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# Progress Report

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## INTRODUCTION

The overall goal of the research program is to determine how plant/herbivore interactions change with increasing atmospheric $CO_2$ concentration and how these changes affect community structure and functioning. Five specific objectives have been developed to approach these goals over the grant period. These objectives are to determine: 1) if specialist feeding herbivores respond to host plant $CO_2$ enrichment in the same manner as generalist feeders, 2) if the impact of defoliation is lessened under enriched $CO_2$ conditions, 3) the biochemical basis for the altered nutritional value of leaves resulting from elevated $CO_2$, 4) the demographic and consumption responses of an herbivore to $CO_2$ regimes, and 5) whether plants with the $C_4$ photosynthetic pathway will also have altered relationships with herbivores. Significant progress has been made on three of these goals (Objectives 1,2 and 3). These results are presented below. Manuscripts derived from objectives 2 and 3 are appended (see Appendices 2 and 3). A revised study plan to complete the remaining objectives is appended (appendix 1).

## CURRENT PROGRESS

### 1. Does a Specialist Herbivore Respond to $CO_2$ Induced Changes in its Host Plant?

Previous studies of herbivore responses to elevated $CO_2$ effects have emphasized insect herbivores with generalized feeding patterns, i.e. feeding on a wide range of host plants (Lincoln et al., 1984, 1986; Butler et al., 1986, Osbrink et al., 1987; Lincoln and Couvet, 1989). The generalists which have been studied were chosen partly because they among the most tractable experimental organisms for laboratory work. However, specialist feeding herbivores, which constitute the majority of insects, are also of interest for assessing $CO_2$-induced effects. Further, because specialist feeding insects are an important herbivore component in many native communities and are often pests in crop and forestry systems, it is of interest to study their responses to $CO_2$ effects. Consequently, an experiment was undertaken to test the response of a specialist feeding caterpillar to its host plant grown under elevated $CO_2$.

The experimental system we was used included _Brassica kaber_, wild mustard, and _Pieris rapae_, cabbage butterfly. This system has several advantages: _Pieris rapae_ feeds solely on members of the mustard family and is a pest on _Brassica_ crops, while _Brassica kaber_, wild mustard, is a widely distributed weed which produces glucosinolates, the characteristic allelochemicals of mustard species. This model system is also advantageous because the chemical basis for this species' specialized feeding behavior is known: the glucosinolates are oviposition and feeding cues for Pieris (David and Gardiner, 1966; Gardiner, 1985).

Specialist herbivores are adapted, via coevolution, to the allelochemical defenses of their particular host plants. Thus, sinigrin, the principal allelochemical of B. kaber has been shown to be a potent toxin to non-adapted insect herbivores (Erickson and Feeny, 1974), even though it is an oviposition and feeding cue for P. rapae.

The glucosinolates, or mustard oils, contain nitrogen and sulfur, as well as carbon, hydrogen and oxygen. We therefore propose to also assay the allelochemical production of Brassica as a part of this experiment. The examination of the glucosinolates will be used to supplement the information on the response of carbon-based allelochemicals in peppermint and sagebrush.

Forty plants were grown in two atmospheric carbon dioxide concentration treatments, 350 and 650 ul $l^{-1}$. The consumption and growth of the caterpillars was measured over a 24 hour period using the standard techniques previously used for caterpillars (Lincoln et al., 1984, 1986).

The significantly increased plant growth in response to the carbon dioxide treatments (Table 1) was in agreement with the numerous previous observations on herbaceous plants. Both shoots and roots increased in biomass in the enriched $CO_2$ treatment. The leaf nitrogen content declined from 2.5% to 1.6%. Because the leaves of this plant species contain secondary chemicals with nitrogen as a structural component, the Kjeldahl total amino nitrogen was measured (Table 6), in lieu of the total nitrogen analysis which has been used in earlier experiments. The Kjeldahl amino nitrogen represents more closely the amount of nutritional protein and amino acids potentially available to herbivores. The leaf water content also declined significantly with higher $CO_2$.

The caterpillars ate at a significantly higher rate on the leaves from the enriched $CO_2$ treatment (Table 2). Further, they did not grow at any higher rate, despite the greater consumption. These results are in agreements with those from previous results for generalist feeding insect (Lincoln et al, 1984, 1986; Lincoln and Couvet, 1989). The measurement of growth and feeding indices were sufficiently reproducible for the rapidly growing insect that digestibility and digestion efficiency could be accurately measured. Digestion efficiency was reduced on the enriched $CO_2$ grown leaves even though the leaf digestibility was not influenced. These results suggest that the gross digestibility of the leaves was not affected by plant growth in elevated $CO_2$. This is an interesting observation because even though the leaf

Table 1. Wild mustard (Brassica kaber) growth responses to atmospheric carbon dioxide concentration regimes. Significance values are for a one-way analysis of variance.

| | Significance of $CO_2$ effect ( P < ) | $350\ ul\ l^{-1}$ | | $650\ ul\ l^{-1}$ | |
|---|---|---|---|---|---|
| | | mean | sd | mean | sd |
| Shoot biomass (g) (g dry wt) | 0.001 | 13.3 | 2.5 | 22.0 | 3.8 |
| Root biomass (g) (g dry wt) | 0.001 | 18.4 | 2.1 | 30.4 | 3.0 |
| Plant biomass (g) (g dry wt) | 0.001 | 31.7 | 4.4 | 52.4 | 6.6 |
| Root/shoot ratio ($g\ g^{-1}$) | ns | 1.40 | 0.14 | 1.40 | 0.15 |
| Leaf water (mg water $g^{-1}$ fr wt) | 0.001 | 817 | 29 | 763 | 46 |
| Leaf nitrogen (mg N $g^{-1}$ dry wt) | 0.041 | 25 | 17 | 16 | 10 |

Table 2. *Pieris rapae* caterpillar feeding characteristics on wild mustard (*Brassica kaber*) grown under two carbon dioxide growth regimes. All values are expressed on a dry weight basis. ns = not significant.

| | Significance of $CO_2$ effect ( P < ) | $350\ ul\ l^{-1}$ | | $650\ ul\ l^{-1}$ | |
|---|---|---|---|---|---|
| | | mean | sd | mean | sd |
| Larval consumption rate (mg eaten $mg^{-1}$ wt $d^{-1}$) | 0.001 | 3.86 | 1.05 | 5.52 | 1.59 |
| Larval growth rate (mg growth $g^{-1}$ wt $d^{-1}$) | ns | 866 | 147 | 878 | 149 |
| Growth efficiency (ECI) (mg growth $g^{-1}$ eaten) | 0.02 | 246 | 115 | 171 | 62 |
| Digestion efficiency (ECD) (mg growth $g^{-1}$ digested) | 0.05 | 536 | 374 | 348 | 153 |
| Digestibility (AD) (mg digested $g^{-1}$ eaten) | ns | 523 | 122 | 540 | 144 |

nitrogen (protein and amino acid content) and leaf water contents declined, the caterpillars were able to digest an equal amount from the leaves. However, the caterpillars expended a greater proportion of the energy and substrate they gained on metabolism and a lesser amount on growth. Thus, the results point to higher costs associated with consumption of leaves grown in elevated $CO_2$ atmospheres, and not to differences in digestibility.

These results unambiguously indicate that for leaf feeding insects, specialist herbivores appear to respond to leaf changes resulting from elevated $CO_2$ in the same manner as expected for generalist feeding insects. Past results have implicated reduced leaf nutritional value in the increased (compensatory) feeding on leaves at higher $CO_2$ levels, along with possible effect from reduced leaf water concentration. The current results are consistent with that interpretation.

These results are the first assessment of the feeding response of a specialist herbivore to the effects of rising atmospheric carbon dioxide. This is important because of the abundance of specialist feeding herbivores in natural and managed communities. In both agricultural and forestry systems, many of the pest insect species have highly restricted host plant ranges, which include the crop and closely related non-crop species. These results suggest that increased feeding may be widespread as the atmospheric concentration of $CO_2$ rises.

The sole additional data which must be collected to complete this experiment is the measurement of leaf glucosinolate (sinigrin) production by the **B. kaber** plants. An enzymatic hydrolysis followed by a gas chromatographic assay of the released odorous products (Louda and Rodman, 1983) will be used. This method is reliable and more rapid than others and is not expected to pose any problems. Measurement of these chemicals is important both because of their potential importance to the experimental caterpillar feeding and because this would be the first measurement of $CO_2$ effects on nitrogen and sulfur containing allelochemicals. The latter aspect has potentially significant implications for the many plants producing alkaloids, cyanogenic glycosides, amines, non-protein amino acids, etc.

## 2. Is the Impact of Defoliation Influenced by Elevated $CO_2$?

This objective has completed with only the exception of manuscript submission. All data has been collected and analyzed and a manuscript prepared (see appendix 2). However, we will obtain internal reviews and revise the manuscript prior to submission.

Our results suggest that sagebrush plants may exhibit greater resistance to chronic herbivory under projected atmospheric concentrations of carbon dioxide. Low leaf nitrogen levels which result from growth in enriched $CO_2$ may be exaggerated at high $CO_2$ levels by the large allocation of resources to shoot regrowth and the static allocation to root mass. The inability of these sagebrush individuals to fully replace lost leaf nitrogen may reduce the nutritional quality of regrowth leaves to subsequent herbivores.

## 3. Determine the Nutritional and Allelochemical Basis for the $CO_2$-Induced Increase in Feeding by Individual Herbivores

One of the goals of the proposed research is understand the underlying biochemical basis for leaf and herbivore responses to $CO_2$, both because of implications for herbivore feeding and to define vegetation responses to elevated $CO_2$. The relationship between reduced nitrogen and enriched $CO_2$ treatment does not conform to the simple prediction that higher carbon equals lower nitrogen, as it does when $CO_2$ is held constant (Figure 1). Under elevated $CO_2$, as leaf carbon content increases there must be additional leaf components which increase in concordance with leaf carbon content and these may influence herbivory.

This objective has been approached by two studies. The first part which assesses allelochemical variation has been completed and the manuscript is appended (Appendix 3). The other portion of this objective is in progress with about one half of the work completed.

We have completed plant growth under two nutrient and carbon dioxide regimes, grasshopper feeding trials and Kjeldahl amino nitrogen analysis. We must still complete the carbon analysis, analysis of other elements with an atomic absorption and carbohydrate and fiber analysis. All of the methods for these analyses have already been conducted in our laboratory to ensure that no problems will be encountered in the completion of these experiments.

The growth of plants to assess changes in allelochemical concentration and its potential effects on herbivore feeding used seed propagated and clonally-propagated plants of Big Sagebrush (<u>Artemisia</u> <u>tridentata</u> var. <u>tridentata</u>) were grown under atmospheric carbon dioxide regimes of 270, 350 and 650 ul $l^{-1}$ and fed them to <u>Melanoplus</u> grasshoppers. Plants grown from seed collected in a single population exhibited a 3-5 fold variation in the concentration of leaf volatile mono- and sesquiterpenes, quaianolide 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. Grasshopper feeding on the seed propagated plants did not differ significantly among the carbon dioxide treatments but varied widely within treatments in relation to the allelochemical differences among plants. Because these plants exhibited a high degree of variation among individuals which could obscure a carbon dioxide enrichment effect, a second experiment was conducted. Even when genotypic variation was controlled by vegetative propagation, allelochemical concentrations did not differ among carbon dioxide treatments. However, leaf nitrogen concentration declined significantly with elevated $CO_2$ and allelochemical contents per leaf increased significantly over the three $CO_2$ treatments. Thus, feeding on sagebrush under carbon dioxide enrichment will require consumption of increased quantities of allelochemicals per amount of nitrogen consumed.

In the second study, whose purpose is to assess how changes in the biochemical and elemental profile of leaves as a result of carbon dioxide enrichment influence herbivorous animals, we grew plants under two levels of nutrients as well as two levels of atmospheric carbon dioxide concentration. Leaf nitrogen contents were significantly depressed by growth under elevated carbon dioxide (Figure 1). As expected growth under low nutrient supply also depressed leaf nitrogen concentrations. Growth was enhanced by carbon dioxide enrichment under the high nutrient availability , but not under low nutrient supply. This result suggests that carbon dioxide effects on sagebrush may be limited if nutrient supplies are in low supply.

Grasshopper consumption was significantly increased by the carbon dioxide treatments as has been observed in studies of butterflies and moths. This study is a significant extension of these previous observations because it clearly suggests that the observations of enhanced feeding under elevated carbon dioxide may be a generalized phenomenon for insects, especially when coupled with the results for the specialist feeding lepidopteran.

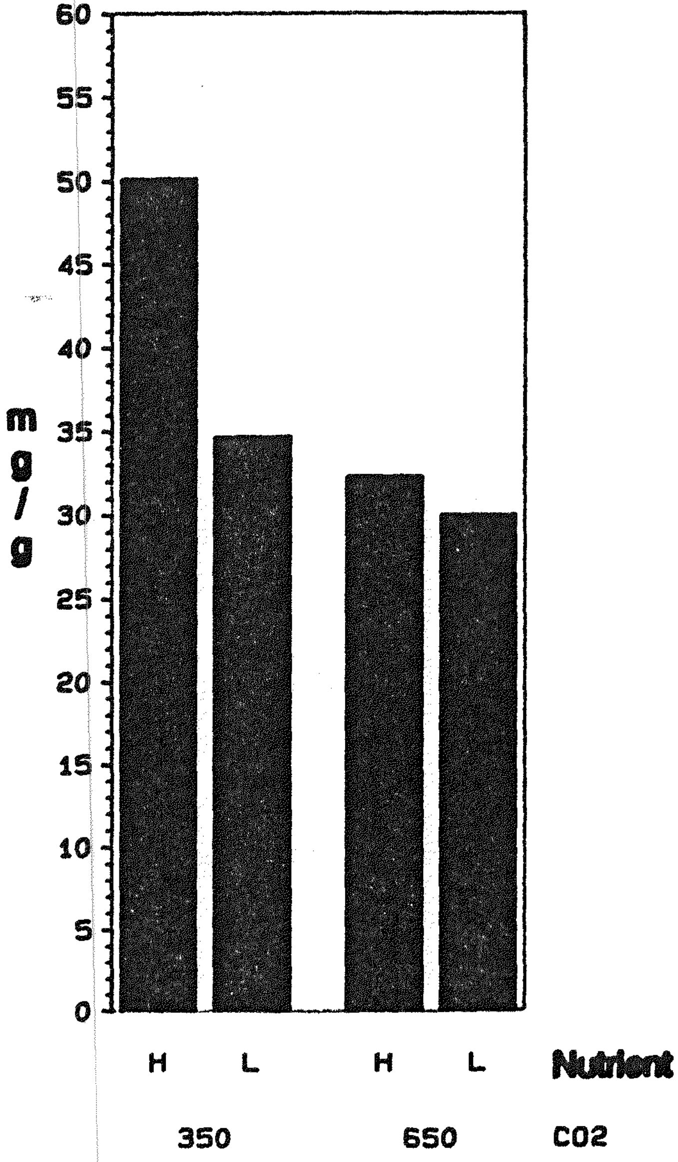
Despite the increased grasshopper consumption of plants grown under elevated carbon dioxide, their growth rates were depressed. Presumably this is due to the reduced leaf nutritional quality reflected by the reduced leaf nitrogen concentration. Grasshopper growth was also reduced under low plant nutrient supply, which also results in reduced leaf nutritional quality for herbivores.

This experiment also allowed us to test the effect of carbon dioxide enrichment on plant growth and allocation. As noted earlier, plant shoot weight was dramatically increased as carbon dioxide supply increased, but only under high nutrient supply. In contrast, leaf specific weight was increased by $CO_2$ treatment and by low nutrient supply. Leaf specific weight commonly reflects leaf thickness and toughness, which affect leaf eating insects. This result suggests that as atmospheric carbon dioxide rises, leaf may become tougher as well as less nutritious.

The root/shoot ratio of these plants was reduced by growth under carbon enrichment. This results is contrary to hypotheses about how plants balance the supplies of carbon from leaves with the supply of other nutrients from the roots (See appendix 2 for a more complete discussion).

Figure 1. Effect of carbon and nutrient supply on theleaf nitrogen concentration of sagebrush plants.

# Mean Leaf Nitrogen Content

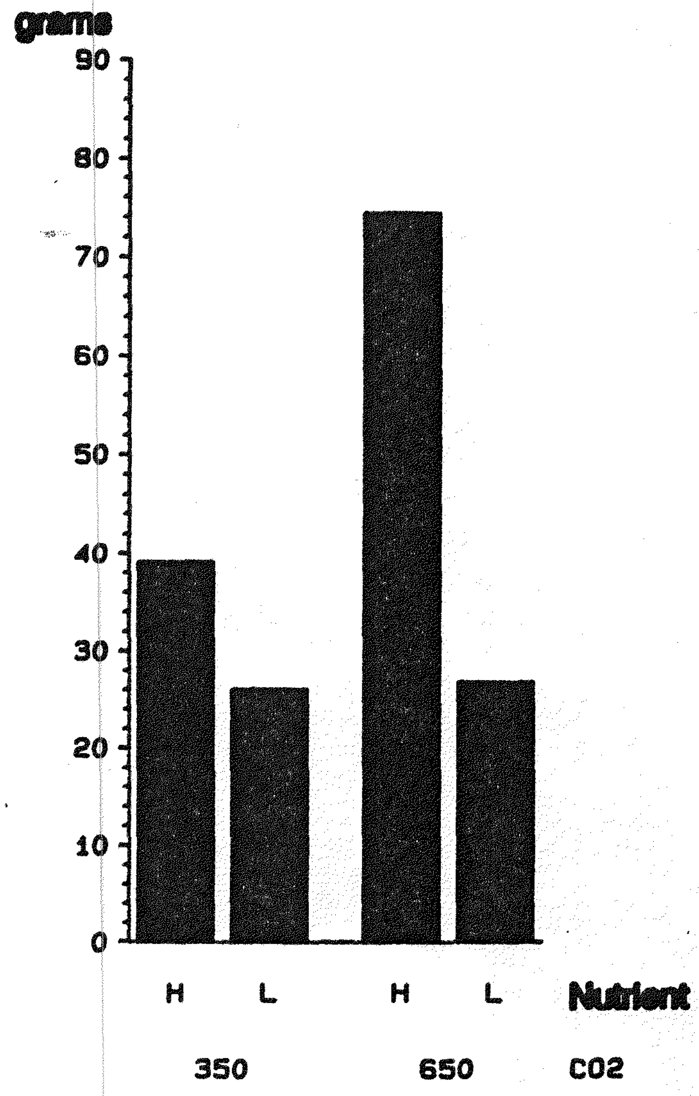


CO2 expressed in ppm, under High and Low nutrient treatments

Figure 2. Growth response of _Artemisia tridentata_ plants to enriched carbon dioxide regime and nutrient supply.

# Mean Sagebrush Shoot Weight

**Under two levels of CO2 and Nutrients**



CO2 expressed in ppm, under High and Low nutrient treatments

Figure 3. Consumption by *Melanoplus differentialis* grasshoppers of *Artemisia tridentata* plants grown under two carbon and nutrient supply rates.

# Grasshopper Relative Consumption

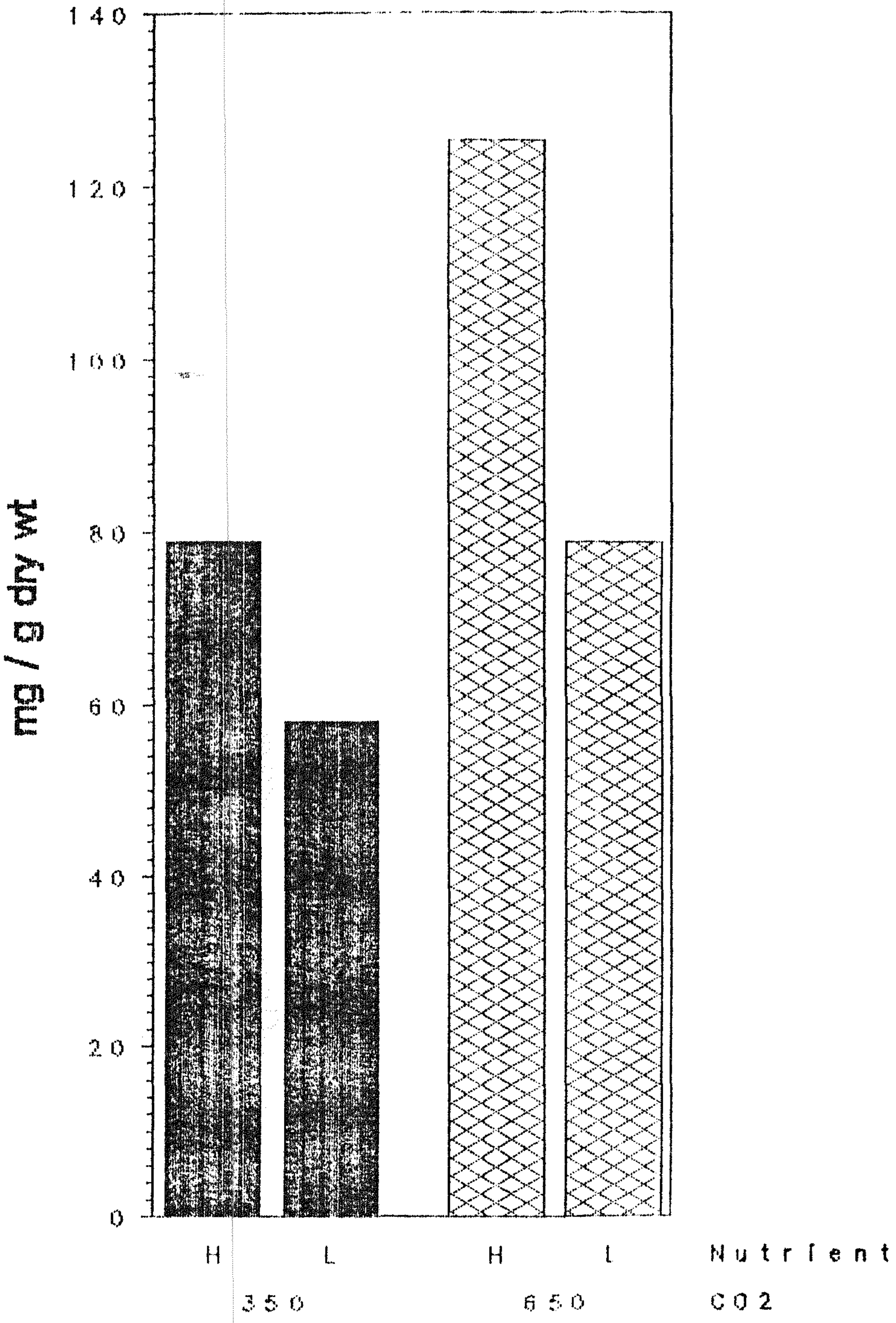
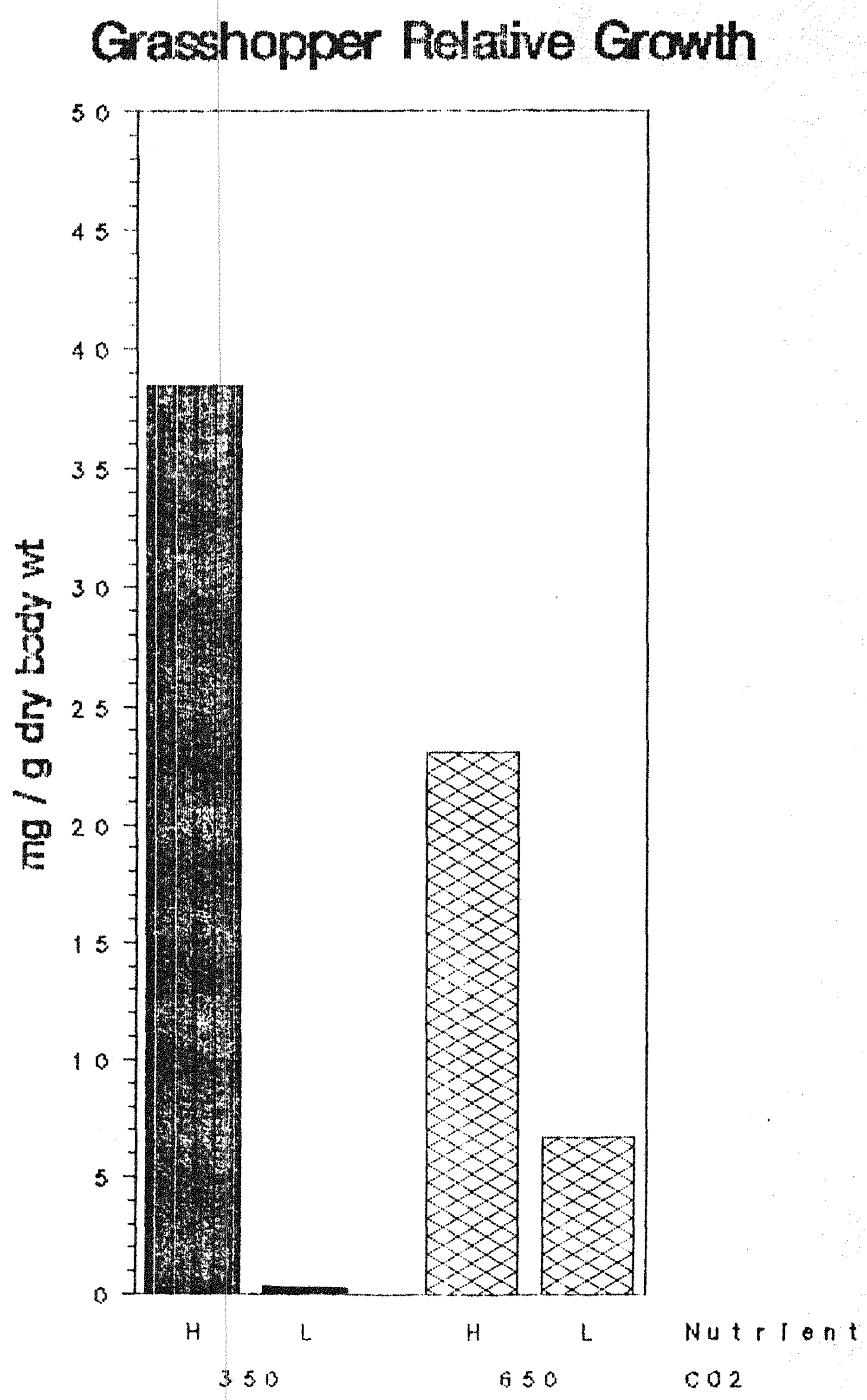


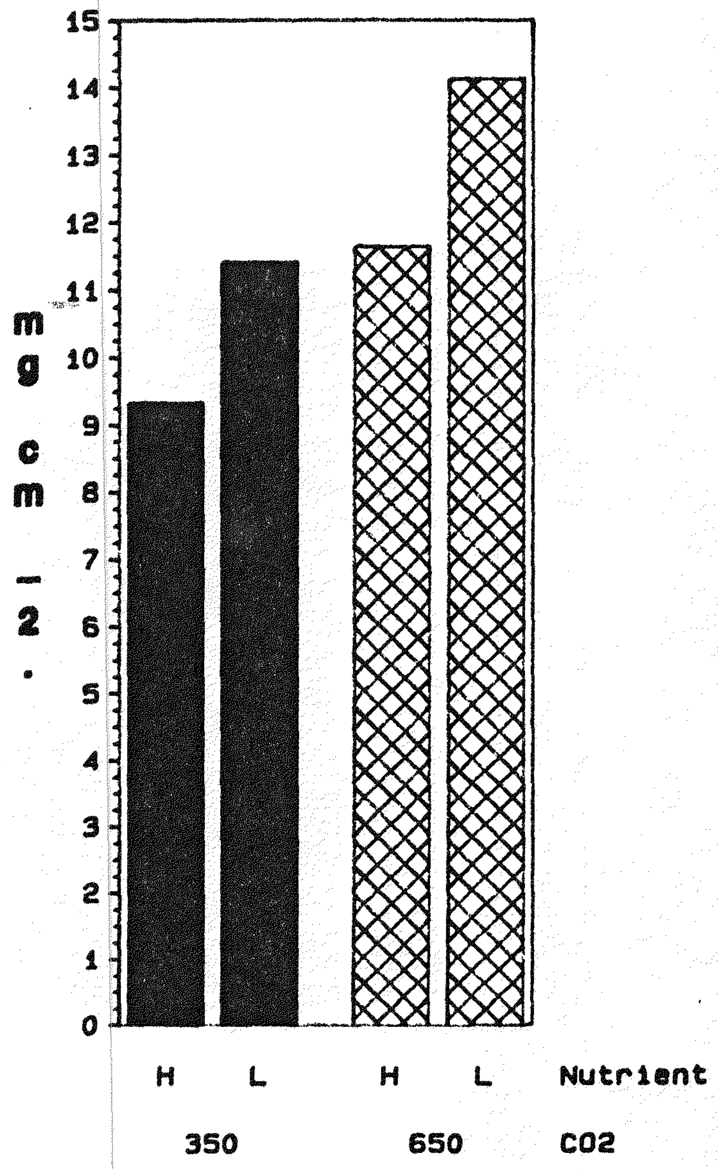
Figure 4. Growth by *Melanoplus differentialis* grasshoppers of *Artemisia tridentata* plants grown under two carbon and nutrient supply rates.



CO2 expressed in ppm under High and Low nutrient

Figure 5. Effect of carbon and nutrient supply on sagebrush leaf specific weight.

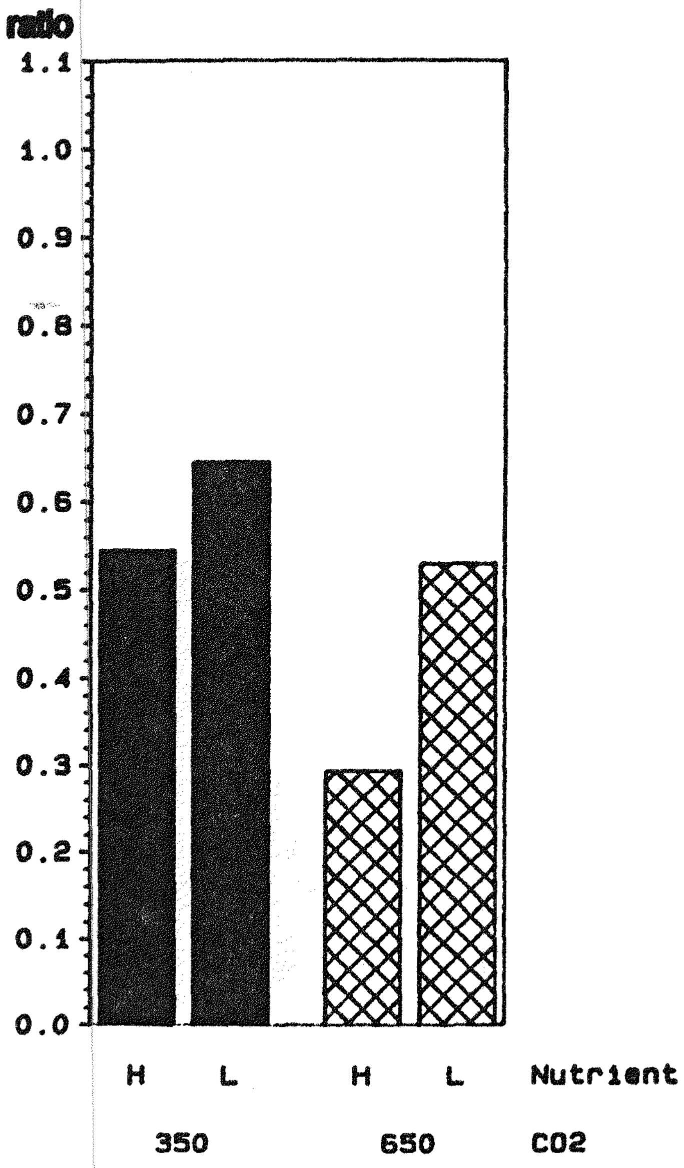
# Mean Sagebrush Leaf Specific – Weight



CO2 expressed in ppm. under High and Low nutrient treatments.

Figure 6. Effect of carbon dioxide concentration and nutrient supply on the root/shoot ratio of sagebrush plants.

# Mean Sagebrush Root/Shoot Ratio



CO2 expressed in ppm, under High and Low nutrient treatments.

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# Appendix 2

Manuscript entitled:

## "CARBON DIOXIDE ENRICHMENT AND DEFOLIATION EFFECTS ON SAGEBRUSH"

# Carbon Dioxide Enrichment and Defoliation Effects on Sagebrush

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## INTRODUCTION

It has long been hypothesized that plant growth is a balanced process between the carbon gaining capacity of the shoot and the nutrient gaining capacity of the root (Chapin, 1984). Understanding of the control and maintenance of this ratio is of particular interest in studying the influence of rising atmospheric carbon dioxide on plants because $CO_2$ supply directly influences the balance between photosynthetic rate, root structure and function, canopy architecture, and biomass allocation (Strain and Cure, 1985). It is well established that plants change their root/shoot ratio in response to changes in soil nitrogen supply, but little is known about their response to $CO_2$. Consumption by leaf eating herbivores is expected to increase with rising atmospheric carbon carbon dioxide (Lincoln et al, 1986). Because increased defoliation has the effect of reducing the rate of carbon gain relative to nutrient uptake while $CO_2$ enrichment has the opposite effect, the interaction between defoliation and $CO_2$ enrichment was investigated.

Although some plants which have been adapted to grazing may produce an 'overcompensating' regrowth (Coughenour et al. 1985; Paige and Whitham, 1987; Brown and Ewel, 1988), many plants experience a decrease in productivity and fitness as a result of leaf loss to herbivores. For plants growing in conditions of nutrient limitation, leaf nitrogen losses to herbivory may be at a higher rate than it can be replaced. The removal of leaf material in some plant species has been found to reduce the nutritional quality of remaining leaves and reduce larval performance of subsequent herbivores (Harrison and Karban, 1986). Thus the lower nitrogen content of regrowth or remaining leaves following defoliation may influence subsequent herbivory (van der Meijden et al. 1988).

Defoliation may alter carbon allocation patterns in some plants leading to increased allelochemical concentrations and inhibiting further loss of carbon gaining organs (Rossiter et al. 1988; Mihaliak and Lincoln, 1989). Bloom et al. (1985) suggest that a plant preferentially allocates it resources to minimize environmental limitations on its growth and function. When defoliation limits carbon gain, plants may be expected to allocate resources to production of new leaves and perhaps to the defense of existing leaves. Thus, the leaves of a defoliated plant growing under carbon dioxide enrichment should more easily replaced compared to growth under current carbon dioxide conditions and would have correspondingly less allocation to chemical defense.

In order to investigate the effects of $CO_2$ and defoliation on sagebrush growth and palatability characteristics, we tested three hypotheses: 1) defoliation should have less effect on plant growth under enriched carbon dioxide conditions, 2) chemical defenses should be reduced on plants grown under carbon dioxide enrichment, and 3) defoliation should lead to lower leaf nitrogen levels under elevated carbon dioxide conditions.

## METHODS

Sixty Four Big Sagebrush plants (Artemisia tridentata var. tridentata) were grown at the Duke University Phytotron for approximately nine weeks in controlled environmental chambers. The carbon dioxide concentration in the two chambers was maintained at 350 and 650 ul $l^{-1}$ (± 20 ul $l^{-1}$) under a 14 hour daylight regime with 27°C and a 23°C night temperature and a relative humidity of 70%. Photosynthetically active irradiance was maintained at 1050 umol $m^{-2}$ $s^{-1}$ photons. All plants received daily waterings of one-half strength Hoagland solution and deionized water. Defoliation treatments of one half of the plants in each $CO_2$ regime removed approximately 40% of expanded leaves in each of three episodes. All plants were moved biweekly both within and between the environmental chambers (with appropriate $CO_2$ adjustment) to minimize possible chamber effects.

Leaf volatile mono- and sesquiterpene concentrations were measured by placing leaves in pentane and storing at -20°C. The fresh frozen leaf material was homogenized in pentane, centrifuged and the volatile supernatant concentrated. Quantitative analysis of the plant volatiles was conducted using a temperature programmed gas chromatograph with a SP-1000 capillary column and flame ionization detector. Tridecane was used as an internal standard. Identities and retention times of the mono- and sesquiterpene peaks were confirmed with GC-mass spectroscopy by comparison with authentic standards and literature spectra. The identical column and temperature program of the quantitative runs was used in making the compound confirmations by GC-MS. After nine weeks all plant material was harvested, dried in a forced air oven and weighed. Leaves (8-10) were dried and assayed for Kjeldahl amino nitrogen content with an autoanalyzer. All statistical analyses were performed using the SAS version 6.03 (SAS Institute Inc. Cary, NC).

## RESULTS AND DISCUSSION

The significant increase in sagebrush above-ground biomass ($P<0.001$) under carbon dioxide enrichment (Figure 1) conforms to the increased growth observed in numerous native and crop species grown under similar carbon regimes (Strain and Cure, 1985). In contrast to $CO_2$ enrichment, chronic artificial defoliation treatments significantly reduced final shoot biomass in both carbon dioxide treatments ($P<0.001$). However, the biomass reduction by defoliation was limited on average to 16% less than the non-defoliated individuals in the high $CO_2$ chamber, compared to a 33% reduction in the ambient $CO_2$ treatment. Although the interaction between carbon dioxide concentration and defoliation treatment was only marginally significant ($P<0.08$), this result suggests that sagebrush may have an enhanced tolerance to chronic herbivory at elevated levels of atmospheric carbon dioxide.

Growth under carbon dioxide enrichment reduced the average root/shoot ratio (Figure 2). On the other hand, chronic defoliation increased the ratio of root biomass to shoot biomass, and this effect was greater in the ambient grown sagebrush plants than those grown under elevated carbon dioxide. The enhanced regrowth of shoot biomass under elevated carbon dioxide allowed these plants to maintain more stable root/shoot ratios in spite of heavy defoliation treatments. Surprisingly, root biomass production was not influenced by either treatment. The plants were grown in high nutrient availability and root production by the plants did not appear to be limited by pot size at the end of the experiment.

Several biotic and abiotic stresses may simultaneously limit plant growth. New plant biomass is expected to be partitioned such that all environmental constraints become equally limiting to plant growth (Bloom et al. 1985). Models of carbon partitioning between root and shoot predict that if carbon acquisition is more limiting to growth than nutrient uptake, then plants will increase the partitioning of biomass to shoots (e.g. Thornley 1972, Reynolds and Thornley 1982, Johnson 1985, Hunt and Nicholls 1986). On the other hand, if below ground resources are more limiting to plant growth, then increased allocation to root growth is expected. If the availability of carbon or nutrients changes, new biomass should be partitioned to achieve a root/shoot ratio which compensates for the shift in availability (Johnson 1985, Bloom et al. 1985). The current results do not agree with these general hypotheses in a simple manner because both the increased carbon supply from $CO_2$ and decreased carbon supply from defoliation lead to lower root/shoot ratios.

Leaf nitrogen concentration exhibited a small decline by growth under carbon enrichment (Figure 3). Defoliation reduced nitrogen concentrations by approximately 10% under ambient conditions and 20% under carbon enrichment. The relatively larger decline in leaf nitrogen by artificial defoliation at high carbon dioxide levels may result in part from the allocation of plant nitrogen to shoot regrowth, without a concomitant increase in nitrogen-gaining tissues. Because allocation to root growth and metabolism is often reduced by defoliation, root surface area and active uptake of nutrients may both be reduced under defoliation (Chapin, 1984).

Increasing carbon dioxide concentration did not affect the yields of sagebrush mono- or sesquiterpene allelochemicals (Figure 4). These results support our previous observations with sagebrush that suggest carbon-based allelochemical production in sagebrush is under genetic control and not regulated by simple carbon fertilization. Defoliated plants under each carbon treatment did exhibit a reduction trend in terpene concentration relative to the non-defoliated plants ($P < 0.055$). Although enhanced carbon availability at projected concentrations did not influence terpene yield, the physical loss of carbon-gaining leaves apparently led to a carbon allocation pattern of enhanced shoot growth at the expense of mono- and sesquiterpene synthesis.

In conclusion, our data suggest that sagebrush plants may exhibit greater resistance to chronic herbivory under projected atmospheric concentrations of carbon dioxide. Low leaf nitrogen levels which result from growth in enriched $CO_2$ may be exaggerated at high $CO_2$ levels by the large allocation of resources to shoot regrowth and the static allocation to root mass. The inability of these sagebrush individuals to fully replace lost leaf nitrogen may reduce the nutritional quality of regrowth leaves to subsequent herbivores.

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Figure 1. Final shoot dry weight of chronically defoliated sagebrush plants grown at two levels of carbon dioxide.

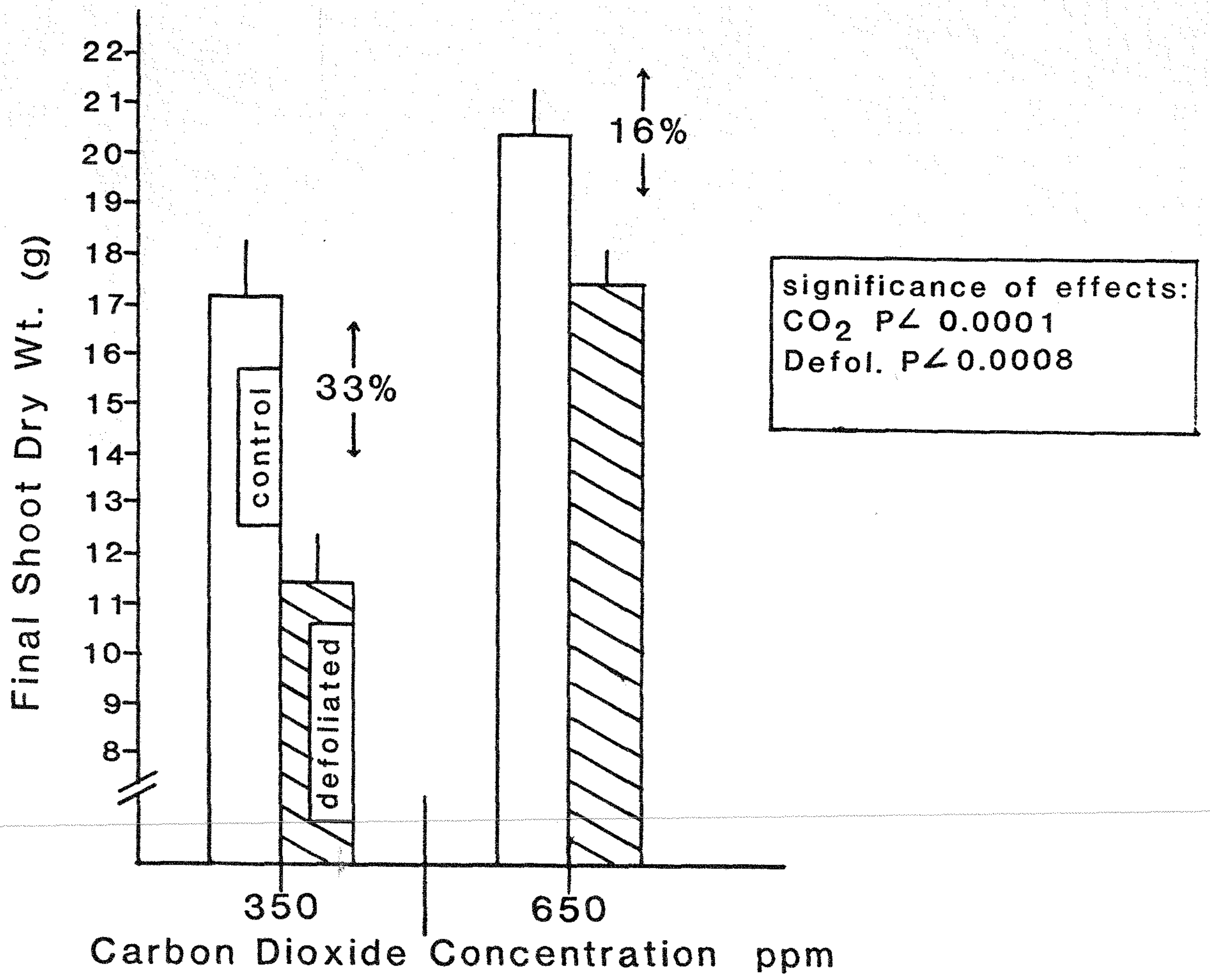

Figure 2. Leaf specific weight and root/shoot ratio of chronically defoliated sagebrush plants grown at two levels of carbon dioxide.

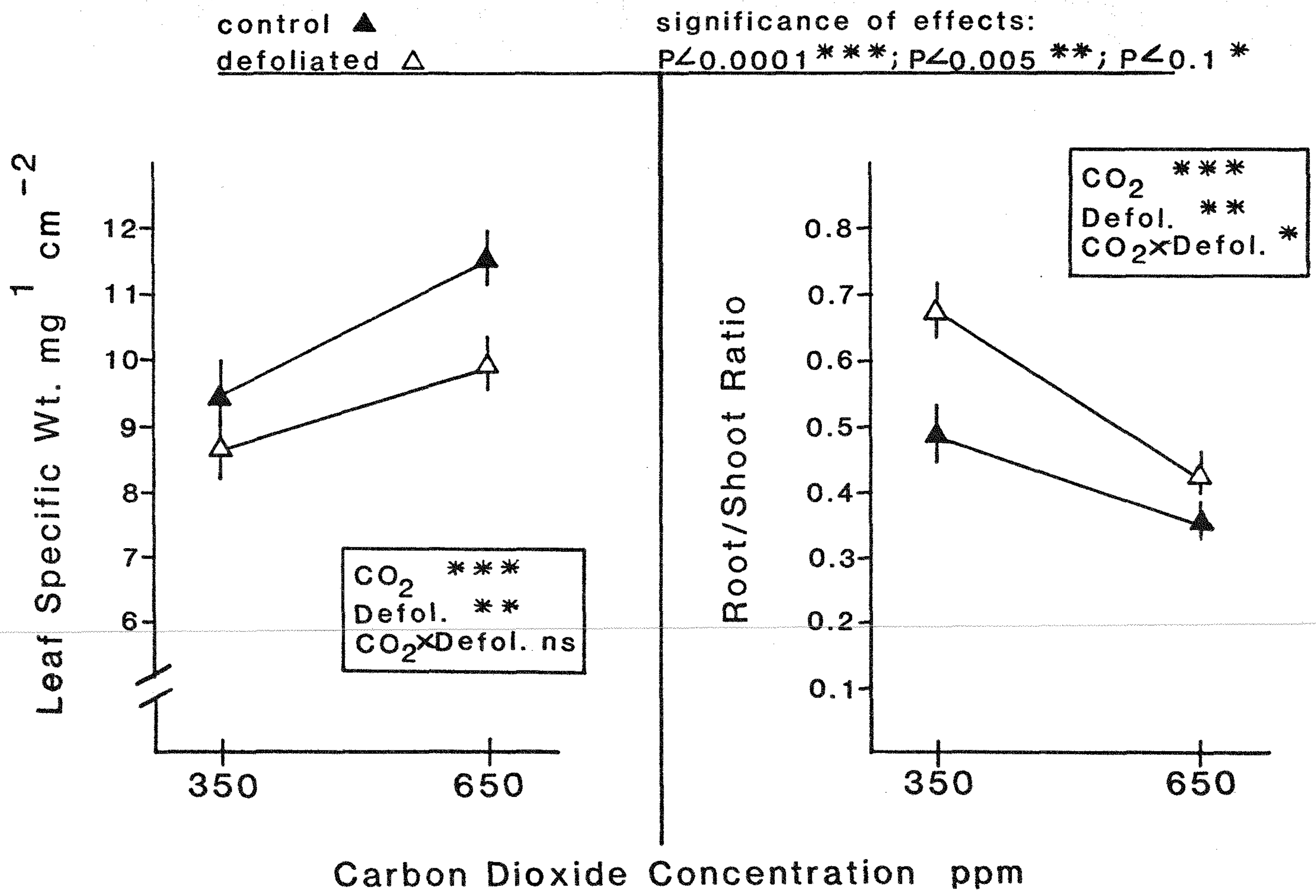

Figure 3. Leaf nitrogen concentration of chronically defoliated and non-defoliated sagebrush plants grown under two levels of carbon dioxide.

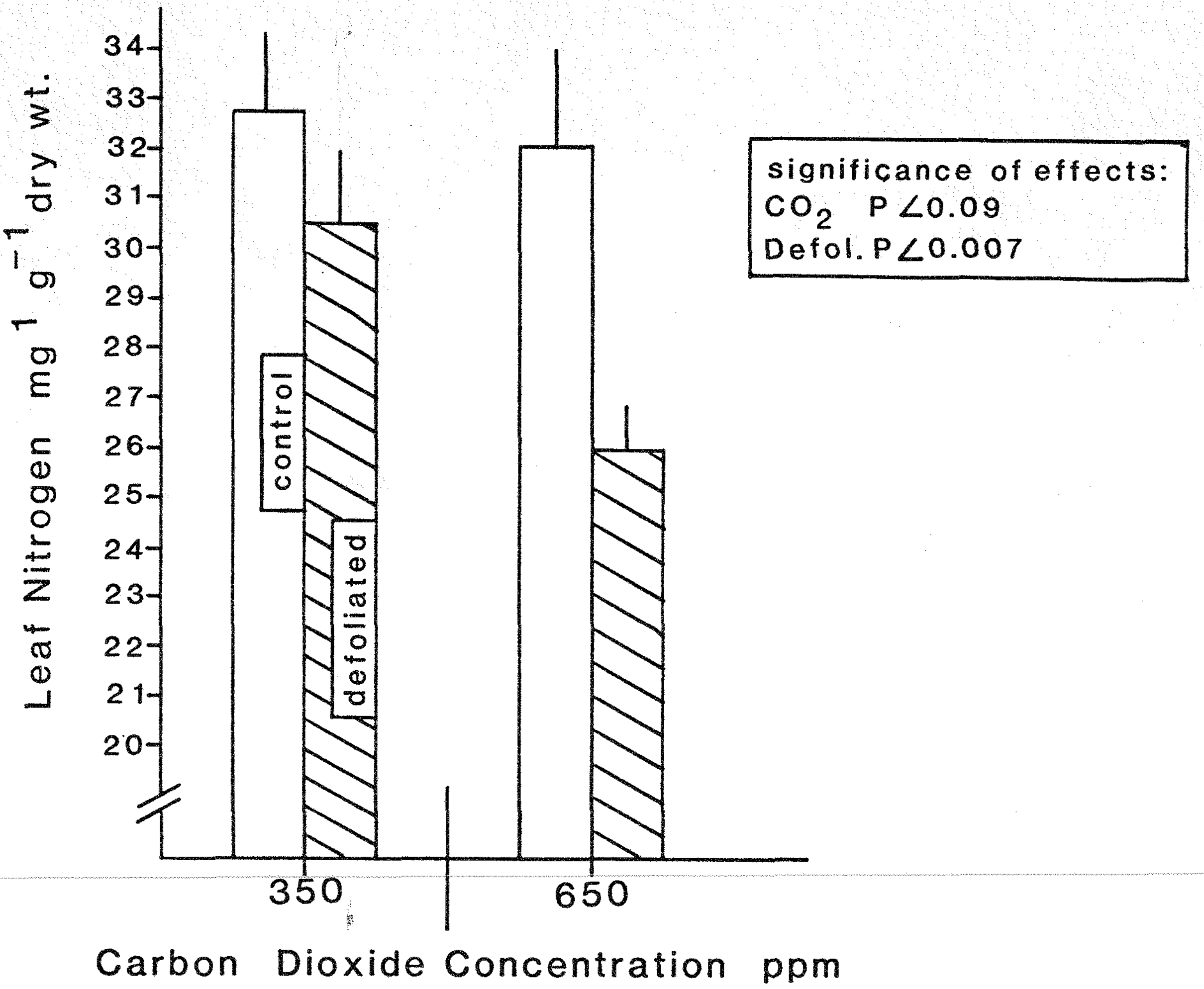

Figure 4. Mono- and sesquiterpene concentration of sagebrush leaves from chronically defoliated and non-defoliated plants grown at two levels of carbon dioxide.

MONO- and SESQUITERPENE YIELDS mg $^{1}$ g$^{-1}$ dry wt.

| | |
|---|---|
| $CO_2$ | ns |
| Defol. | $P \angle 0.06$ |

| | | Minimum | Maximum | Mean | Std Dev |
|---|---|---|---|---|---|
| 350 ppm | Control | 19.76 | 88.79 | 40.74 | 17.85 |
| | Defoliated | 17.08 | 64.25 | 33.71 | 13.62 |
| 650 ppm | Control | 17.86 | 83.54 | 44.43 | 18.04 |
| | Defoliated | 19.12 | 63.60 | 36.26 | 11.68 |

# Appendix 3

Manuscript entitled:

# BASIN BIG SAGEBRUSH AND <u>MELANOPLUS</u> GRASSHOPPER RESPONSES TO ATMOSPHERIC CARBON DIOXIDE CONCENTRATION

# **Basin Big Sagebrush and _Melanoplus_ Grasshopper Responses to Atmospheric Carbon Dioxide Concentration**

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## 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 ul $l^{-1}$ and fed to Melanoplus grasshoppers. Total shrub biomass significantly increased as carbon dioxide levels increased, as did the mean 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, quaianolide 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, even when genotypic variation was controlled by vegetative propagation, allelochemical concentrations did not differ among carbon dioxide treatments. However, leaf nitrogen concentration declined significantly with elevated $CO_2$ and allelochemical contents per leaf increased significantly over the three $CO_2$ treatments. Thus, feeding on sagebrush under carbon dioxide enrichment will require consumption of increased quantities of allelochemicals per amount of nitrogen consumed. Grasshopper feeding did not differ significantly among the carbon dioxide treatments but varied widely within treatments in relation to the allelochemical differences among plants.

KEYWORDS: Carbon dioxide;; Artemisia tridentata; Sagebrush; Melanoplus; Grasshopper; Allelochemical; Nutritional quality

## INTRODUCTION

Atmospheric carbon dioxide concentration has been projected to increase from the ambient average of 345 ul $l^{-1}$ to 650 ul $l^{-1}$ or greater within the next 50-75 years (Strain 1987). Numerous studies using laboratory and field chambers have documented an overall trend of enhanced plant growth with increased $CO_2$ concentrations (Strain and Cure 1985). Investigations into the fate of "extra" carbon fixed in enriched atmospheres indicate additional allocation into structural and storage carbohydrates (Nafziger and Koller 1976; Cave et al. 1981). In addition, recent studies using several legume species have shown that plant leaves grown under carbon enriched conditions contain proportionally less nitrogen than ambient leaves (Lincoln et al. 1984 1986; Osbrink et al. 1988). Feeding trials on this plant material using three species of noctuids demonstrated as much as a 30% increase in consumption on the plants grown at the highest carbon dioxide concentrations (650 ul $l^{-1}$) relative to the ambient.

Leaf nitrogen is a limiting nutrient in many insect diets (Mattson 1980; Slansky and Rodriguez 1986) and that many insect herbivores respond to protein poor food sources by increased feeding or reduced growth (Scriber and Slansky 1981). Thus 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 scenario of increased 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 1988), mono- and sesquiterpenes (Langenheim and Hall 1986; Mihaliak et al. 1987) and sesquiterpene lactones (Pickman 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 compensatory consumption of leaf monoterpenes increased the insect's metabolic cost of allelochemical detoxification which stimulated further feeding. These data indeed suggest that herbivore feeding behavior predicted 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 (Astoraceae) plants were grown under 270 ul $l^{-1}$ (historical), 350 (ambient) and 650 ul $l^{-1}$ (projected future) carbon dioxide regimes and fed to tow 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 and they are suitable for experimental feeding trials.

## MATERIALS AND METHODS

Seed propagated plants: Sixty-eight sagebrush 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 ul $l^{-1}$ (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 ± 20 ul $l^{-1}$. The chambers were maintained at 70% relative humidity, 14 hour light period with 1050 umol $m^{-2}$ $s^{-1}$ 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. Three week old seedlings were established in the environmental chambers where they grew for nine weeks under the treatment conditions.

Clonal propagations: Twenty six sagebrush plants were propagated using stem cuttings from four genotypes using the methods of Alvarez-Cordero and McKell (1979). The plants were grown as above but in carbon dioxide regimes of 350 and 650 ul $l^{-1}$. When root initation was apparent, cuttings were put in the treatment conditions and allowed to grow for eight weeks. The plants were moved biweekly within chambers and exchanged between chambers, with the $COC_2$ concentration adjusted, in order to minimize potential chamber effects.

Leaf volatile analysis: Fresh-frozen leaf material was homogenized in pentane, centrifuged and the supernatant concentrated under nitrogen. Quantitative analysis was conducted using a temperature programmed gas chromatograph with a SP-1000 capillary column, flame ionization detector and a digital integrator. Tridecane was used as an internal standard. Identities of the mono- and sesquiterpene peaks were confirmed with GC-mass spectroscopy by comparison with authentic standards and literature spectra. Total volatile yield from each plant was determined from the sum of all peaks, with the following major compounds being identified: alpha-pinene, santolina triene, camphene, beta-pinene, beta-phellandrene, artemisia triene, arthole, 1, 8-cineole, santolina epoxide, camphor, beta-terpineol, linalool, bornyl acetate, caryophylene, Z-lyratyl acetate, alpha-santolina alcohol, alpha-terpineol, neryl acetate and geranyl acetate.

Sesquiterpene lactone and flavonoid analysis: Eight dried leaves were homogenized in 3 ml of chloroform and further blended with addition of three ml of methanol. The resulting slurry was centrifuged and dried *in vacuo*. The residue was suspended in 1 ml aqueous acetonitrile (1:1 v/v) and analyzed by HPLC with a UV detector (270 nm) and a digital integraator. Compounds were separated with a C-18 reverse phase column using an increasing gradient of acetonitrile with 1% aqueous acetic acid. Identification was confirmed by isolation from 20 g of dry sagebrush leaves using an HPLC with a semi-preparatory 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-chromatography 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 quaianolides: matricarin, deacetylmatricarin and deacetoxymatricarin, because of their relatively high concentration and strong absorption in the 250 - 270 nm range.

<u>Coumarin analysis</u>: The sesquiterpene lactone quantitative sample from each plant was also analyzed for coumarin content using reverse phase HPLC with florescence 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-chromatrography with authentic standards. A concentration response curve for each coumarin was determined from standards. Leaf and total plant measurements: Approximately 14 leaves were used for fresh weight/dry weight/area conversions. Three leaves, randomly selected from the dry control leaves were diced to a fine powder and assayed with a CHN analyzer. Statistical analysis: All analyses were performed using the Statistical Analysis System (SAS) for Personal Computers version 6.03 (SAS Institute Inc. Cary, NC). All data were tested for normality and transformed where appropriate.

## RESULTS

### Allelochemical Responses

Total volatile concentration among seed-propagated individuals exhibited a high degree of variability within each treatment (Table 1). For example, the range of variability within the the 350 ul $l^{-1}$ $CO_2$ treatment ranged from a minimum concentration of 10.1 mg/g dry leaf and a maximum concentration of 36.6 mg/g. Mean allelochemical concentration for each chemical class as well as significance values for carbon dioxide effect from each treatment are listed in Table 1. Guaianolide sesquiterpene lactone concentration of the seed propagates paralleled the variability found with leaf volatiles in each carbon dioxide treatment. THus, allelochemical concentrations did not differ significantly among the $CO_2$ treatments (Table 2).

Leaf phenolic constituents, including the flavonoid luteolin and the coumarins esculin and umbelliferone, also exhibited a high degree of variability in their concentration. Coumarins were the allelochemicals found in the lowest concentration.

The high degree of chemical variability found in the seed-propagated individuals demonstrated the need to control genetic influences if subtle carbon dioxide effects on allelochemicals were to be detected. Thus we analyzed the same volatile terpenes, sesquiterpene lactones and the coumarin umbelliferone from clonal sagebrush plants. 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. The use of clonal propagations reduced the standard deviations from the means of all the allelochemicals we tested (Figure 1). Even when genetic variability was controlled, an analysis of variance for carbon dioxide and plant genotype did not reveal significant effects for any class of compound.

### Plant and Leaf Responses

The leaf nitrogen concentration declined significantly as atmospheric $CO_2$ increased (Table 2), in agreement with previous observations (Lincoln et al. 1984, 1986; Lincoln and Couvet, 1989). Unexpectedly however, a concurrent decrease in elemental carbon content was also seen with $CO_2$ enrichment.

Both above and below ground plant biomass was significantly greater in treatments with increased $CO_2$ as were mean leaf weights and areas (Table 2). Root/shoot ratios and leaf water content did not change significantly.

Only in the 650 ul $l^{-1}$ carbon dioxide treatment did all the original 22 seed propagated plants survive and grow to sufficient size to provide leaf material for feeding trials and experimental analysis. Of the 22 plants started in the 350 ul $l^{-1}$ $CO_2$ regime, 20 survived (9% mortality) the 9 week growing period and 19 provided sufficient leaf material for trials and analysis. 17 of the 23 original plants survived (26% mortality) the growth period under the 270 ul $l^{-1}$ carbon dioxide regime. Of the surviving 17, only 14 plants were large enough to provide sufficient leaf material for feeding trials and analysis.

Grasshopper Feeding and Growth

Grasshopper consumption differed when fed leaves from the three $CO_2$ treatments (Table 1). 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 current ambient $CO_2$ treatment than on leaves from the other two treatments, although the $CO_2$ treatment effect for M. sanguinipes was significant at only the $P < 0.076$ level. These results are in agreements 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, 1986; Lincoln and Couvet 1989; Osbrink 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 $CO_2$ concentration.

Grasshopper growth was significantly influenced by the host plant $CO_2$ regime for M. differentialis, but not for M.

sanguinipes. However, the growth rates are very low for both species. This low growth rate was expected because the grasshoppers were starved for 18 hours before and after the feeding trials to ensure motivation to feed and accurate 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. Although relative growth rate is often closely related to relative consumption rate ($P<0.008$ for M. differentialis), the carbon dioxide effect remains significant ($P<0.05$) when differences in relative consumption are included in an analysis of covariance.

The carbon dioxide regime significantly influenced leaf nitrogen content, as has been observed in previous studies (Lincoln et al., 1984, 1986; Lincoln and Couvet, 1989). However, consumption by both species of grasshoppers was significantly influenced by the leaf allelochemical content (Figures 2 and 3), but not by leaf nitrogen content. On the other hand, the effect of the carbon dioxide treatments on sagebrush allelochemicals was not significant for any of the allelochemicals in the leaves (Table 2). However, consumption by the grasshoppers was very significantly influenced by the leaf content of volatile monoterpenes and sesquiterpene lactones, but not by flavonoids or coumarins. Both species of grasshopper responded in the same manner (Figures 2 and 3). When the leaf content of allelochemicals was high, grasshoppers consumed only small amounts of leaf tissue. When allelochemicals were low, some grasshoppers consumed large quantities, while other consumed little. Thus, the allelochemicals are inhibitory to feeding, but other factors may limit feeding when it is not precluded by allelochemicals.

Although $CO_2$ treatments had no direct effect on proportional allocation to allelochemicals, i.e. proportion of leaf weight, the differences in consumption among the $CO_2$ regimes may nevertheless be due to the differences in allelochemical content among the plants in the treatments. For example, although not significantly different, the allelochemical content of plants in the ambient $CO_2$ treatment was higher than those form plants in either of the other two $CO_2$ treatments. Indeed, the ambient $CO_2$ treatment has the highest allelochemical contents, both in respect to total allelochemicals and in respect to each allelochemical group, as well as sustaining the lowest consumption rates.

When the various leaf characteristics are combined in an analysis of covariance, none of the factors could account for the $CO_2$ effect on relative consumption of <u>Melanoplus differentialis</u>. On the other hand, leaf nitrogen concentration reduced the carbon dioxide effect on the growth of <u>M. differentialis</u> to non-significance. In other words, variation in leaf nitrogen concentration may account for the Carbon dioxide treatment effect on <u>M. differentialis</u> relative growth rate. The phytochemicals did not have any such effects, i.e. when combined in an analysis of covariance with carbon dioxide, they did not account for the same variation as carbon dioxide. Thus, in this experiment carbon dioxide effects appear to be separate from allelochemical differences.

The lack of effect of carbon dioxide enrichment on allelochemical production is in general agreement with the previous results on peppermint (Lincoln and Couvet, 1989). Although the allelochemical content per leaf dry weight did not vary in response to $CO_2$, leaf nitrogen content decreased and leaf specific weight increased significantly with increasing $CO_2$. Hence, an herbivore feeding on A. tridentata growing in elevated atmospheric carbon dioxide must consume a greater amount of leaf tissue to obtain the same amount of nitrogen (or protein) and in turn will encounter greater amounts of allelochemicals.

## DISCUSSION

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 also 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 herbivores.

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 enahnced by elevated atmospheric carbon during the brief periods of water availability that are characteristic of its native environment. In addition, the enhanced tolerance to water stress that often occurs in elevated $CO_2$ atmospheres may lead to enhanced growth under partially water limiting conditions (Tolley and Strain 1985).

The depressed growth of the 270 ul $l^{-1}$ 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 ul $l^{-1}$ 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 ul $l^{-1}$ conditions.

The concentrations of <u>Artemisia</u> allelochemicals did not vary significantly among carbon dioxide treatments for either seed or clonally propagated plants. 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 et al. 1981). Thus, our data suggest that the potential effects carbon dioxide enrichment may have on the leaf allelochemical concentration of individuals within other species may be obscured by the chemical variability of its members and shifts among genotypes represented in populations.

The volatile mono- and sesquiterpenes exhibited a highly significant correlation with variation in the sesquiterpene lactones over all treatments (Table 3). 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. Phenolic glycosides (e.g. esculin) usually occur within the cytosol or vacuole and aglycones (e.g. luteolin and umbeliferone) are usually associated with secretory structures or leaf surfaces (Wollenweber and Dietz 1981). 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 high volatile yield individuals in this population can be expected to have a broad spectrum of chemical defense.

The increase in total terpenoids per leaf was apparently due to the increase in leaf size under carbon enrichment. Although environmental conditions are known to affect allelochemical production (Gershenzon 1984), it has been hypothesized that metabolic pathways and sites of synthesis as well as their associated secretory or storage structures are under genetic control (Berenbaum et al. 1986; Coley 1986; Haslam 1986). Total increase in leaf size may increase the number of glandular trichomes per leaf. In Artemisia, the glandular trichomes develop from basal, head and stalk epidermal cells. Apparently, the contents and walls of the head cells decompose and the resulting space fills with a high proportion of terpenes (Kelsey and Shafizadeh 1980). Although allelochemical concentration exhibited no significant carbon dioxide effect among either the seed or the clonally propagated plants, mono- and sesquiterpene content on a per leaf basis did increase over the three treatments in the seed propagates (Figure 1).

The decline in leaf nitrogen resulting from plant growth in increased carbon dioxide atmospheres reduces the nutritional quality. Nitrogen is commonly limiting to growth and reproduction of many insects (Mattson 1980). Lincoln et al. (1984, 1986) and Osbrink et al.(1988) showed that insects feed at an increased rate on plants grown at above ambient levels of carbon dioxide. The offered leaves grown under $CO_2$ enrichment contained proportionally less nitrogen than ambient grown and the herbivores exhibited increased consumption rates to meet necessary metabolic levels of nitrogen.

Under future projected carbon dioxide regimes, as insect herbivores attempt to adjust their consumption rates to compensate for the reduced nutritional quality, plant chemical defense may play an increasingly important role. Because leaf allelochemical concentrations are apparently not affected by carbon fertilization per se, compensatory feeding strategies would require an insect herbivore to encounter a greater quantity of allelochemicals for each unit of nitrogen consumed. This potential increase in the ratio of leaf allelochemical to nitrogen in herbivore diets may have chronically deleterious effects on nutrient use efficiency. Insect metabolism may also be affected as more energy and nitrogen resources are utilized to detoxify or sequester the chemicals. Further, the greater allocation of resources to nitrogen rich mixed function oxidases and transferases to detoxify the chemicals and fatbody proteins to sequester them may place additional metabolic demands for an insect to increase its nitrogen intake.

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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.

| | Significance of $CO_2$ effect ($P <$ ) | 270 ul $l^{-1}$ | | 350 ul $l^{-1}$ | | 650 ul $l^{-1}$ | |
|---|---|---|---|---|---|---|---|
| | | mean | sd | mean | sd | mean | sd |
| Leaf nitrogen concentration (mg N $g^{-1}$ dry wt) | 0.001 | 37.0 | 3.3 | 31.6 | 5.5 | 29.5 | 6.2 |
| Leaf carbon content (mg C $g^{-1}$ dry wt) | 0.001 | 441 | 18 | 439 | 15 | 420 | 15 |
| Leaf water content (mg water $g^{-1}$ fr wt) | 0.255 | 763 | 32 | 733 | 15 | 750 | 59 |
| Plant biomass (g) | 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 |
| Leaf biomass (mg) | 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 |

Table 2. Effect of carbon dioxide treatments on leaf alleochemical concentration in seed propagated *Artemisia tridentata*. Significance values are for a one-way analysis of variance.

| | Significance of $CO_2$ effect ($P <$ ) | 270 ul $l^{-1}$ | | 350 ul $l^{-1}$ | | 650 ul $l^{-1}$ | |
|---|---|---|---|---|---|---|---|
| | | mean | sd | mean | sd | mean | sd |
| 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 3. Correlation matrix of seed propagated sagebrush allelochemicals. Significance probabilities: *** = $P < 0.0001$, ** = $P < 0.005$, * = $P < 0.05$.

| | **Volatiles** | **Guaianolides** | **Coumarins** |
|---|---|---|---|
| **Guaianolides** | 0.517<br>*** | | |
| **Coumarins** | 0.336<br>* | 0.031<br>ns | |
| **Luteolin** | 0.408<br>** | 0.674<br>*** | -0.048<br>ns |

Table 4. 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.

| | | VOLATILES | | SESQUITERPENE LACTONES | | COUMARIN | |
|---|---|---|---|---|---|---|---|
| Genotype | number of indiv. | 350 ul $l^{-1}$ | 650 ul $l^{-1}$ | 350 ul $l^{-1}$ | 650 ul $l^{-1}$ | 350 ul $l^{-1}$ | 650 ul $l^{-1}$ |
| A | 5 | 40.3 | 40.7 | 20.4 | 24.6 | 0.25 | 0.23 |
| B | 6 | 36.2 | 44.3 | <0.1 | <0.1 | 0.16 | 0.22 |
| C | 5 | 34.7 | 36.9 | 11.7 | 21.5 | 0.52 | 0.18 |
| D | 4 | 52.0 | 46.8 | <0.1 | <0.1 | 0.90 | 1.42 |
| E | 3 | 54.4 | 47.5 | 32.9 | 30.6 | 0.74 | 1.23 |

Table 5. Response of _Melanoplus_ grasshoppers to leaves of sagebrush plants grown under three carbon dioxide concentration regimes. Significance values are for one-way analysis of variance.

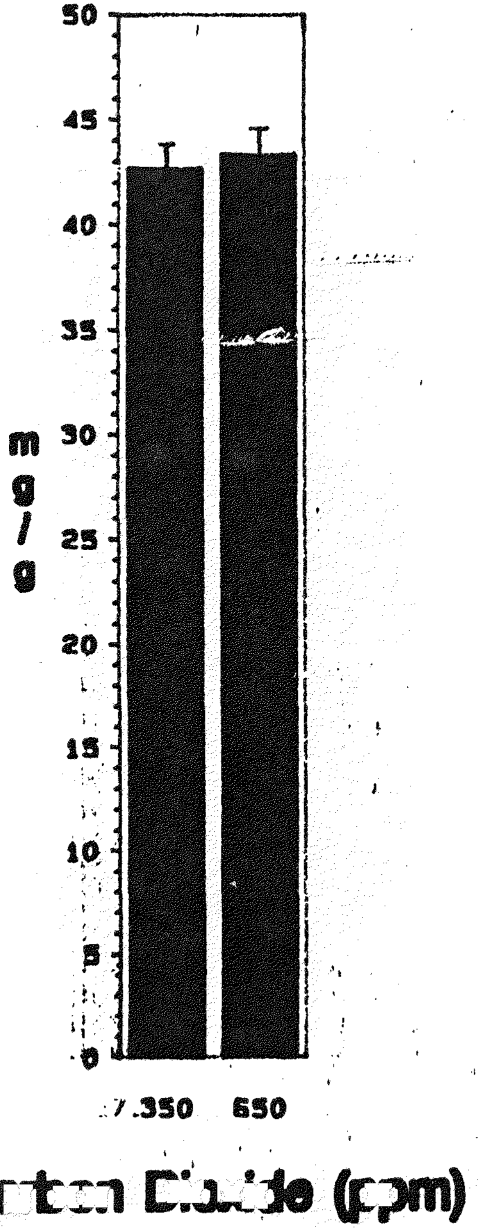
| | Significance of $CO_2$ effect (P < ) | 270 ul $l^{-1}$ | | 350 ul $l^{-1}$ | | 650 ul $l^{-1}$ | |
|---|---|---|---|---|---|---|---|
| | | mean | sd | mean | sd | mean | sd |
| _Melanoplus differentialis_ | | | | | | | |
| Relative consumption rate (mg eaten $g^{-1}$ dry wt $d^{-1}$) | 0.001 | 491 | 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_ | | | | | | | |
| 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 6. Significance of _Melanopus_ grasshopper responses to leaf characteristics of _Artemisia_ _tridentata_ (linear regression of all plants). * =P < 0.05, ** =P < 0.01, *** =P < 0.005, **** =P < 0.001. ns = not significant.

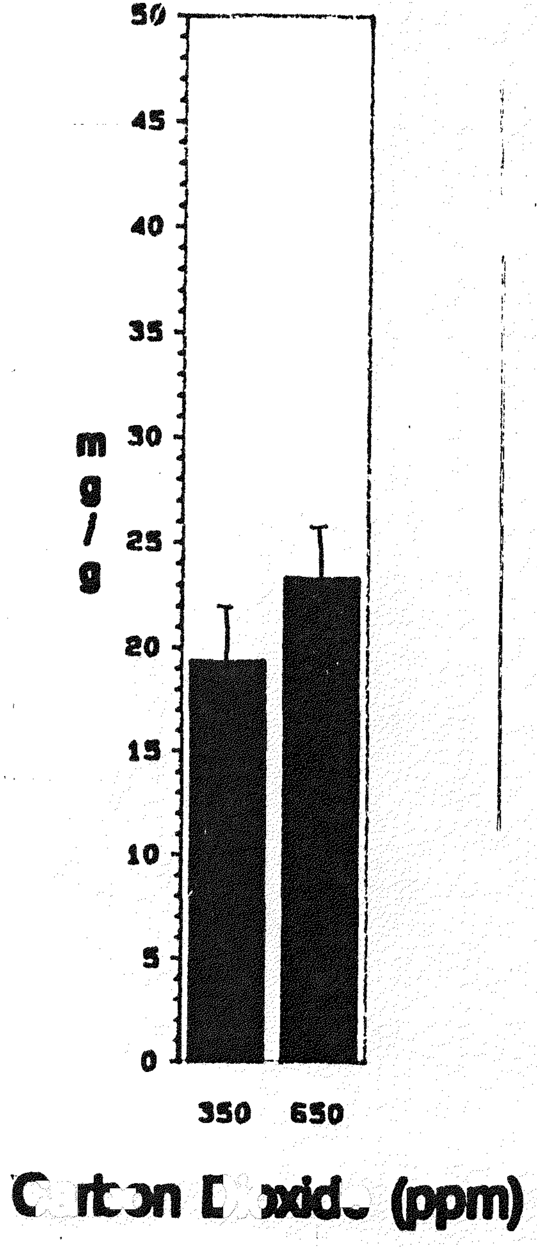
| | _Melanoplus differentialis_ | | _Melanoplus sanguinipes_ | |
|---|---|---|---|---|
| | relative consumption | relative growth | relative consumption | relative growth |
| Volatile concentration | **** | ns | ** | ns |
| Sesquiterpene lactone concentration | **** | ** | **** | * |
| Luteolin concentration | 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 |

Figure 1. Mean leaf content of seed-propagated sagebrush volatile terpenes and sesquiterpene lactones under three carbon dioxide regimes. Vertical bars indicate standard error of the mean. Significance of carbon dioxide treatment in a one-way analysis of variance is $P < 0.015$ for volatiles and $P < 0.025$ for sesquiterpene lactones.

**Volatile terpenes**



**Sesquiterpene lactones**



**Umbelliferone**

(coumarin)

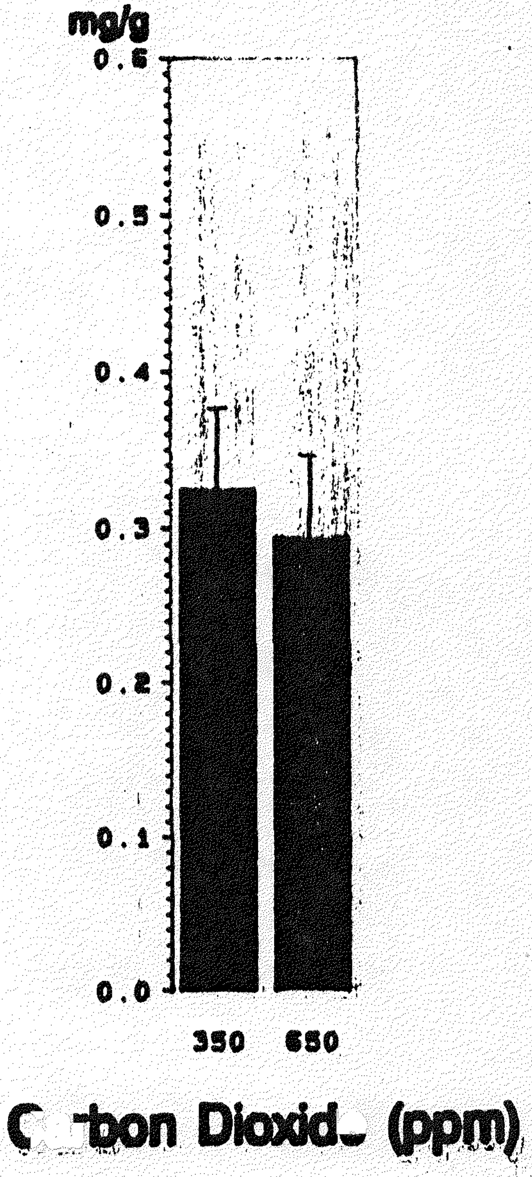


Figure 2. Mean leaf allelochemical concentrations of clonally-propagated sagebrush plants grown under two levels of carbon dioxide. A) Volatile terpenes. B) Sesquiterpenes. C) Coumarin. Vertical lines indicate standard error. Carbon dioxide effect not significant as a main effect nor in interactions with plant genotype in a two way analysis of variance.

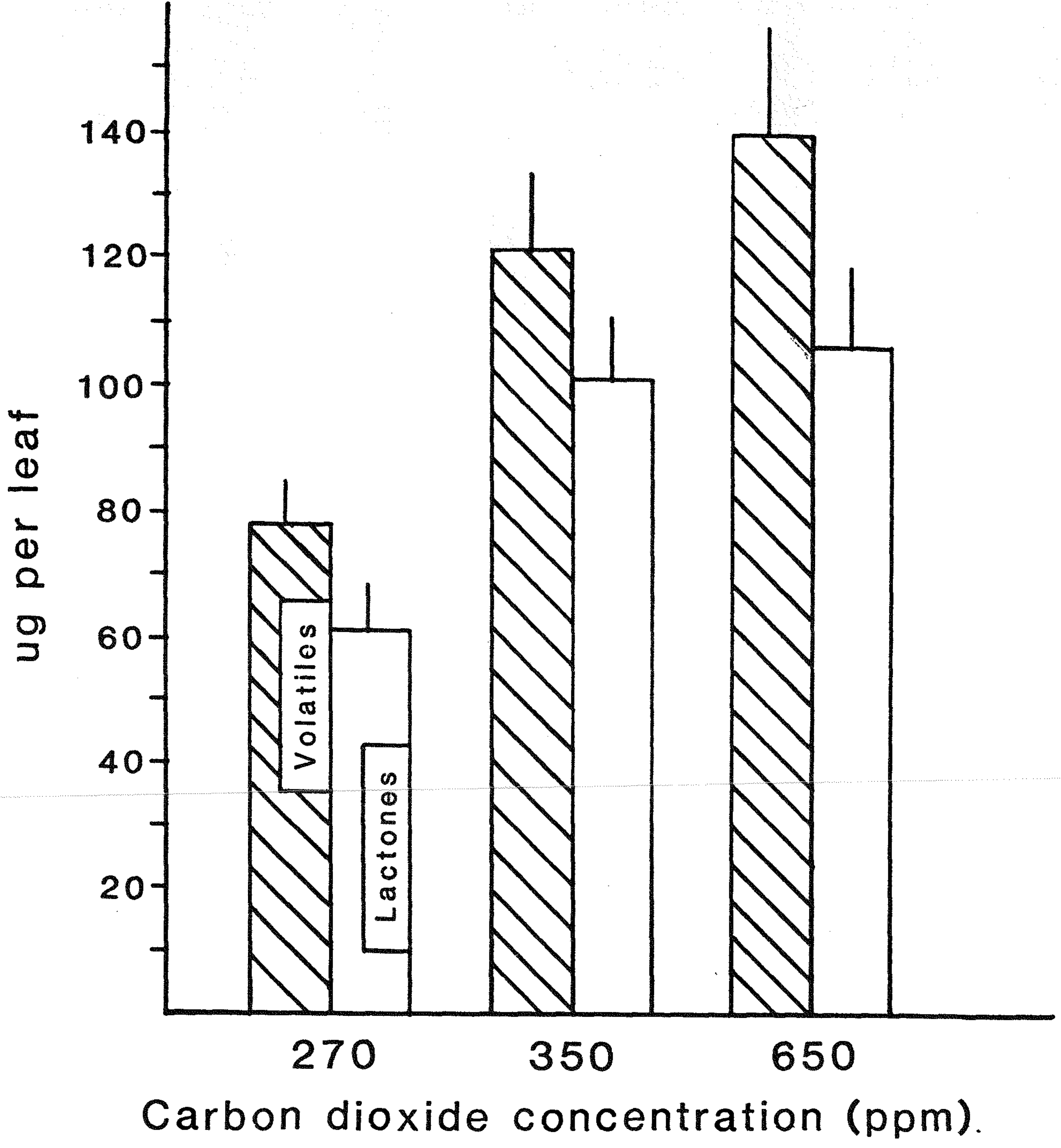
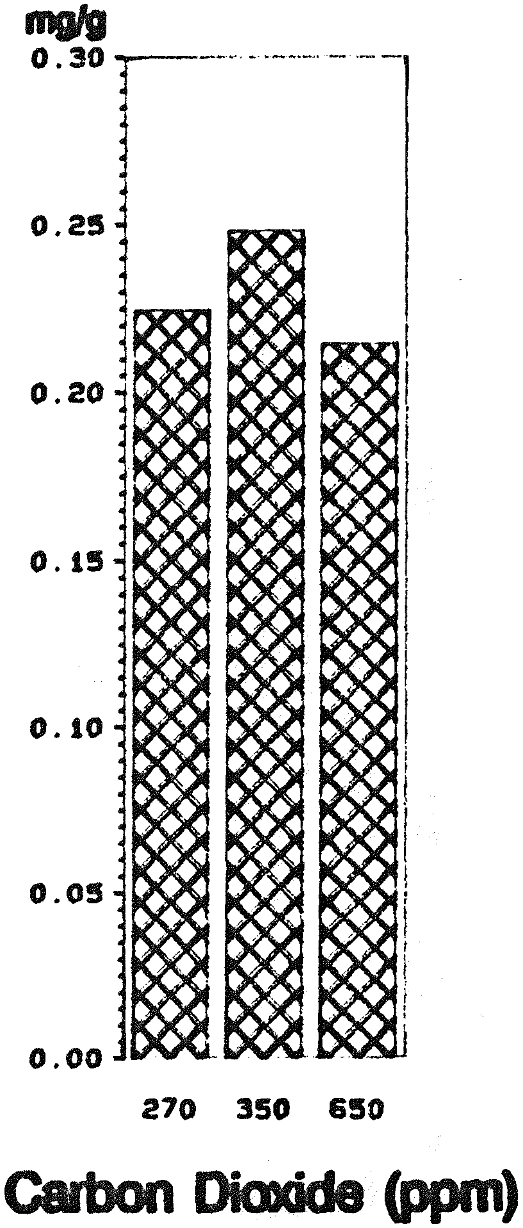
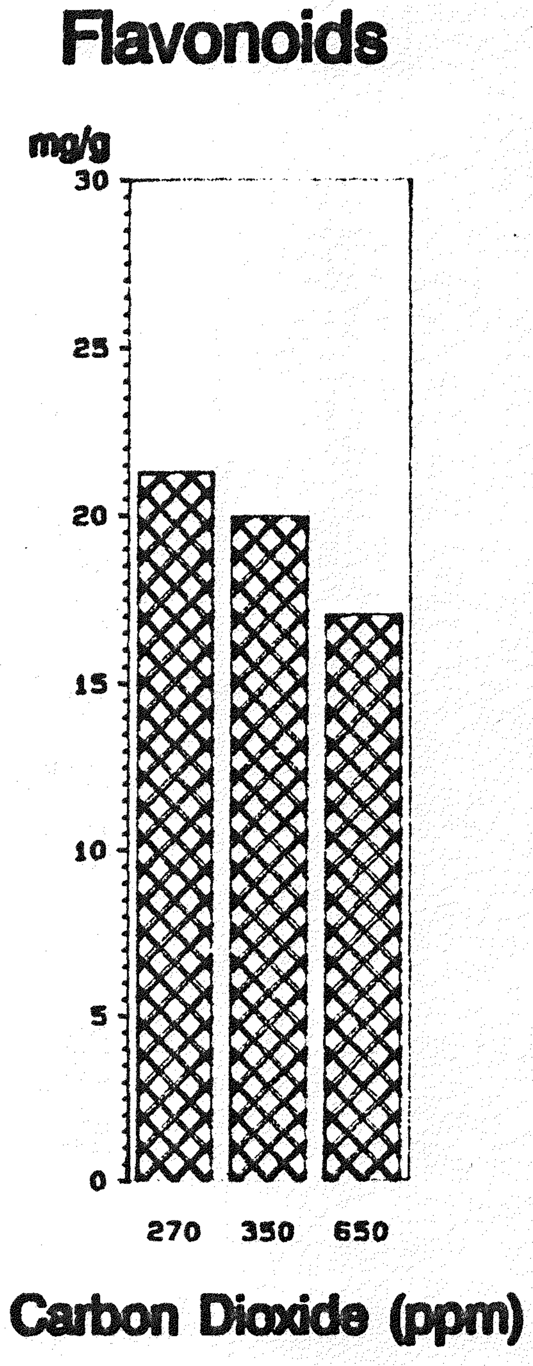

ug per leaf
140
120
100
80
60
40
20
Volatiles
Lactones
270
350
650
Carbon dioxide concentration (ppm).

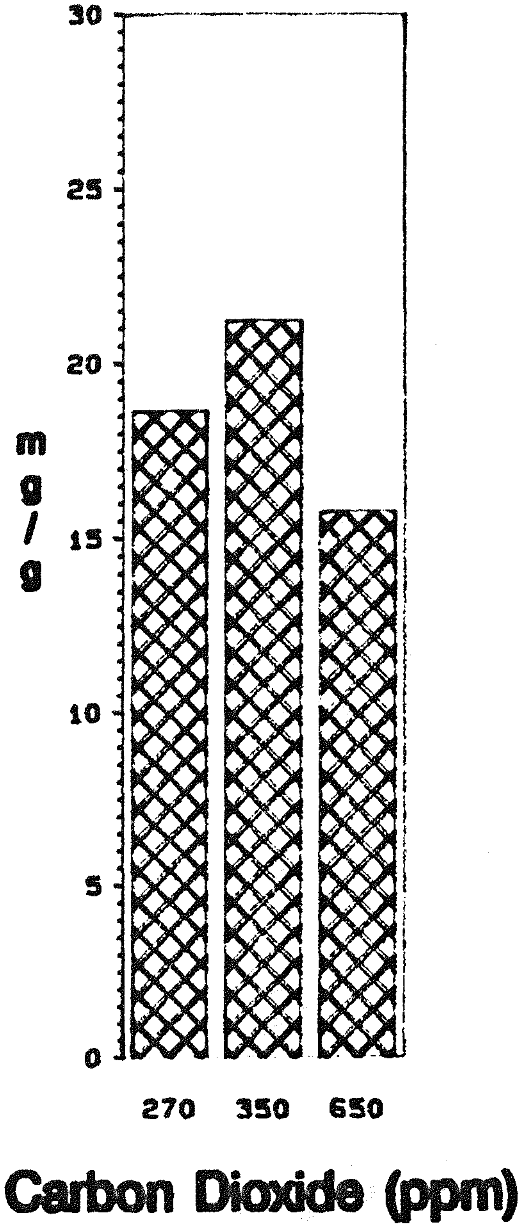
Figure 3. Mean leaf allelochemical concentrations of seed-propagated sagebrush plants grown under three levels of carbon dioxide concentration.

# Coumarins




Flavonoids
mg/g
30
25
20
15
10
5
0
270
350
650
Carbon Dioxide (ppm)

# Sesquiterpene lactones



# Volatile terpenes