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Elevated Atmospheric Carbon Dioxide Alters the Effects of Allelochemicals Produced by Tall Fescue on Alfalfa Seedlings

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ABSTRACT

Aqueous extracts of tall fescue *(Festuca arundinacea* Schreber) leaf tissues were applied to alfalfa (*Medicago sativa* L.) seedlings grown under ambient or double ambient atmospheric CO_2 (approximately 700 μL L^{-1}). Exposure to elevated CO_2 for 2 weeks stimulated the growth of alfalfa seedlings. More importantly, however, there was a significant interaction between the extract concentration and the CO_2 level on plant height and root dry weight, indicating that extract concentrations which were stimulatory under ambient conditions, had little effect or became slightly toxic at the higher CO_2 level. This result is important given the currently increasing level of CO_2 and the unknown effect it will have on plants exposed to allelochemicals. Our data demonstrated that elevated levels of atmospheric CO_2 enhanced the phytotoxicity of tall fescue in alfalfa.

INTRODUCTION

Several studies have reported on the altered effect of allelochemicals (Toai and Linscott, 1979; Stowe and Osborn, 1980) and allelochemical production (Tang et al., 1995; Sterling et al., 1987) by environmental stress. Normally, the amount or toxicity of allelochemicals are increased in the producing plant, and the receiving species becomes more intolerant with stress. Despite the demonstrated effects of environmental stress on allelopathy, little work has been done to study the effect of elevated atmospheric CO_2.

Since 1750, atmospheric CO_2 concentration has risen from 280 μL L^{-1} to 345 μL L^{-1} (Neftel et al., 1985). As the trend continues, the importance of determining the consequences to plant growth increases. Several authors have found varying effects of ele-

vated CO_2 on the production of secondary metabolites (Fajer et al., 1992; Stuhlfauth et al., 1987). However, no predictions regarding the ecological role of allelochemicals can be made until the effect on receiving species is determined.

The allelopathic potential of the forage grass tall fescue has been previously demonstrated (Chung and Miller, 1995; Smith and Martin, 1994; Peters and Mohammed Zam, 1981). Identification of inhibitory substances by gas-liquid chromatography of the active anion fraction of aqueous extracts detected eight organic acids and four unknowns. Of these, lactic and succinic acid were the most inhibitory to birdsfoot trefoil (*Lotus corniculatus* L.) seedlings (Luu et al., 1989). Legumes, such as alfalfa, are commonly used as target species because of the difficulty in establishing legumes in tall fescue pastures (Smith and Martin, 1994). The objective of this study was to ascertain the effect of elevated CO_2 on alfalfa, a species known to be sensitive to allelochemicals produced by tall fescue (Smith and Martin, 1994; Chung and Miller, 1995).

MATERIALS AND METHODS

We obtained aqueous extracts from leaf tissue clipped from mature tall fescue ('Kentucky 31') plants which had been grown in a greenhouse. These plants were all infected by the endophyte *Neotyphoium coenophialum* (Morgan-Jones & W. Gams) Glenn, Bacon & Hanlin. The clippings were freeze-dried and ground in a Wiley mill with a 40-mesh screen. We used three extract concentrations: 5 g/L, 3 g/L, and 1 g/L dried and ground tall fescue leaves in distilled water. Concentrations were based on an estimation by Smith and Martin (1994) that aqueous extracts from tall fescue leaf tissue of 4.4 (±0.3) g dry wt./L would result in 50% inhibition of the growth of germinating alfalfa seed. Ten, 6, or 2 grams of dried material were placed in 200 mL of distilled water, and allowed to stand for 48 to 72 hours in the laboratory at room temperature. We then filtered the extracts with Whatman no. 2 paper and diluted them to 2 L. The extract was kept up to 6 days in the laboratory and the procedure was repeated as more extract was needed (usually every four days).

Two to three seeds of alfalfa (*M. sativa* 'WL-322 HQ') were planted in each of eighty 10.2 cm diameter pots filled with a soilless growth media consisting of two parts peat moss, one part vermiculite, and one part perlite by volume. After 6 days, we thinned the alfalfa seedlings to the largest plant per pot.

Twenty open-topped CO_2 chambers were constructed according to the design of Ashenden et al. (1992) and placed in a field at the Touch of Nature Environmental Center, Carbondale, IL. The experiment took place in July 1996. Ambient air was pumped into ten of the chambers, and the other ten chambers were maintained at 700 μL L^{-1}. We placed four alfalfa plants in each chamber; a control treated with distilled water and one seedling each treated with one of the three extracts (1 g/L, 3g/L, and 5 g/L). Seedlings were treated to saturation with extract/water every other day and all plants were fertilized on alternate days with half strength 20-10-20 Peters® professional general purpose fertilizer (Scotts-Sierra Horticultural Products Company, Marysville, OH). Alfalfa plants received supplemental watering from rain, or twice daily as needed to avoid water stress. After 1 week, we measured shoot height of the plants in the chambers and re-randomized pot placement. After 2 weeks, we determined shoot height and leaf number in the cham-

bers and removed the plants. We rinsed the roots and measured their length. Plants were dried at 57° C, cut into root and shoot, and weighed.

Height data were analyzed with a split-plot design using SAS (SAS Institute, Cary, NC). CO_2 concentration and block were the whole plot factors and extract concentration and time were the subplot factors. The twenty chambers were arranged so that one fan ventilated four chambers: two ambient and two elevated. Within a block, each ambient chamber was paired with an elevated chamber. This design gave us five blocks of four chambers each. Leaf number, root length, and dry weight data were analyzed in SAS using separate non-parametric two-way ANOVAs (treatments were extract concentration and CO_2 level) on ranks. Plants were subject to insect herbivory while in the chambers. The data for six plants with severe herbivore damage were not used in the analysis. Eight plants included in the data set had visible leaf damage. There was no apparent pattern of damage either between chambers or among extract concentrations.

RESULTS

There was a significant interaction between extract concentration and atmospheric CO_2 level on the height of the alfalfa seedlings (DF=3/48, F=3.09, P=0.04) (Figure 1). Under ambient atmospheric CO_2, aqueous tall fescue extracts stimulated growth of alfalfa seedlings. However, under elevated CO_2 there was no apparent affect on the height of alfalfa stems at the concentrations studied. As expected, time had the greatest influence on height (DF=1/18, F=947.82, P=0.0001) but did not interact with either CO_2 level or extract concentration. Remaining treatments and interactions were not significant (Table 1).

Other measurements on the alfalfa seedlings taken in addition to height at the end of the experiment demonstrated differential sensitivity towards the treatments (Table 2). Root biomass displayed an interaction effect similar to, but stronger than, that of height (DF=3/48, F=3.52, P=.02) (Figure 2). Under ambient CO_2 increasing extract concentration stimulated root growth. However, root biomass was greater under elevated CO_2 and not affected by extract concentration except at the highest concentration at which root biomass was decreased. Root length was the only measurement to exhibit a significant effect of extract concentration independent of CO_2 level (DF=3, F=3.00, P=.04; data not shown). Leaf number and shoot biomass were significantly affected by CO_2 level, but not extract concentration, with larger values under elevated CO_2 (Table 2).

DISCUSSION

The allelochemical effects of tall fescue extract on alfalfa seedlings reported here are not as dramatic as those observed by Smith and Martin (1994). However this variation could be attributed to the developmental stage of seedlings at the time of extract application (germinating seeds in their study vs. three week seedlings in ours) or varying environmental conditions (laboratory environment in their study vs. field conditions in our study). Also, allelopathic potential has been shown to vary between tall fescue genotypes (Peters and Mohammed Zam, 1981). Smith and Martin (1994) did not report which genotype or cultivar of tall fescue they used and it is not known whether their plants were endophyte infected as were ours. Differences have been shown among cultivars of cotton

and soybeans in their ability to cope with allelochemicals (Hicks et al., 1989; Herrin et al., 1986), but alfalfa has not been tested for a similar response. Finally, the endophytic fungus of tall fescue which was included in this study, may produce alkaloids or other chemicals which could alter the effect of the normally occurring allelochemicals produced by non-endophyte infected tall fescue.

The nature of the interaction between extract concentration and CO_2 level (Figure 1) was unexpected considering CO_2 often stimulates many of the mechanisms considered as possible means of inhibition by allelochemicals. Elevated CO_2 often increases photosynthesis of C3 plants, at least temporarily (Körner, 1993). Suppressed photosynthesis may be a mechanism of allelochemical growth inhibition (Bhomik and Doll, 1984). Bhomik and Doll (1984) demonstrated decreased nutrient uptake/accumulation in some species exposed to allelochemicals. Conversely, increased CO_2 aids in nutrient use efficiency (Luxmoore et al., 1986; Norby et al., 1986). In general, dark respiration increases with increased CO_2 levels, and photorespiration decreases (Körner, 1993). Reaction of respiration to allelochemicals varies between studies, but most often respiration is stimulated, especially in mature tissue as opposed to germinating seeds and juvenile tissue (Weaver and Klarich, 1977; McCahon et al., 1973).

The significant change in root length from extract treatments is consistent with the results of Chung and Miller (1995) who found radicle length in alfalfa to be more sensitive to allelochemicals than hypocotyl length. The significant effects of elevated CO_2 on leaf number, shoot weight, and root weight are consistent with the stimulatory effects of increasing carbon dioxide on growth demonstrated by Macdowall (1983) with alfalfa.

This study introduces a new and important effect of elevated atmospheric CO_2 levels. If rising atmospheric CO_2 levels affect the response of a plant to allelochemicals then significant consequences could be seen in vegetational patterning and agro-ecosystems. Just as elevated CO_2 may affect evolution in a community, although indirectly (Bazzaz et al., 1995); so have allelochemicals been shown to induce genetic diversity (Lawrence et al., 1991) thereby making predictions regarding the long term effects of a CO_2 and allelochemical interaction in nature difficult and uncertain.

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Table 1. Split-plot analysis of height. The whole plot factors are identified in parentheses. F-values with * are significant at $\alpha \leq 0.05$, ** are significant at $\alpha \leq 0.01$. The interaction between CO_2 and extract concentration is depicted in Figure 1.

Source	DF	MS	F Value
Block	4	11.86	
CO_2	1	49.70	3.83
Chamber (CO_2, Block)	14	12.97	
Extract	3	4.04	0.38
CO_2 x Extract	3	32.61	*3.09
Chamber (CO_2, Block) Extract	48	10.56	
Time	1	5043.21	**947.82
Time x CO_2	1	19.08	3.59
Chamber (CO_2, Block) Time	18	5.32	
Extract x Time	3	0.80	0.15
Extract x Time x CO_2	3	8.12	1.56
Chamber (CO2, Block) Extract x Time	48	5.20	

Table 2. Non-parametric two-way ANOVAs. The whole plot factors are identified in parentheses. F-Values with * are significant at $\alpha \leq 0.05$, ** are significant at $\alpha \leq 0.01$.

		Leaf Number		Root Length		Shoot Biomass		Root Biomass	
Source	DF	MS	F Value	MS	F Value	MS	F Value	MS	F Value
Block	4	891.82		507.73		746.47		1251.96	
CO_2	1	7198.49	**12.95	102.42	0.23	7174.89	**16.00	10110.95	**24.65
Chamber(CO_2, Block)	14	555.97		451.09		448.56		410.23	
Extract	3	45.93	0.15	1341.01	*3.00	120.50	0.38	118.03	0.56
Extract x CO_2	3	284.91	0.93	361.01	0.81	408.44	1.28	738.58	*3.52
Chamber(CO_2, Block) Extract	48	306.63		447.38		319.73		209.96	

Figure 1. The interaction of atmospheric CO_2 levels and concentration of aqueous extracts from tall fescue leaf tissue upon height (mean ± S.E.) of alfalfa seedlings (P=0.036).

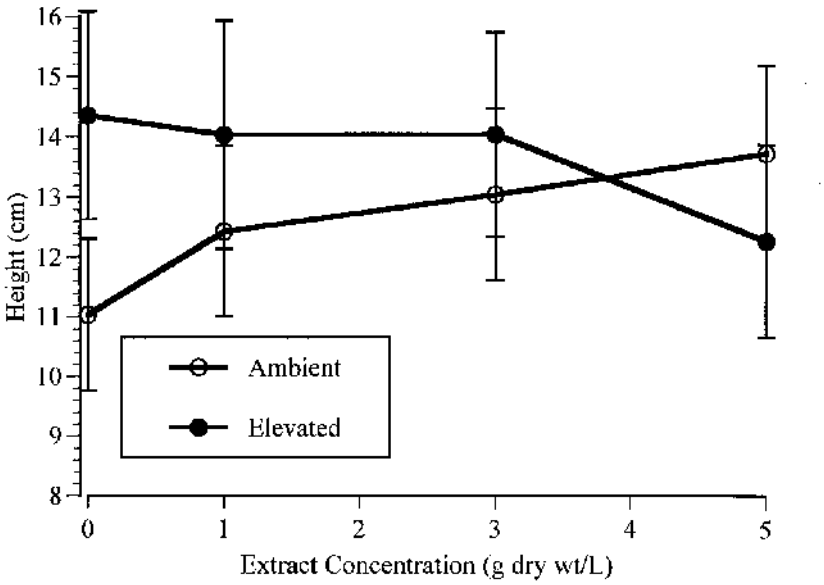

Figure 2. The interaction of atmospheric CO_2 levels and concentration of aqueous extracts from tall fescue leaf tissue upon root biomass (mean ± 1 S.E.) of alfalfa seedlings (P=0.022).