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Effects of CO_2 enrichment, nutrient addition, and fungal endophyte-infection on the growth of two grasses

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Summary. Increasing atmospheric carbon dioxide (CO_2) concentration is expected to increase plant productivity and alter plant/plant interactions, but little is known about its effects on symbiotic interactions with microorganisms. Interactions between perennial ryegrass, *Lolium perenne* (a C3 plant), and purpletop grass, *Tridens flavus* (a C4 plant), and their clavicipitaceous fungal endophytes (*Acremonium lolii* and *Balansia epichloe*, respectively) were investigated by growing the grasses under 350 and 650 $\mu l\, l^{-1}$ CO_2 at two nutrient levels. Infected and uninfected perennial ryegrass responded with increased growth to both CO_2 enrichment and nutrient addition. Biomass and leaf area of infected and uninfected plants responded similarly to CO_2 enrichment. When growth analysis parameters were calculated, there were significant increases in relative growth rate and net assimilation rate of infected plants compared to uninfected plants, although the differences remained constant across CO_2 and nutrient treatments. Growth of purpletop grass did not increase with CO_2 enrichment or nutrient addition and there were no significant differences between infected and uninfected plants. CO_2 enrichment did not alter the interactions between these two host grasses and their endophytic-fungal symbionts.

Key words: Grasses – Fungal infection – Growth – CO_2 – Nutrients

The concentration of atmospheric carbon dioxide (CO_2) (currently 350 $\mu l\ l^{-1}$) has been increasing since late in the 19th century and is predicted to reach twice the pre-industrial concentration before the end of the next century (Trabalka et al. 1985). This increase may affect terrestrial ecosystems indirectly, through global climate changes (Manabe and Wetherland 1975; Manabe and Stouffer 1979), and directly, through effects on plant growth and plant interactions with other organisms (Strain and Cure 1986).

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Symbiotic interactions with microorganisms can play an important role in the growth, establishment and competition of plants. The presence of mycorrhizal fungi can be critical in the uptake of nutrients that would otherwise not be available to plants (Melin 1953; Allen et al. 1981; Bajwa and Read 1986), and they can have important effects on the balance of plant competitive interactions (Hall 1978; Grime et al. 1987; Allen and Allen 1990). A large number of grasses, including the cultivated species *Lolium perenne* (perennial ryegrass, C3 photosynthesis) and the wild grass *Tridens flavus* (purpletop grass, C4 photosynthesis) are infected by systemic clavicipitaceous fungal endophytes (Clay 1988). Endophyte-infected grasses often exhibit increased survival, growth, and resistance to herbivory compared to uninfected conspecifics (Clay 1984; Cheplick and Clay 1988). Differences in the vigor and growth of infected versus uninfected plants can affect competitive abilities, and thus persistence and dominance in communities (Read and Camp 1986; Kelley and Clay 1987; Clay 1990; Marks et al. unpublished work).

While several studies have examined the effects of CO_2 enrichment on plant/insect interactions (Lincoln et al. 1986; Butler et al. 1986; Osbrink et al. 1987; Lincoln and Couvet 1989; Fajer et al. 1989), except for plant/mycorrhizal fungi interactions (Telson et al. 1980; Norby et al. 1986; O'Neill et al. 1987b), there have been no studies examining the effects of increased atmospheric CO_2 concentration on plant-fungal interactions (e.g. pathogens, endophytes). Both carbohydrate and nutrient availability appear to have important effects on the intensity and direction of the interaction between grass hosts and their endophytic fungal symbionts. When infected and uninfected *Festuca arundinacea* and *Lolium perenne* plants were grown under reduced nutrient levels, the stimulatory effect of the fungus on plant growth was reduced or negated (Cheplick et al. 1989). This same effect was seen when these species were grown under reduced light (Marks, unpublished data).

The purpose of this paper is to describe the results of experiments examining the effect of elevated CO_2 and nutrient addition on symbiotic interactions between two

grasses and their fungal endophytes. In *Lolium perenne*, the symbiotic interaction is often mutualistic (Hardy et al. 1985; Clay 1987b); plant reproduction, growth, survival and herbivore resistance can be enhanced by infection. In *Tridens flavus*, the interaction appears to be more pathogenic as infected plants rarely, if ever, flower and set seed. The effect of the fungal endophyte on growth and survival has not been previously examined although there is evidence of enhanced herbivore resistance (Cheplick and Clay 1988). Changes in CO_2 or nutrient levels that influence the grass-endophyte symbiosis could influence not only plant growth but a range of community interactions such as interspecific competition and herbivory.

Materials and methods

Experimental material

Perennial ryegrass (*Lolium perenne* L., C3 plant) is infected by the imperfect fungus *Acremonium lolii* Latch, Christensen and Samuels. This systemic endophyte produces intercellular hyphae in host leaves and stems and is transmitted to the next generation when it grows into the ovules and seeds of infected plants (Clay 1988). It is believed to be derived from *Epichloe typhina* (Pers.) Tul. in the tribe Balansieae, family Clavicipitaceae (Ascomycetes) (Bacon et al. 1977; Siegel et al. 1987).

Seeds of endophyte-infected perennial ryegrass cv. Repell, were obtained from a commerical seed company (Loft's Seed Co., Bound Brook, NJ, USA). In September, 1988, endophyte-free seedlings were created by placing seeds in 60 C water for 15 min and then planting in sand saturated with Benomyl fungicide. For endophyte-infected seedlings, seeds were planted directly in sand saturated with water (Williams et al. 1984). There was no effect of the hot water treatment on growth of perennial ryegrass seedlings, cv. Yorktown, which is endophyte-free (Marks et al., submitted). Treated plants and controls were allowed to grow in the Indiana University greenhouses until December, 1988, when tillers were collected, root washed and weighed before transport to the Duke University Phytotron in North Carolina.

Purpletop grass (*Tridens flavus* (L.) Mitch., C4 plant) is often infected by *Balansia epichloe* (Weese) Diehl (Diehl 1950). This fungus is also systemic, endophytic and intercellular within leaves and stems. However, it produces fruiting structures (stromata) which are borne on adaxial leaf surfaces coincident with flowering of healthy plants in the same population. Infected plants rarely, if ever, produce inflorescence. The mode of infection of new hosts is not known but may occur when ascospores and/or conidia infect seeds through the stigma or plants through wounds (Diehl 1950; Western and Cavett 1959).

In September, 1988, an average of 12 infected and 12 uninfected plants of *Tridens flavus* were collected from each of three field sites in Monroe Co., Indiana for a total of 36 infected and 36 uninfected genotypes. These were broken into tillers, planted in the Indiana University greenhouses, and allowed to propagate. In December, 1988, new tillers were collected, root washed and weighed and then transported to the Duke University Phytotron, Durham, NC, USA. All tillers of both perennial ryegrass and purpletop grass were then planted in pots with a volume of 500 cm^3 in a standard inert Phytotron substrate mixture (Wray and Strain 1986).

Experimental setup

The experiments were conducted in four replicated, controlled-environment chambers in the Duke University Phytotron. Temperatures were 26/25° C day and 20/18° C night with a 14 hour photoperiod and a vapour pressure deficit of 1.0 kPa during the day. The photosynthetic photon flux density (PPFD) at the pot surface was 350 ± 15 μmol m^{-2} sec^{-1}. Atmospheric CO_2 concentrations in two of the chambers were maintained at 350 ± 15 μl l^{-1} CO_2, and in the other two at 650 ± 20 μl l^{-1} CO_2 by a computer-controlled injection system (Hellmers and Giles 1979). Plants were watered daily with either a 1/8 strength or 1/2 strength modified Hoagland's nutrient solution (Downs and Hellmers 1975). Thus, for each species there were two infection types (infected and uninfected), two nutrient levels (1/8 and 1/2 strength nutrient) and two CO_2 levels (350 and 650 μl l^{-1} CO_2) for a total of eight different combinations. Both species were in each chamber in all combinations of nutrient level and infection type.

In perennial ryegrass, 15 pots per treatment were harvested after 5 and 10 weeks of growth in the chambers. Purple-top grass had relatively low initial survival after planting in the Phytotron so only one harvest was made after 10 weeks in the chambers. Replicates varied from 9 to 14 pots per treatment. Leaf area for both species was measured with a LiCor model 3100 leaf area meter. Above- and below-ground biomass was dried to constant weight at 65° C.

Statistical analyses

Mathematical growth analysis (Kvet et al. 1971, Hunt 1978) was used with perennial ryegrass to evaluate the effects of CO_2 enrichment, nutrient level and infection status on relative growth rate (RGR), dry matter production (DMP) and its components, net assimilation rate (NAR) and leaf area duration (LAD). Dry matter production is approximately equal to the product of net assimilation rate and leaf area duration for a defined time interval, where:

$NAR = [(W2/A2\text{-}W1/A1)]\ [\alpha/(\alpha-1)]/\Delta T$ in g cm^{-2} day^{-1},
$\alpha = \ln (W2/W1)/\ln (A2/A1)$,
$LAD = (A2\text{-}A1)/[\ln (A2/A1)]\ \Delta T$ in cm^2 days, and
$RGR = \ln (W2/W1)/\Delta T$ in g g^{-1} day^{-1}.

W1 and W2 are total plant dry weight and A1 and A2 are total leaf area at the beginning and end of the interval, respectively. This relationship can be used to assess the relative importance of physiological changes in rates of dry weight production per unit leaf area (NAR) and morphological changes in amounts of leaf area present (LAD) in accounting for the dry weight produced. Relative growth rate is defined as the rate of increase in biomass per unit biomass present, and is a measure of the efficiency of dry matter production.

Percentage response to CO_2 enrichment was equal to mean measurement at high CO_2 less mean measurement at low CO_2 divided by mean measurement at low CO_2. Specific leaf area was calculated on a per plant basis by dividing plant leaf area by total leaf dry weight (shoot dry weight).

Where data were not normally distributed, normally distributed log-transformed data were used in data analyses. The responses of host plants to CO_2 enrichment, nutrient level, and infection status were tested with a nested, split-plot analysis of covariance (Kirk 1982) where chamber was nested within CO_2 concentration and the covariate was the initial fresh weight of the tiller at planting (SAS Insitute 1985). When the covariate was significant, adjusted means were calculated and used in data presentation.

Results

Perennial ryegrass

In general, CO_2 and nutrient main effects, but not endophyte infection, were significant for perennial ryegrass

Table 1. P values for main effects from analysis of co-variance for perennial ryegrass and purple-top grass for leaf area (LA), shoot dry weight (SDW), root dry weight (RDW), total dry weight (TDW), specific leaf area (SLA), root/shoot ratio (RS). See results for interactions

	LA	SDW	RDW	TDW	SLA	RS
perennial ryegrass, harvest 1						
CO_2 concentration	0.0034	0.0408	0.0033	0.0246	0.1285	0.6622
Nutrient addition	0.0004	0.0021	0.1736	0.0226	0.0222	0.1273
Infection	0.8749	0.9665	0.1928	0.2660	0.6587	0.0461
Covariate	0.0001	0.0001	0.0243	0.0011	0.0006	0.2744
perennial ryegrass, harvest 2						
CO_2 concentration	0.1470	0.1239	0.0065	0.0435	0.6326	0.7987
Nutrient addition	0.0160	0.0182	0.1483	0.0505	0.0109	0.0763
Infection	0.6376	0.7250	0.7591	0.8429	0.4731	0.9231
Covariate	0.1361	0.0526	0.9532	0.3091	0.6859	0.0312
purple-top grass						
CO_2 concentration	0.7390	0.6258	0.7380	0.9629	0.2684	0.0252
Nutrient addition	0.9287	0.8337	0.2885	0.5589	0.6911	0.0367
Infection	0.3131	0.9133	0.2400	0.5267	0.0571	0.0071
Covariate	0.1801	0.0593	0.2749	0.0956	0.0501	0.1329

(Table 1). The only significant interaction in perennial ryegrass was at the first harvest, where there was a significant $CO_2 \times$ nutrient interaction for leaf area. The only other interaction with a P value less than 0.20 ($P = 0.176$) was the $CO_2 \times$ nutrient interaction for specific leaf area.

There were significant effects of both CO_2 and nutrient level on leaf area at harvest 1. Plants with either CO_2 enrichment or high nutrient levels had more leaf area than plants at low CO_2 and low nutrient. Plants with both CO_2 enrichment and high nutrient levels had the greatest leaf area (Fig. 1A). Nutrient addition had a greater effect than CO_2 enrichment on plant growth. Leaf area was approximately 120% greater at high nutrient as compared to low nutrient, compared to a 35% increase with CO_2 enrichment.

There were also significant CO_2 and nutrient effects on dry weight of ryegrass (Table 1). CO_2 enrichment and nutrient addition had equivalent effects with similar means at low CO_2, high nutrient and high CO_2, low nutrient (Fig. 1C, E). Plants with both high CO_2 and high nutrient were the heaviest. Means of shoot dry weight are very similar for infected and uninfected plants under all treatments. Means of root dry weight showed more variability between infected and uninfected plants across all treatments.

Main effects were generally similar at harvest 2 as at harvest 1 for perennial ryegrass (Table 1). Only one interaction ($CO_2 \times$ nutrient $\times$ infection for shoot dry weight) had a P value less than 0.20 ($P = 0.161$). Nutrient addition had a greater effect on leaf area than CO_2 enrichment, with the greatest leaf area with both increases in atmospheric CO_2 concentration and nutrient level (Fig. 1B). There was no significant effect of infection, although infected plants had 29% more leaf area than uninfected plants at high CO_2, high nutrient.

CO_2 enrichment significantly affected root and total dry weight, and nutrient addition significantly affected

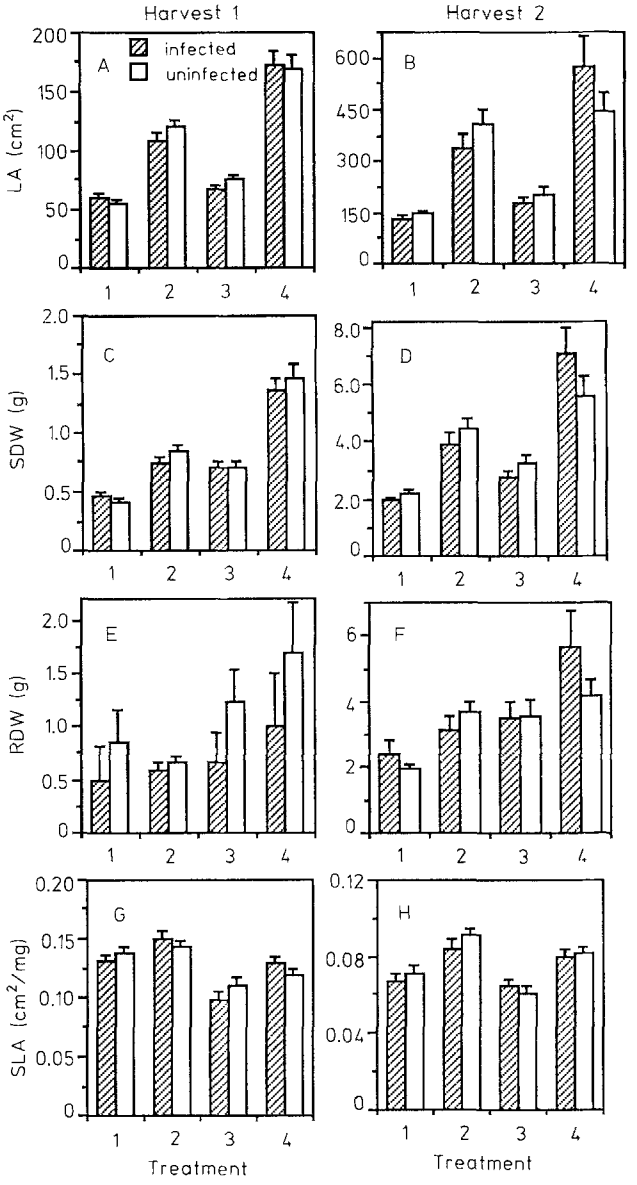

Fig. 1A–H. Leaf area (LA), shoot dry weight (SDW), root dry weight (RDW), and specific leaf area (SLA) for perennial ryegrass at harvest 1, five weeks, and harvest 2, ten weeks after beginning of treatments. Treatments are 1, low CO_2, low nutrient; 2, low CO_2, high nutrient; 3, high CO_2, low nutrient; and 4, high CO_2, high nutrient. Note differences in vertical axes between harvests 1 and 2. Error bars are ± 1 standard error

shoot and total dry weight (Table 1). Plants at high CO_2, high nutrient had the greatest dry weight (Fig. 1D, F). Again, there was no significant effect of infection, but infected plants with high CO_2, high nutrient had 26% more shoot dry weight and 35% more root dry weight than uninfected plants.

Specific leaf area was significantly affected by nutrient addition at both harvests (Table 1). Specific leaf area increased with nutrient addition at both CO_2 concentrations (Fig. 1G, H).

Although there were no significant interactions between CO_2 enrichment and infection, there were differences in the percentage increases with CO_2 enrichment of biomass and leaf area for perennial ryegrass (Table

Table 2. Percentage response of perennial ryegrass and purpletop grass to CO_2 enrichment under low and high nutrient after 10 weeks of treatment. See methods for definition of percentage response

	Perennial ryegrass		Purpletop grass	
	infected	uninfected	infected	uninfected
leaf area				
low nutrient	36%	35%	13%	−11%
high nutrient	70%	10%	−30%	2%
biomass				
low nutrient	45%	64%	19%	4%
high nutrient	80%	21%	−20%	−20%

Table 3. P values from analysis of co-variance for perennial ryegrass growth analysis for relative growth rate (RGR), dry matter production (DMP), net assimilation rate (NAR), and leaf area duration (LAD)

	RGR	DMP	NAR	LAD
CO_2 concentration	0.0239	0.0005	0.0822	0.0001
Nutrient addition	0.2944	0.0001	0.0001	0.0001
Infection	0.0425	0.1214	0.0363	0.8826
CO_2 × nutrient	0.0598	0.5330	0.0118	0.0143
CO_2 × infection	0.1223	0.2109	0.9100	0.3584
Nutrient × infection	0.8462	0.4101	0.4578	0.6534
CO_2 × nutrient × infection	0.3039	0.0312	0.2807	0.0910
Covariate	0.9924	0.0969	0.2811	0.0470

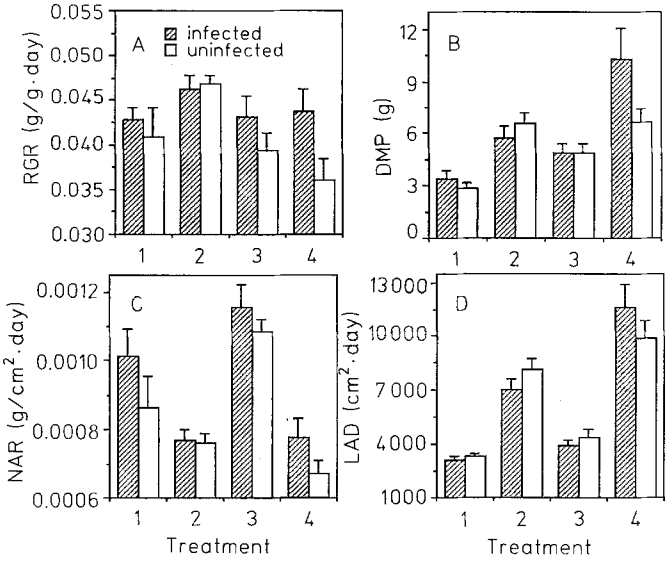

Fig. 2A–D. Relative growth rate (RGR), dry matter production (DMP), net assimilation rate (NAR), and leaf area duration (LAD) for perennial ryegrass for the period from five to ten weeks of the treatment period. Treatments are 1, low CO_2, low nutrient; 2, low CO_2, high nutrient; 3, high CO_2, low nutrient; and 4, high CO_2, high nutrient. Error bars are ±1 standard error

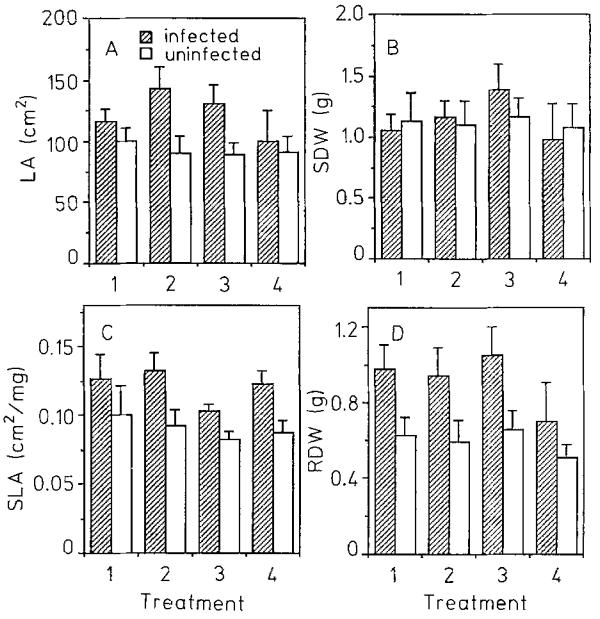

Fig. 3A–D. Leaf area (LA), shoot dry weight (SDW), root dry weight (RDW), and specific leaf area (SLA) for purpletop grass at harvest 2, ten weeks after beginning of treatments. Treatments are 1, low CO_2, low nutrient; 2, low CO_2, high nutrient; 3, high CO_2, low nutrient; and 4, high CO_2, high nutrient. Error bars are ±1 standard error

2). At low nutrient, infected and uninfected perennial ryegrass had the same difference in leaf area between low and high CO_2 levels, about a 35% increase. However, at high nutrient, infected plants were able to use the additional carbon to better advantage, with a 70% increase in leaf area as compared to only a 10% increase for uninfected plants. The same pattern was observed in the percentage increases for biomass.

In growth analysis calculations, dry matter production (DMP) and its morphological parameter, leaf area duration (LAD), were significantly affected by CO_2 enrichment and nutrient addition, but not infection (Table 3, Fig. 2B, D). There was a significant interaction between CO_2 and nutrient in LAD and a significant three-way interaction between CO_2 enrichment, nutrient addition and infection for DMP.

Net assimilation rate (NAR), the physiological parameter of DMP, differed in its responses to the experimental variables. There was no effect of CO_2 enrichment, but nutrient addition significantly affected NAR (Table 3). Infected plants also had a significantly greater NAR than uninfected plants (Table 3, Fig. 2C). There was a significant CO_2 × nutrient interaction for NAR as for LAD. Several other interactions had P values between 0.05 and 0.10.

Relative growth rate (RGR) was also significantly greater for infected plants as compared to uninfected plants, and there was a small but significant effect of CO_2 enrichment on RGR (Table 3, Fig. 2A).

Purpletop grass

In purpletop grass, means of variables of infected plants were frequently larger than those of uninfected plants (Fig. 3), although overall differences were not signifi-

cant. While leaf area of infected plants varied with treatment, it was always greater than that of uninfected plants, and it was significantly greater than uninfected plants in both the low CO_2, high nutrient (treatment 2) and high CO_2, low nutrient (treatment 3) treatments (Fig. 3A).

There were also differences between infected and uninfected plants in specific leaf area, with infected plants having greater average specific leaf areas than uninfected plants (Fig. 3C). As with leaf area, there was more variation among treatments in infected as compared to uninfected plants. Root/shoot ratios were significantly affected by CO_2, infection and nutrient (Table 1). Root/shoot ratios tended to decrease in infected plants as resources were increased, while ratios of uninfected plants were unaffected (Fig. 3B, D).

There was no apparent pattern in leaf area and biomass changes with CO_2 enrichment (Table 2), as would be expected for a plant with the C4 photosynthetic pathway. There were no significant interactions of any main effects for purpletop grass. The lowest P value ($P = 0.195$) occurred for the $CO_2 \times$ nutrient interaction for specific leaf area.

Discussion

The two grasses used in this experiment, perennial ryegrass and purpletop grass, were chosen for the differences in their symbiotic relationship with their fungal endophytes. Perennial ryegrass is infected by an endophyte that enhances growth and insect resistance, with no suppression of host flowering. It is a C3 plant and known to respond to CO_2 enrichment with increased growth (Goudriaan and de Ruiter 1983; Overdieck and Reining 1986). Perennial ryegrass is an agricultural important grass, requires high levels of nutrient input, and is generally found in fertilized areas. Although no previous studies have compared growth of infected and uninfected purpletop grass, its fungal endophyte suppresses host flowering, and, if only for this reason, is presumed to be more pathogenic. As a C4 plant, it was not expected to respond to CO_2 enrichment. In contrast to perennial ryegrass, purpletop grass occurs in natural communities on poor soils. It may therefore be less sensitive to availability of nutrients than perennial ryegrass (Chapin 1985; Coley et al. 1985). Thus, if there were interactions between fungal-endophyte infection, CO_2 enrichment, and/or nutrient addition, they should be apparent in one of these two species.

In this study, there were significant responses of both infected and uninfected perennial ryegrass to CO_2 enrichment and nutrient addition. The percentage increase with CO_2 enrichment in biomass or leaf area depended both on nutrient level and infection status. The greatest increases were in infected plants at high nutrient, the lowest were in uninfected plants at high nutrient, and both infected and uninfected plants at low nutrient had similar intermediate responses. In another study where perennial ryegrass (var. Printo) was grown in competition with white clover there was enhancement of both leaf area and biomass of perennial ryegrass with a CO_2 concentration of 620 $\mu l\, l^{-1}$ as compared to 300 $\mu l\, l^{-1}$ (Overdieck and Reining 1986). When perennial ryegrass was grown under differing levels of atmospheric carbon dioxide concentration and nitrogen, the response of perennial ryegrass to CO_2 enrichment was dependent on nitrogen availability with the greater response under higher nitrogen levels (Goudriaan and de Ruiter 1983). Endophyte status of the experimental plants was not reported; our results would suggest that the plants were infected. The response of other species to CO_2 enrichment can also depend on nutrient or nitrogen availability. Patterson and Flint (1982) found greater responses to CO_2 enrichment at higher nutrient levels in soybean, *Cassia obtusifolia* L. (sicklepod), and *Crotalaria spectabilis* Roth (showy crotalaria). As in our experiment, there were still effects of CO_2 enrichment at the low nutrient level. Interactions between CO_2 enrichment and nutrient level were also observed in wheat (Sionit et al. 1981) and cotton (Wong 1979). However, the growth response of the Australian weed *Xanthium occidentale* Bertol. to CO_2 enrichment was similar at all nitrogen levels (Hocking and Meyer 1985).

There was no response of purpletop grass, a C4 plant, to CO_2 enrichment in this study. Other studies also have documented the non-response of C4 plants to CO_2 enrichment (Wong 1979; Patterson et al. 1984; Wray and Strain 1986; Strain and Cure 1986). There also was no response of purpletop grass to nutrient addition. Purpletop grass grows in poor soils and may be unable to exploit nutrients over some minimal level. Many slow-growing plants from resource-poor habitats are unresponsive to nutrient additions (Chapin 1980; Coley et al. 1985). Although the fungal endophyte of purpletop grass suppresses flowering of its host plants, growth of infected plants was greater over all CO_2 and nutrient conditions. The same response was observed in the grass *Panicum agrostoides* Spreng, where tillers infected by *Balansia henningsiana* (Moell.) Diehl did not reproduce sexually, but had greater growth than uninfected tillers in both field and greenhouse studies (Clay et al. 1989).

The relationship between many fungal endophytes and their host grasses is considered to be mutualistic because the plants provide the fungi with nutrients and/or carbohydrates and infected grasses grow faster than uninfected grasses (Clay 1984; Latch et al. 1985; Clay 1987b; Clay et al. 1989; Marks et al., submitted). The mechanism of enhanced plant growth is unknown and different hypotheses have been proposed (Latch et al. 1985; Bacon and Siegel 1988; Clay 1990). The degree of mutualism is dependent on the plant environment. When plants are grown under stressful conditions such as low nutrient levels (Cheplick et al. 1989) or low light levels (Marks, unpublished data), uninfected plants tend to do as well or better than infected plants. Thus, we expected to find significant interactions of infection with both nutrient and CO_2 level. However, significant interactions between nutrient, CO_2 level and infection were few, and there appeared to be no general trends. Interac-

tions were only significant in perennial ryegrass at harvest 1, and in the growth analysis data where there was a significant $CO_2 \times$ nutrient $\times$ infection interaction for dry matter production. There were no significant interactions at harvest 2 for perennial ryegrass or for purpletop grass.

Growth analysis revealed differences between infected and uninfected perennial ryegrass plants over the five week period between the harvests. While there was no effect of infection on morphological parameters such as dry weight, leaf area or leaf area duration during the length of experiment, there were significant differences in physiological parameters. Relative growth rate, the efficiency of dry matter production, and net assimilation rate, a whole plant measure of carbon gain, were greater in infected plants as compared to uninfected plants. Differences in relative growth rate and net assimilation rate were particularly apparent in the high CO_2, high nutrient treatment. Dry matter production at the high CO_2, high nutrient treatment, where dry matter production of infected plants was over 50% greater than that of uninfected plants ($P<0.08$). If the experiment were continued over a growing season or for a longer period of time, there would be differences in dry matter of infected and uninfected plants.

Several previous studies have examined the possible effects of CO_2 enrichment on interactions between plants and other microorganisms (Lamborg et al. 1983). Total nodule activity of soybean (Finn and Brun 1982) and other nitrogen-fixing woody plants (Norby 1987) are increased with CO_2 enrichment. In both cases, activity appeared to be the result of increases in plant growth and the number of nodules, and not an increase in the nitrogenase activity of the individual nodules. Population density of rhizosphere bacteria of *Quercus alba* L. also did not increase with CO_2 enrichment, although it was assumed that total populations increased along with a large increase in fine root dry weight (Norby et al. 1986). In contrast, O'Neill et al. (1987a) found a decrease in rhizosphere bacterial populations of *Liriodendron tulipifera* L. after 24 weeks in elevated CO_2 concentrations.

Considering mycorrhizae, CO_2 enrichment appears to increase the rate of colonization, but not final mycorrhizal density (O'Neill et al. 1987b; Norby et al. 1987). Early in the experiment, when there were significant differences in mycorrhizal densities between CO_2 treatments, seedlings of *Pinus echinata* Mill. with the greater mycorrhizal density had significantly increased growth with CO_2 enrichment (O'Neill et al. 1987b). However, differences between inoculation treatments disappeared with time, and there were significant CO_2 effects on all seedlings by the end of the experiment (O'Neill et al. 1987b). Differences in growth between mycorrhizal and non-mycorrhizal *Pinus sylvestris* was also greater under CO_2 enrichment than under ambient conditions (Telson et al. 1980). Changes in mycorrhizal interactions with CO_2 enrichment are time-dependent; it is unknown what the cumulative effect could be over several seasons.

Other studies have looked at plant-insect interactions under CO_2 enrichment. For example, no difference was found between populations of *Bemisia tabaci* Gennadius (sweet potato whitefly) in ambient versus high CO_2 chambers (Butler et al. 1986). Similarly, the growth and development of *Pectinophora gossypiella* Saunders (pink bollworm) reared on cotton bolls grown with and without CO_2 enrichment were not significantly different (Akey et al. 1988). However, carbon to nitrogen ratios in leaves can increase with CO_2 enrichment, decreasing nitrogen concentrations in plant tissues and altering insect feeding patterns. *Pseudoplusia includens* Walker (soybean looper) larvae consumed more leaf tissue grown at 650 $\mu l\, l^{-1}$ but gained only the same amount of nitrogen as loopers fed tissue grown at 350 $\mu l\, l^{-1}$ (Lincoln et al. 1986). *Trichoplusia ni* Hubner (cabbage looper) consumed more dry weight and leaf area of lima bean leaves grown under CO_2 enrichment but because plants were larger, the percent leaf area consumed remained constant (Osbrink et al. 1987). In all three experiments there were no significant effects of CO_2 enrichment on the growth or developmental rate of the insects. However, there could be a significant impact of herbivores on plants as their consumption of leaf tissue increases with an increase in CO_2 concentration.

Fungal endophytes can affect plant-herbivore interactions by their production of ergot and other alkaloids (Hardy et al. 1985; Clay 1987a; Bacon and Siegel 1988; Cheplick and Clay 1988). If the production of alkaloids was altered by an increased carbon source, plant-insect interactions could be affected. In one study there were no significant effects of CO_2 on production of iridoid glycosides in *Plantago lanceolata* L. although there were significant effects of CO_2 on development time of the buckeye butterfly (*Junonia coenia*) (Fajer et al. 1989). Another study used the southern armyworm (*Spodoptera eridania Cramer*) and peppermint (*Mentha piperita* L.), which produces allelochemicals (mono- and sesquiterpenes) (Lincoln and Couvet 1989). They found increased consumption of plant tissue with CO_2 enrichment, but no effects on insect growth. Plant volatile content did not change with increased CO_2 level although there was a decrease in nitrogen content (Lincoln and Couvet 1989). It seems unlikely that N-rich, fungal endophyte-produced ergot alkaloids will increase with increasing atmospheric CO_2 concentration. In contrast, alkaloid content, like nitrogen content, may be diluted by the increasing carbohydrates, increasing insect consumption of infected plants.

This study was conducted in a controlled environment where CO_2, nutrient, and endophyte infection were the only variables manipulated. Although there were not dramatic changes in the symbiotic interaction with CO_2 enrichment, changes have been observed in plant competition and plant-insect interactions with CO_2 enrichment in other studies (Lincoln et al. 1986; Osbrink et al. 1987; Fajer et al. 1989). Fungal endophytes can affect both competitive ability and herbivore resistance (Hardy et al. 1985; Clay 1987a; Kelley and Clay 1987; Clay 1990). Increasing CO_2 concentration in nature where herbivory, competition and fungal symbiosis occur simultaneously may result in changes in both the magnitude and direction of multispecies interactions.

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