (1971)

Wilson, J., Haydock, K.: The comparative response of tropical and temperate grasses to varying levels of nitrogen and phosphorous nutrition. Aust. J. Agric. Res. **22**, 573–587 (1971)

Wong, W., Benedict, C., Kohel, R.: Enzymic fractionation of the stable carbon isotopes of carbon dioxide by RuDP carboxylase. Plant Physiol. **63**, 852–856 (1979)

Received November 11, 1979