Oecologia (1990) 84:103–110

Oecologia

Sagebrush and grasshopper responses to atmospheric carbon dioxide concentration

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Received September 12, 1989 / Accepted in revised form March 13, 1990

Summary. Seed- and clonally-propagated plants of Big Sagebrush (*Artemisia tridentata* var. *tridentata*) were grown under atmospheric carbon dioxide regimes of 270, 350 and 650 μl l^{-1} and fed to *Melanoplus differentialis* and *M. sanguinipes* grasshoppers. Total shrub biomass significantly increased as carbon dioxide levels increased, as did the weight and area of individual leaves. Plants grown from seed collected in a single population exhibited a 3–5 fold variation in the concentration of leaf volatile mono- and sesquiterpenes, guaianolide sesquiterpene lactones, coumarins and flavones within each CO_2 treatment. The concentration of leaf allelochemicals did not differ significantly among CO_2 treatments for these seed-propagated plants. Further, when genotypic variation was controlled by vegetative propagation, allelochemical concentrations also did not differ among carbon dioxide treatments. On the other hand, overall leaf nitrogen concentration declined significantly with elevated CO_2. Carbon accumulation was seen to dilute leaf nitrogen as the balance of leaf carbon versus nitrogen progressively increased as CO_2 growth concentration increased. Grasshopper feeding was highest on sagebrush leaves grown under 270 and 650 μl l^{-1} CO_2, but varied widely within treatments. Leaf nitrogen concentration was an important positive factor in grasshopper relative growth but had no overall effect on consumption. Potential compensatory consumption by these generalist grasshoppers was apparently limited by the sagebrush allelochemicals. Insects with a greater ability to feed on chemically defended host plants under carbon dioxide enrichment may ultimately consume leaves with a lower nitrogen concentration but the same concentration of allelochemicals. Compensatory feeding may potentially increase the amount of dietary allelochemicals ingested for each unit of nitrogen consumed.

Key words: Carbon dioxide – Nutritional quality – Allelochemcials – *Artemisia tridentata* – *Melanoplus*

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Atmospheric carbon dioxide concentration has been projected to increase from the ambient average of 345 μl l^{-1} to 650 μl l^{-1} or greater within the next 50–75 years (Hansen et al. 1981). Numerous studies using laboratory and field chambers have documented an overall trend of enhanced photosynthetic rates and plant growth with increased CO_2 concentrations (Strain and Bazzaz 1983; Strain and Cure 1985). The additional carbon fixed by plants growing under carbon dioxide enrichment may also be allocated to pools of storage carbohydrate (Nafziger and Koller 1976; Cave et al. 1981). Recent studies using weed, crop and woody plant species have also shown that leaves produced under carbon enriched conditions contain proportionally less nitrogen than ambient leaves (Lincoln et al. 1984; Lincoln et al. 1986; Williams et al. 1986; Osbrink et al. 1987; Fajer et al. 1989). Leaf nitrogen is a limiting nutrient in many insect diets (Mattson 1980; Slansky and Rodriguez 1986) and many insect herbivores will respond to protein poor food sources by increased feeding or reduced growth (Scriber and Slansky 1981). Feeding trials on legume crop plants using three species of noctuids demonstrated a 20–40% increase in consumption of the leaves grown at elevated carbon dioxide concentrations (650 μl l^{-1}) relative to the ambient (Lincoln et al. 1984; Lincoln et al. 1986; Osbrink et al. 1987). Lincoln et al. (1986) postulated that the greater proportion of carbon per leaf, due to growth under carbon enriched conditions, tended to dilute the leaf nitrogen, forcing the herbivore to consume more leaf material (compensatory feeding) to obtain necessary metabolic levels of nitrogen. The buckeye butterfly, a specialist on *Plantago lanceolata*, was even found to have prolonged larval development times when reared on host plants grown under carbon dioxide enrichment (Fajer et al. 1989).

The scenario of nutritional effects on insect feeding at future levels of carbon dioxide may, however, be complicated by natural plant defenses. Plants have evolved a wide variety of allelochemicals in response to herbivore pressure (Rosenthal and Janzen 1979; Spencer 1988), with phenolics (Feeny 1970; Lindroth and Peterson

1988), mono- and sesquiterpenes (Langenheim and Hall 1986; Mihaliak et al. 1987) and sesquiterpene lactones (Picman 1986) being among the important plant defenses against herbivores. In an initial study, Lincoln and Couvet (1989) tested the effect of carbon dioxide enrichment on peppermint leaf monoterpenes. Their study showed that leaf nitrogen followed the predicted dilution effect, but monoterpene concentration remained unchanged. Lincoln and Couvet (1989) suggested that the increased consumption of leaf monoterpenes that they observed, also increased the insect's metabolic cost of allelochemical detoxification which stimulated further feeding. These data indeed suggest that herbivore feeding behavior under future carbon dioxide regimes may be mediated by the defensive chemistry of their host plants.

To better understand the effects of increasing atmospheric carbon dioxide on both leaf allelochemical and nitrogen contents and its potential influence on herbivory, *Artemisia tridentata* var. *tridentata* (Asteraceae) plants were grown under 270 $\mu l\, l^{-1}$ (historical), 350 (ambient) and 650 $\mu l\, l^{-1}$ (projected) carbon dioxide regimes and fed to two species of grasshopper, *Melanoplus differentialis* and *Melanoplus sanguinipes*. Their responses to preindustrial carbon dioxide conditions was also tested to further examine responses to changes in carbon regimes.

Basin sagebrush *Artemisia tridentata* was selected for this study because of its wide range and abundance, and because it produces a wide array of biologically active phytochemicals including volatile terpenes, sesquiterpene lactones, flavonoids and coumarins. The quantitative analysis of these four major phytochemical classes provide a comprehensive test of carbon-based allelochemical production in response to carbon dioxide concentration in plants from a natural population. *Melanoplus* grasshoppers were selected for study because they are an occasional herbivore of *A. tridentata* (Sheldon and Rogers 1978) and they are suitable for experimental feeding trials.

Materials and methods

Seed-propagated plants

Sixty-eight plants were grown from seed collected in a native population (Washoe County, Nevada, USA) at the Duke University Phytotron under carbon dioxide regimes of 270, 350 and 650 $\mu l\, l^{-1}$ (ppm). The CO_2 concentration in each of the three environmental chambers was monitored by an infrared gas analyzer and maintained within an accuracy of $\pm 20\ \mu l\, l^{-1}$. The chambers were maintained at 70% relative humidity, 14 hour light period with 1050 $\mu mol\ m^{-2}\ s^{-1}$ photons and temperatures of 32° C (day) and 25° C (night). Each plant received daily waterings of one-half strength Hoagland solution and deionized water. This nutrient-water regime was utilized to prevent nitrogen limitation from constraining potential carbon dioxide effects (Cure et al. 1988). Seedlings were three weeks old at the start of the experiment and grew for nine weeks under the treatment conditions.

Clonally-propagated plants

Twenty six plants were propagated using stem cuttings from five genotypes using the methods of Alvarez-Cordero and McKell (1979). The plants were grown as above but in CO_2 regimes of 350 and 650 $\mu l\ l^{-1}$. When root initiation was apparent, cuttings were grown in the treatment conditions for eight weeks. The plants were moved biweekly within chambers and exchanged between chambers, with the CO_2 concentration adjusted, in order to minimize potential chamber effects.

Leaf volatile analysis

Volatile terpenes were extracted into pentane from 8–10 fresh-frozen leaves from each plant and quantified using capillary column (SP-1000) gas chromatography with flame ionization detector. The identities of twenty-six mono- and sesquiterpene peaks were confirmed with GC-mass spectroscopy by comparison with authentic standards and literature spectra (Jennings and Shibamoto 1980). Total volatile yield from each plant was determined from the uncorrected sum of all peaks. The major compounds consisted of santolina triene, camphene, arthole, 1,8-cineole, camphor and caryophylene, which comprised 40–50% of the total concentration of leaf volatile terpenes.

Sesquiterpene lactone and flavonoid analysis

Non-volatile allelochemicals were extracted from 8 dried leaves by homogenization in methanol-chloroform. The extract was centrifuged, dried *in vacuo* and resuspended in aqueous acetonitrile. Analysis utilized reverse phase (C-18) HPLC and UV detector (270 nm) with an increasing elution gradient (20–50%) of acetonitrile in 1% aqueous acetic acid (Strack et al. 1980).

Identities were confirmed by isolation from 20 g of dry sagebrush leaves using an HPLC with a semi-preparative C-18 column and an increasing gradient of acetonitrile with water. The isolated sesquiterpene lactones were identified using UV-visible scanning, mass and H-NMR spectroscopies and comparison with literature data (Yoshoika et al. 1973). The flavonoid luteolin was identified by UV-visible scanning spectroscopy with shift reagents (Mabry et al. 1970) and confirmed by co-chromatograhy with an authentic standard.

The major sesquiterpene lactone (deacetoxymatricarin) was crystallized and used to produce a concentration response curve for quantifying the sesquiterpene lactone peaks. Flavonoid peaks were quantified using a concentration curve produced from a luteolin standard. The sesquiterpene lactones identified from the Nevada sagebrush included ridentin, parishin A, matricarin, costunolide, deacetylmatricarin, arbusculin, deacetoxymatricarin and achillin. Quantitative analysis however was limited to the following guaianolides: matricarin, deacetylmatricarin and deacetoxymatricarin, because of their relatively high concentration and strong absorption in the 250–270 nm range.

Coumarin analysis

The sesquiterpene lactone sample from each plant was also analyzed for coumarin content, using an isocratic reverse phase HPLC (15% acetonitrile in 1% aqueous acetic acid) with a fluorescence detector (350 nm excitation and 540 nm emission filters). The coumarins were isolated following the semi-preparatory HPLC procedure with the fluorescence detector and identified by mass spectroscopy and co-chromatography with authentic standards. A concentration response curve for each coumarin was determined from standards.

Leaf and total plant measurements

Approximately 14 leaves were used to measure fresh weight/dry weight/area conversion factors. Three leaves, randomly selected from the dry control leaves were diced to a fine powder and assayed for total nitrogen and carbon content with a CHN analyzer.

Grasshopper feeding trials

Trials were conducted on fifth instar *M. sanguinipes* and *M. differentials* larvae. The feeding episode consisted of presenting 8–10 fresh leaves from each plant to larvae of each species for a period of 24 hours. Leaf petioles were inserted into a moist cheese cloth wick to insure their turgor and placed into a capped 4 ounce plastic cup for the trial. All insects were weighed before and after the trial following an 18 h fast to insure an empty gut. Relative consumption and growth was determined using the calculations of Waldbauer (1968).

Statistical analysis

All analyses were performed using SAS for Personal Computers, ver. 6.03 (SAS Institute Inc. Cary, NC). Unless otherwise stated, the significance of main effects was tested by one-way ANOVA. Chemical characteristics from the vegetatively propagated plants were tested by two-way ANOVA using CO_2 and plant genotype as class variables with an interaction term. Analysis of covariance (GLM procedure) was used as a test to determine if the variance accounted for by the class variable (CO_2) on grasshopper feeding and growth could be explained by continuous variables (leaf allelochemical, nitrogen and carbon concentrations). All data were tested for normality and log transformed where appropriate.

Results

Plant and leaf responses

The overall leaf nitrogen concentration declined significantly as atmospheric CO_2 increased (Table 1). The 6.6% decline in foliar nitrogen concentration as carbon dioxide growth conditions increased from the ambient to the 650 µl l^{-1} level was a lower response than that seen for other woody plants (Williams et al. 1986), but followed a similar pattern. Nonetheless, the trend of nitrogen dilution by carbon accumulation was evident over all three carbon dioxide treatments (Fig. 1). Increased carbon dioxide concentrations over the three treatments acted to steadily reduce the foliar nitrogen least-squares means at the grand mean carbon concentration (Table 2). A test for the homogeneity of slopes

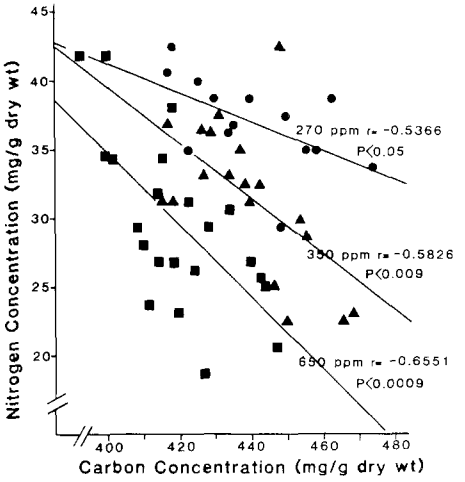

Fig. 1. Regression analysis of the effect of carbon dioxide concentration on the balance of leaf elemental carbon versus nitrogen

Table 2. Mean leaf nitrogen concentration (computed by least squares at a carbon grand mean concentration of 432 mg g^{-1}) in leaves grown in three concentrations of atmospheric carbon dioxide. All treatments significantly different ($P<0.001$) for pairwise comparisons

CO_2 Treatment	Nitrogen concentration (mg g^{-1} dry wt)	
	LS Mean	Std Err
270	38.8	1.2
350	33.0	1.0
650	27.2	1.0

Table 1. Effect of carbon dioxide enrichment on seed-propagated *Artemisia tridentata* leaf and growth characteristics. Significance values are for a 1-way analysis of variance (df = 2)

	Significance of CO_2 effect ($P<$)	270 µl l^{-1}		350 µl l^{-1}		650 µl l^{-1}	
		mean	sd	mean	sd	mean	sd
		$n=14$		$n=19$		$n=22$	
Leaf nitrogen concentration (mg N g^{-1} dry wt)	0.001	37.0	3.3	31.6	5.5	29.5	6.2
Leaf water content (mg water g^{-1} fr wt)	0.255	763	32	733	15	750	59
Leaf biomass (mg dry wt)	0.001	3.50	1.12	4.84	1.69	7.07	2.43
Leaf area (cm^2)	0.001	0.46	0.17	0.49	0.20	0.82	0.39
Specific leaf weight (mg cm^{-2})	0.017	7.83	1.12	10.60	2.94	9.47	3.02
Plant biomass (g dry wt)	0.002	15.8	3.1	25.1	7.8	27.5	12.1
Root/shoot ratio (g g^{-1})	0.068	1.33	0.58	0.86	0.48	0.96	0.64

also suggested that the magnitude of the dilution response of leaf nitrogen by carbon marginally increased ($P<0.056$) as the carbon dioxide concentration increased. Thus, as carbon dioxide growth conditions increased, the percentage of leaf nitrogen decreased as a proportion of leaf carbon and the relationship became increasingly negative.

Both above and below ground plant biomass was significantly greater in treatments with increased CO_2 as were mean leaf weights and areas (Table 1). Root/shoot ratios and leaf water content did not change significantly.

Table 4. Correlations among allelochemical concentrations for seed-propagated sagebrush plants

	Volatiles	Guaianolides	Coumarins
Guaianolides	0.517 ***		
Coumarins	0.336 *	0.031 ns	
Luteolin	0.408 **	0.674 ***	−0.048 ns

Significance probabilities: *** = $P<0.0001$, ** = $P<0.005$, * = $P<0.05$

Allelochemical responses

There was no significant effect of carbon dioxide enrichment on leaf allelochemical concentration (Table 3). Total volatile concentration among seed-propagated individuals exhibited a high degree of variability within each treatment. For example, the variability within the 350 µl l^{-1} CO_2 treatment ranged from a minimum concentration of 10.1 mg/g dry leaf and a maximum concentration of 36.6 mg/g. Guaianolide sesquiterpene lactone concentration of the seed propagates paralleled the variability found with leaf volatiles in each carbon dioxide treatment, as did the leaf phenolic constituents. Coumarins were the allelochemicals found in the lowest concentration.

The volatile mono- and sesquiterpenes exhibited a highly significant correlation with variation in the sesquiterpene lactones over all treatments (Table 4). These compounds share biogenic precursors (Croteau 1984) and appear to be sequestered together in leaf glandular trichomes (Kelsey and Shafizadeh 1980). The flavonoid luteolin and the two coumarins which have cinnamic acids as precursors were not correlated with each other. Luteolin production was significantly correlated with both presence of leaf volatiles and sesquiterpene lactones; whereas the coumarins were significantly correlated with only volatile content. Thus, plants having high volatile yields had correspondingly high yields of the other allelochemical classes. In general, the individuals with high volatile yields in this population can be expected to have a broad spectrum of chemical defense.

The high degree of chemical variability observed among the seed-propagated individuals demonstrated a need to control genetic influences if carbon dioxide effects on allelochemicals were to be readily detected. Thus, the same volatile terpenes, sesquiterpene lactones and the coumarin umbelliferone from clonal plants were analyzed to observe how five sagebrush genotypes may respond to increasing carbon dioxide concentration (Table 5). Two independent sample replicates were taken from each clonal plant for analysis; average coefficients of variation of 10.8% for pentane extractables and 9.34% for acetonitrile extractables confirmed the precision of our chemical assays. Although the concentrations of volatiles (df=4, $P<0.001$), sesquiterpene lactones (df=4, $P<0.06$) and umbelliferone (df=4, $P<0.07$) differed between plant genotypes, the use of clonal propagations helped reduce the variability in measured allelochemical concentrations. Even with genetic variability controlled, carbon dioxide was not significant as a main effect (df=1; volatiles $P=0.316$, sesquiterpene lactones $P=0.55$, umbelliferone $P=0.74$) nor in interactions with plant genotype in a two-way analysis of variance.

Table 3. Effect of carbon dioxide treatments on leaf allelochemical concentration in seed-propagated *Artemisia tridentata*. Significance values are for a one-way analysis of variance (df=2)

	Significance of CO_2 effect ($P<$)	270 µl l^{-1}		350 µl l^{-1}		650 µl l^{-1}	
		mean	sd	mean	sd	mean	sd
		$n=14$		$n=19$		$n=22$	
Volatile concentration (mg volatiles g^{-1} dry wt)	0.169	24.2	10.2	25.4	7.2	20.3	9.0
Sesquiterpene lactone concentration (mg guaianolides g^{-1} dry wt)	0.150	20.0	10.2	23.1	8.1	17.2	9.6
Coumarin concentration (mg coumarins g^{-1} dry wt)	0.846	0.22	0.17	0.25	0.21	0.21	0.18
Flavonoid concentration (mg luteolin g^{-1} dry wt)	0.356	21.3	8.3	19.9	7.4	17.0	10.6

Table 5. Mean leaf allelochemical concentrations (mg g^{-1} dry wt) for clonally-propagated genotypes grown under two levels of carbon dioxide. Individuals of each genotype were evenly distributed between two environmental chambers, uneven numbers due to mortality

Genotype	Number of indiv.	Volatiles		Sesquiterpene lactones		Coumarin	
		350 μl l^{-1}	650 μl l^{-1}	350 μl l^{-1}	650 μl l^{-1}	350 μl l^{-1}	650 μl l^{-1}
A	5	40.3	40.7	20.4	24.6	0.20	0.32
B	6	36.2	37.1	< 0.1	< 0.1	0.45	0.42
C	5	34.7	36.9	11.7	21.5	0.48	0.22
D	4	52.0	46.8	< 0.1	< 0.1	0.13	0.26
E	3	54.4	47.5	32.9	30.6	0.25	0.18
Mean ± se		41.5 ± 1.7	41.8 ± 1.2	9.6 ± 3.0	13.2 ± 3.7	0.33 ± 0.04	0.29 ± 0.03

Grasshopper feeding and growth

Grasshopper consumption differed when fed leaves from the three CO_2 treatments (Table 6). *Melanoplus differentialis* fed at a higher mean rate on leaves from the historical and projected CO_2 regimes than they did on plants from the current ambient CO_2 treatment. *Melanoplus sanguinipes* also fed at a lower rate on the ambient CO_2 grown leaves than on those from the other two treatments, although the CO_2 treatment effect for *M. sanguinipes* was marginally significant ($df=2$, $P<0.07$). These results are in agreement with previous studies in that herbivores fed at higher rates on leaves from elevated carbon dioxide conditions than on those from current CO_2 conditions (Lincoln et al. 1984; Lincoln et al. 1986; Lincoln and Couvet 1989; Osbrink et al. 1987), but the observed increase when feeding on 270 ppm treated leaves relative to current CO_2 levels is the first observation of feeding rates on leaves grown at historical CO_2 concentrations.

Grasshopper growth was significantly influenced by the host plant CO_2 regime for *M. differentialis*, but not for *M. sanguinipes* (Table 6). However, the growth rates are very low for both species. This low growth rate was expected because the grasshoppers were starved for 18 h before and after the feeding trials to ensure motivation to feed and enhance the accuracy of measurement of consumption, i.e. the gut was empty at the beginning and end of the feeding trial. Consequently, grasshoppers commonly lost weight over the entire trial period, despite their consumption of often substantial amounts of *A. tridentata* leaves. Previous experiments using caterpillars have found little change in herbivore growth on elevated CO_2 grown leaves, presumably because of the compensatory increase in feeding. To examine the effect of CO_2 treatment on nymphal weight gain while accounting for changes in consumption, we used RCR as a covariant in an analysis of covariance and found that the carbon dioxide effect on relative growth remained significant ($P<0.05$).

Consumption by both species of grasshoppers was significantly influenced by the allelochemical concentration of the leaves (Table 7, Figs. 2 and 3), but not by foliar nitrogen concentration. For both species, their relative consumption exhibited a negative correlation with the concentration of volatile terpenes and sesquiterpene lactones. *M. sanguinipes* is known to be sensitive to the toxic effects of a sesquiterpene lactone (Picman et al. 1981). *M. sanguinipes* consumption was negatively correlated with luteolin concentration and was the only instance of a correlation between feeding and phenolic concentration.

Table 6. Response of *Melanoplus* grasshoppers to leaves of seed-propagated sagebrush plants grown under three carbon dioxide concentration regimes. Significance values are for one-way analysis of variance ($df=1$)

	Significance of CO_2 effect ($P<$)	270 μl l^{-1}		350 μl l^{-1}		650 μl l^{-1}	
		mean	sd	mean	sd	mean	sd
Melanoplus differentialis		($n=19$)		($n=18$)		($n=19$)	
Relative consumption rate (mg eaten g^{-1} dry wt d^{-1})	0.001	491	179	247	125	431	229
Relative growth rate (mg growth g^{-1} dry wt d^{-1})	0.006	134	75	50	78	67	87
Melanoplus sanguinipes		($n=15$)		($n=15$)		($n=18$)	
Relative consumption rate (mg eaten g^{-1} dry wt d^{-1})	0.068	307	166	212	122	367	237
Relative growth rate (mg growth g^{-1} dry wt d^{-1})	0.714	177	103	162	70	192	127

Table 7. Significance of *Melanopus* grasshopper responses to leaf characteristics of *Artemisia tridentata* (linear regression of all plants)

	Melanoplus differentialis		*Melanoplus sanguinipes*	
	relative consumption	relative growth	relative consumption	relative growth
Volatile concentration	****	ns	**	ns
Sesquiterpene lactone concentration	****	**	****	*
Luteolin concentration	ns	ns	***	ns
Coumarin concentration	ns	ns	ns	ns
Nitrogen concentration	ns	****	ns	ns
Carbon concentration	*	ns	***	**
Water concentration	ns	**	ns	ns
Specific weight	ns	***	ns	ns

* = $P<0.05$, ** = $P<0.01$, *** = $P<0.005$, **** = $P<0.001$. ns = not significant

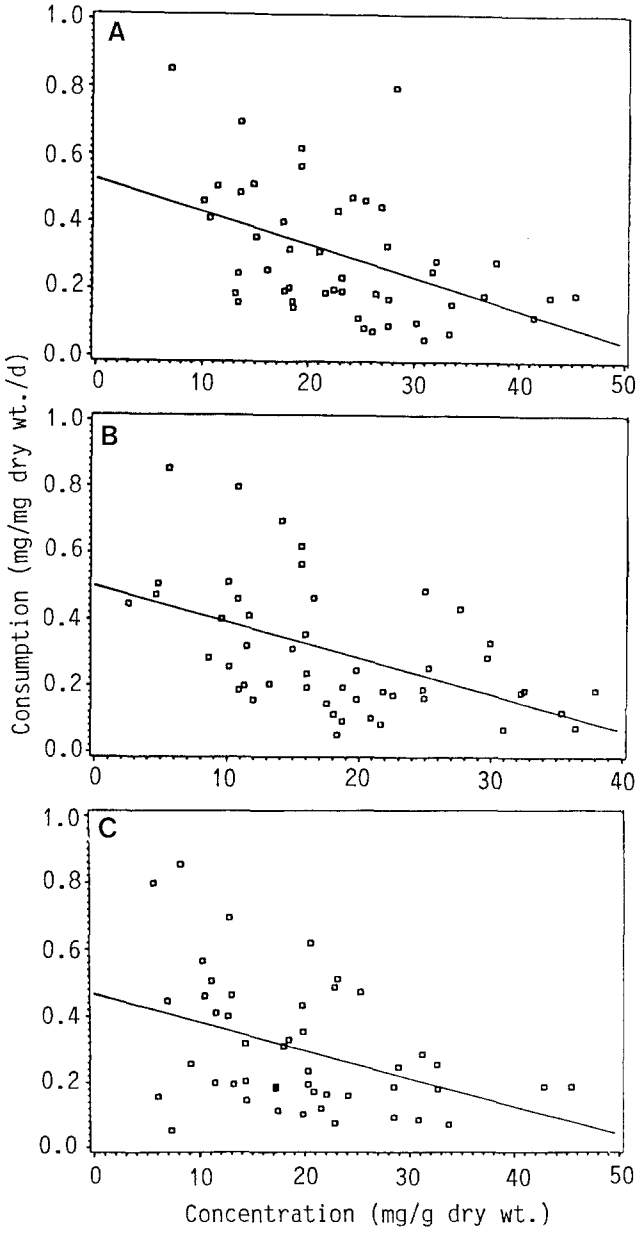

Fig. 2A–C. The effect of sagebrush leaf allelochemical concentration on *Melanoplus sanguinipes* consumption. Negative slopes of linear regression lines are all significantly different from zero ($P<0.01$). **A** Volatile terpenes; **B** *Sesquiterpene lactones;* **C** Flavonoid

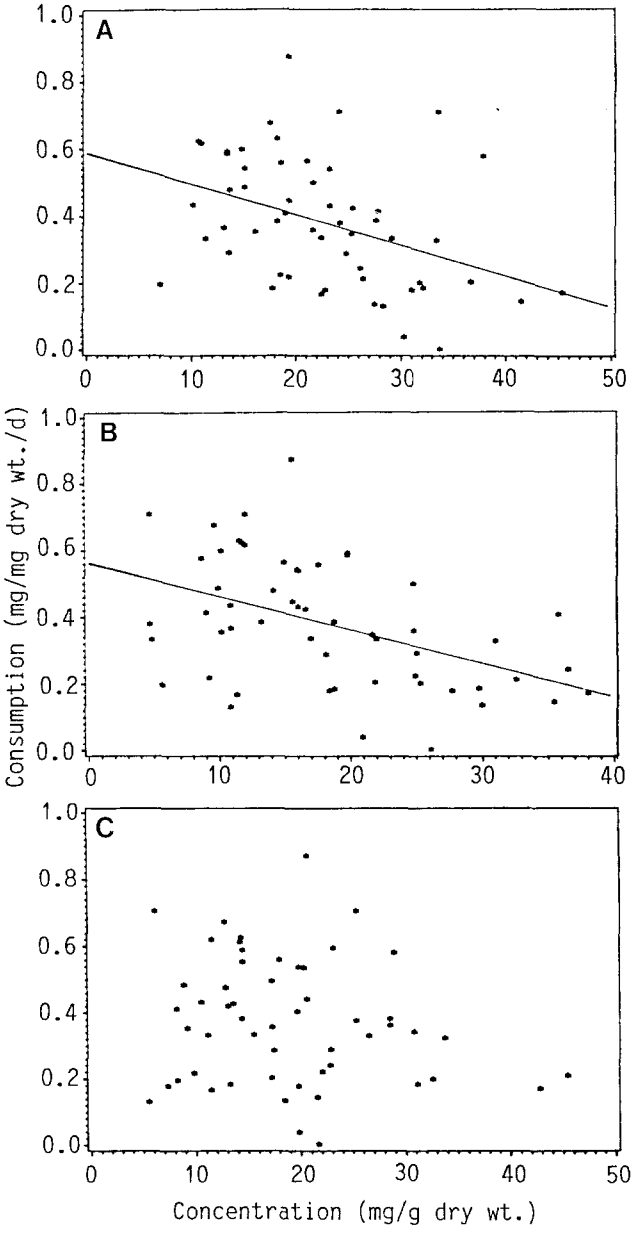

Fig. 3A–C. The effect of sagebrush leaf allelochemical concentration on *Melanoplus differentialis* consumption. Negative slopes of linear regression lines for volatile and sesquiterpene lactone concentration are significantly different from zero ($P<0.001$); flavonoid concentration was not significantly different. **A–C** see Fig. 1

When leaf nitrogen and allelochemical concentrations were individually combined in an analysis of covariance with CO_2, none of the factors could completely account for the CO_2 effect on relative consumption of *Melanoplus differentialis*. In these tests CO_2 remained significant at $P<0.005$ (df=2). On the other hand, leaf nitrogen concentration (df=2, $P<0.013$) reduced the carbon dioxide effect on the growth of *M. differentialis* to non-significance. In other words, variation in leaf nitrogen concentration may account for the carbon dioxide treatment effect on *M. differentialis* relative growth rate. The phytochemicals did not have the same effect, i.e. when combined in an analysis of covariance with carbon dioxide, they did not account for the same variation in relative growth as carbon dioxide (which remained significant at $P<0.006$, df=2). Although allelochemicals were seen to be inhibitory to feeding, other factors may also limit feeding when it is not precluded by allelochemicals.

Discussion

The lack of a carbon dioxide enrichment effect on allelochemical concentration in either seed or clonally propagated plants is in general agreement with previous results on peppermint (Lincoln and Couvet 1989). In spite of the increasing balance of leaf carbon versus nitrogen under carbon dioxide enrichment, the carbon allocation pattern to allelochemicals remained unchanged. These data suggest that allelochemical production in sagebrush plants is under strong genetic control and not influenced by simple carbon fertilization. However, plant responses to carbon dioxide in combination with other resources, e.g. excess nutrients in experiments such as this or limiting constraints under field conditions, are not understood.

The observed differences in grasshopper consumption among the CO_2 regimes was presumably due to the natural variability in allelochemical concentration among the plants in the treatments. Although not significantly different, the allelochemical concentration of plants in the ambient CO_2 treatment was higher than those from plants in either of the other two CO_2 treatments, and generally sustained the lowest consumption rates. The relatively small decline in leaf nitrogen concentration from the ambient to the enriched CO_2 treatment may have acted to minimize the grasshoppers need to increase feeding rates over the 24 h trial. Leaf quality characteristics that were not measured may have been important in determining consumption; however, potential compensatory consumption by these generalist grasshoppers was also apparently limited by sagebrush allelochemicals. Alternatively, insects with a greater ability to feed on chemically defended host plants could ultimately consume leaves (grown under CO_2 enrichment) that have a lower nitrogen concentration but the same allelochemical concentration. Compensatory feeding in these insects could potentially increase the amount of dietary allelochemicals ingested for each unit of nitrogen consumed.

How dietary content of terpene allelochemicals may affect insect performance is not well understood. However, evidence exists which suggests that quantitative plant defenses (i.e. phenolics) may act in a dietary content or dose-dependent manner (Feeny 1976). Several plant phenolics are generally thought to interact directly with leaf proteins to reduce their digestibility. Lincoln et al. (1982) simultaneously manipulated the protein and resin content of artificial diets fed to checkerspot butterfly larvae. Their tests found that larvae increased their feeding on low nitrogen diets but exhibited reduced growth; growth was lowest on diets with greater resin contents. A simple dose-dependent interaction was observed between the effects of leaf protein and resin on relative growth and larval weight. Single and multi-generational studies are now needed to assess insect metabolic responses to shifts in the ratio of dietary nitrogen and allelochemics.

The significant increases in total sagebrush biomass with increasing carbon dioxide concentration followed documented responses for other C_3 plants (Patterson and Flint 1980; Strain and Cure 1985). The perennial *A. tridentata* can accumulate increased levels of biomass, even in relatively short growing spans, as has been shown for annual crop and weedy species. The large increases in mean leaf weight and area with carbon dioxide enrichment suggest that much of the accumulated sagebrush biomass at future projected CO_2 levels may be in leaf tissue, which represents an important food source to phytophagous hervivores. The increases in plant biomass over a relatively short growing span under carbon dioxide enrichment and favorable water and nutrient conditions indicate that sagebrush may be enhanced by elevated atmospheric carbon during the brief periods of water availability that are characteristic of its native environment.

The depressed growth of the 270 $\mu l\ l^{-1}$ carbon dioxide grown plants relative to ambient grown, followed the results for soybeans grown at subambient CO_2 levels (Allen 1984) and suggest that in these genotypes, historical levels of atmospheric CO_2 may have imposed a strongly carbon limiting environment. Unexpectedly, the root/shoot ratio was highest in the 270 $\mu l\ l^{-1}$ carbon dioxide treatment. Numerous models of plant growth suggest that higher shoot production (lower root/shoot ratio) should occur under the more carbon limiting conditions of the 270 $\mu l\ l^{-1}$ conditions.

Three to five-fold differences in chemical yields among seed-propagated individuals within each treatment was observed. Wide ranges in allelochemical yields and composition are common among plants within natural plant populations (Morrow and Fox 1980), including some sagebrush populations (Kelsey et al. 1983; Welch and McArthur 1981). Thus, our data suggest that the potential effects carbon dioxide enrichment may have on plant herbivore interactions may be obscured by the chemical variability among individuals and changes in frequencies of genotypes in populations.

Acknowledgments. Funds for this study were provided by DOE Grant DE-FG09-84ER60255. Sagebrush seeds were supplied by James Young, USDA-ARS Reno. We are grateful to Elaine Oma,

USDA Rangeland Insect Laboratory for generously providing grasshopper eggs and advice. We thank Eric Fajer, Brenda Young and Bruce Welch for comments on the manuscript and the Duke University Phytotron staff for their continuing assistance. Technical assistance by RL Liu, Shanxi University, PRC is greatly appreciated.

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