VARIABLE PERFORMANCE OF OUTBREAK DEFOLIATORS ON ASPEN CLONES EXPOSED TO ELEVATED CO_2 AND O_3

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Abstract: Increasing atmospheric concentrations of ozone and CO_2 affect many aspects of tree physiology. However, their effects on tree resistance to insects have received relatively little attention. The objectives of this study were to test the effects of elevated CO_2 and ozone on the resistance of three quaking aspen (*Populus tremuloides*) clones (216, 259, and 271) to first and fourth instars of four Lepidoptera species: gypsy moth (*Lymantria dispar)*, forest tent caterpillar *(Malacosoma disstria*), large aspen tortrix *(Choristoneura conflictana*), and whitemarked tussock moth *(Orgyia leucostigma*). Larval survival, growth rates, and nutritional indices were quantified. There were no treatment effects on larval survival. Elevated CO_2 decreased the growth rates of both instars of all species, except that of first instar forest tent caterpillar on aspen clone 216, which was increased. Elevated ozone increased the growth of first and fourth instars of all insect species tested. The treatment effects on growth rate were generally caused by their effects on the ability of larvae to convert digested food to biomass (ECD). Elevated ozone increased ECD. The effects of elevated CO_2 on ECD were clone dependent: elevated CO_2 decreased ECD on clones 271 and 259, but increased ECD on clone 216. Ozone had no effect on larval consumption rates. Elevated CO_2 decreased the consumption rate of large aspen tortrix but had no effect on the other species. This contrasts with other studies, in which elevated CO_2 generally increased insect consumption. There were no statistically significant interactions between the CO_2 and ozone treatments for any of the variables measured.

INTRODUCTION

Elevated concentrations of atmospheric CO_2 and ozone alter many aspects of tree physiology including gas exchange, growth, and carbon allocation (Pye 1988, Jarvis 1989). Environmentally-induced variation in tree resistance to insects and other herbivores results primarily from effects on plant nutrient and secondary metabolite concentrations, which are intimately connected to whole-plant patterns of resource acquisition and allocation (Herms and Mattson 1992). Hence, elevated CO_2 and ozone may influence interactions between plants and herbivores, and therefore their distribution and abundance (Ayres 1993, Williams and Liebhold 1995). In turn, community composition and insect-mediated ecosystem processes such as nutrient cycling may be affected (Ayres 1993, Lambers 1993). Despite this potential, few studies have addressed the effects of increased atmospheric CO_2 or ozone on tree/insect interactions, and to our knowledge, no study has examined both factors simultaneously.

Elevated CO_2 generally decreases the nutritional quality of plants for leaf-feeding insects. In some cases, this translates into decreased insect growth and survival, but in other cases insects compensate for decreased foliage

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quality by increasing their consumption rate such that elevated CO_2 has no effect on growth (Lincoln 1993, Lincoln and others 1993). The only study with forest insects suggests that effects of elevated CO_2 are likely to be dependent on the species involved (Lindroth and others 1993). The growth of gypsy moth larvae (*Lymantria dispar*) fed foliage from trees grown under elevated CO_2 was decreased on quaking aspen (*Populus tremuloides*), was unaffected on sugar maple (*Acer saccharum*), and increased on red oak (*Quercus rubra*). In the same experiment, elevated CO_2 decreased the growth of forest tent caterpillar (*Malacosoma disstria*) on quaking aspen and sugar maple, but did not affect its growth on red oak.

The effects of ozone on plant/herbivore interactions have received less attention, but some studies have found elevated ozone to enhance insect performance on crop plants (Chappelka and others 1988, Reimer and Whittaker 1989, Lin and others 1990). Only a few studies have investigated ozone effects on tree resistance to insects. In a study with cottonwood (Populus *deltoides*), ozone exposure had no effect on the aphid *Chaitophorus populicola* (Coleman and Jones 1988a).

Although ozone decreased the growth and fecundity of the cottonwood leaf beetle (*Plagiodera versicolora*), this insect preferred to feed on, and consumed more, foliage exposed to elevated ozone than foliage exposed to ambient air (Coleman and Jones 1988b, Jones and Coleman 1988). Gypsy moth preferred to feed on white oak (*Quercus alba*) foliage exposed to the highest concentration of ozone (about 3X ambient), but preferred foliage exposed to ambient air over that exposed to intermediate levels of ozone (Jeffords and Endress 1984).

Objectives

Quaking aspen is the most widely distributed tree species in North America. Forest tent caterpillar, gypsy moth, large aspen tortrix (*Choristoneura conflictana*), and whitemarked tussock moth (*Orgyia leucostigma*) can be important outbreak defoliators and dominant lepidopteran folivores of aspen. Quaking aspen displays genetic variation in ozone tolerance (Berrang and others 1989). Hence, ozone effects on insect performance may be dependent on host genotype. However, to our knowledge no studies have examined genetic variation in the effects of ozone or elevated CO_2 on tree resistance to insects. The objectives of this study were to test the effects of elevated CO_2 and ozone on the nutritional suitability for these four insect species of three aspen clones (*Populus tremuloides*) that differ in their sensitivity to ozone. The aspen genotypes tested were clone 216 (Bayfield Co. Wisconsin, ozone-tolerant), clone 259 (Porter Co. Indiana, ozone sensitive), and clone 271 (Porter Co. Indiana, intermediate tolerance of ozone).

METHODS

Production of Experimental Plant Material

Softwood cuttings taken from greenhouse-grown stock plants were dipped in 5000 mg/l IBA and rooted in 65 cm^3 Leach cells in vermiculite-peat (3:1) mix with intermittent misting. Rooting occurred in four weeks, after which the plants were hardened on the greenhouse bench, then transplanted into 38 cm deep x 15 cm plastic pots in a media of peat-sand-vermiculite (2:1:1), supplemented with 8 g of Sierra Osmocote (17-6-10 formulation), plus micronutrients (8-9 month release time). Two days after transplanting, plants were moved to test chambers and exposed to experimental treatments for five weeks before use in insect bioassays.

Insect Culture

Insects of all four species were obtained as newly molted fourth instars and newly eclosed first instars from Forest Pest Management Institute, Canadian Forest Service, Sault Ste. Marie, Ontario. All four species were tested as fourth instars. All but large aspen tortrix were tested as first instars. Bioassays were initiated within 24 hours of obtaining the insects.

Implementation of Ozone and CO_2 Treatments

Gaseous treatments were applied in 1.7m^3 exposure chambers constructed from mylar covered aluminum angle frame. Ozone was produced using an electric discharge generator (GTC-0.25, Griffin Technics, Inc. Lodi, New Jersey) from bottled oxygen delivered through proportional control valves that were automatically adjusted to maintain 150 nmol/mol by a CR-10 datalogger (Campbell Scientific, Logan, UT). Ozone was maintained at the set point between 900 and 1700 hours each day, and returned to ambient for the remained of the day. The ozone application resulted in 10 ppm h accumulated each week, compared to 3 ppm h for the controls exposed to ambient levels. Elevated CO_2 was manually dispensed 24 hr per day through needle valves. The ambient concentrations (approximately 400 umol/ mol) were supplemented with 350 umol/mol. Ozone and CO_2 monitors were calibrated weekly. Hourly treatment concentrations were recorded for each chamber by the datalogger.

Experimental Design

Elevated and ambient ozone and CO_2 were applied to four chambers in factorial combination. Each CO_2/ozone treatment combination was replicated twice, for a total of eight chambers in the experiment. Five replicate individuals of all three aspen clones were grown in each chamber, for a total of 15 trees in each chamber. Thus, the experiment constituted a 2 x 2 x 3 x 4 nested factorial, with two levels of ozone (ambient and elevated), two levels of CO_2 (ambient and elevated), three aspen clones (216, 259, 271), and four insect species (three in the case of first instars with large aspen tortrix omitted from the test). We measured the growth of four individual larvae (fourth instars) or four groups of larvae (first instars) within each four-way treatment combination. Each of the four replicate larvae or larval groups received leaves from a separate tree so that individual trees were the experimental unit. First and fourth instar bioassays were treated as separate experiments.

Foliage Sampling and Leaf Assignments

To control for effects of tree ontogeny on leaf chemistry and insect performance, larvae were assigned leaves of a specific physiological age as determined by their leaf plastichron index (LPI) (Larson and Isebrands 1971). Leaves were numbered sequentially with LPI one designated as the youngest leaf on the tree that was at least 3 cm in length. Assignments were based on judgements of leaf ages that larvae were likely to feed on under field conditions. First instar forest tent caterpillar, gypsy moth, and whitemarked tussock moth were fed LPI two, four, and seven, respectively. Gypsy moth and forest tent caterpillar both were fed LPI 10 and 11, whitemarked tussock moth were fed leaf plastichron 13. Each large aspen tortrix replicate was randomly assigned either LPI 10, 11, 13, or 15.

First Instar Bioassay Procedures

Ten larvae were confined to petri dish an (8.5 cm diam. by 2 cm deep) with a base of plaster-of-Paris and charcoal. Water added to the plaster base provided a high humidity environment that maintained the turgor of detached leaves fed to larvae. Simultaneous measurements of leaves from trees exposed to the same treatments but not fed to insects showed no main or interacting effects of any of the treatments on fresh weight or respiratory losses of dry mass of leaves over the 48-hour bioassay period. Larvae were reared in a growth chamber at 25°C, under an 18:6 light:dark cycle.

All 10 larvae from each dish were weighed as a group prior to initiation of the bioassay. After 48 hours of feeding the bioassay was terminated and the number of surviving larvae was recorded. Duration of the bioassay was quantified to the nearest 15-minute interval. Surviving larvae were then flash frozen, oven-dried to constant weight, and then weighed as a group. In order to estimate initial weights, twenty 10-larvae samples were weighed for each of the three species, then immediately frozen at -40°C, dried to constant weight and reweighed. The relationship of fresh mass to dry mass of these insects was determined from these data using linear regression. These regression equations were then used to estimate initial dry mass of the bioassay insects based on their initial fresh masses. We calculated percent survival and relative growth rate for each of the three species.

Fourth Instar Bioassay Procedures

Bioassays with fourth instars were conducted as above, with the following alterations. Only one larva was used per petri dish, and the petri dishes were larger: 11.5 cm diam. by 2.5 cm deep. The initial dry mass of each insect was estimated from initial fresh mass as above, except that regression equations were developed from 20 individual larvae. Total leaf area consumed during the bioassay was determined by measuring the area of the leaf with a digital image analyzer before and after the bioassay period. Frass was collected, dried to constant weight, and weighed. The portions of the leaves not consumed were collected, and their cumulative area and dry mass determined. Initial dry mass was then calculated as:

initial dry mass = (initial leaf area * final dry mass) / final leaf area

The amount of foliage consumed was estimated by subtracting final leaf dry mass from initial leaf dry mass. Relative growth rate (RGR), relative consumption rate (RCR), approximate digestibility (AD), and efficiency of conversion of digested food (ECD) were calculated from gravimetric measurements following Ayres and McLean (1987) (note: AD=the percentage of food consumed that is digested; ECD=the percentage of digested food that is converted to biomass; RGR=RCR*AD*ECD).

Data Analysis

The data were analyzed by ANOVA (SAS, Proc GLM; Type III sum of squares). Aspen clone and insect species were nested within the CO_2 and ozone treatments. CO_2, ozone, aspen clone, insect species, and chamber were all treated as fixed effects, and were tested over residual error (MSE). Chamber was considered a fixed effect because the chambers used in the study were not selected at random from a larger population of chambers. Treating chamber as a fixed effect increases the power of the experiment for detecting treatment effects, but limits the scope of inference from the analysis to this study (see Bennington and Thayne 1994 for a discussion of fixed vs. random effects in ANOVA). Means were separated using the protected LSD test.

RESULTS

Larval Survival

First instar survival over the 48-hour bioassay period was high: 84 percent, 92 percent, and 99 percent for first instar gypsy moth, forest tent caterpillar, and whitemarked tussock moth, respectively, and over 90 percent for fourth instars of all species. Survival was not affected by any of the treatments.

CO_2 Effects on Insect Performance

Elevated CO_2 decreased the growth of first instars of all three species, but only on two of the three aspen clones tested (Table 1, significant CO_2*clone interaction). Overall, elevated CO_2 decreased the relative growth rates of first instar forest tent caterpillar, gypsy moth, and whitemarked tussock moths by 65 percent, 32 percent, and 48 percent, respectively (Figure 1). However, the growth rates of none of the insect species were decreased by elevated CO_2 when feeding on aspen clone 216, and elevated CO_2 actually increased the growth rate of forest tent caterpillar by 80 percent on this clone (Table 1, significant CO_2*clone interaction). Elevated CO_2 decreased the relative growth rates of fourth instars of all species on all clones (Table 1, Figure 2). Percent decreases in growth rate ranged from 51 percent for gypsy moth to 26 percent for large aspen tortrix.

Table 1. F-values from ANOVA of performance of first and fourth instar gypsy moth, forest tent caterpillar, whitemarked tussock moth, and fourth instar large aspen tortrix on three aspen clones exposed to two levels of CO2 and ozone. Statistical significance is shown as follows: **** indicates P≤ 0.0001; *** indicates P ≤ 0.001; ** indicates P ≤ 0.01; * indicates P ≤ 0.05.

	1st Instar		4th Instar				
Source	df	RGR	df	RGR	RCR	ECD	AD
CO_2	1	25.8 ****	1	34.3 ****	7.0 ***	1.7	5.1 *
O_3	1	31.4 ****	1	14.8 ****	0.2	14.4 ***	35.5 ****
CO_2*O_3	1	2.9	1	0.4	0.1	0.1	0.6
chamber(CO_2*O_3)	4	10.4 ***	4	5.9 ***	2.0	0.7	5.9 ***
aspen clone	2	19.0 ****	2	0.7	0.1	2.9	13.5 ****
CO_2*clone	2	5.5 ***	2	3.7 *	2.1	5.1***	2.1
O_3*clone	2	0.4	2	3.8 *	1.2	0.8	5.0 **
CO_2*O_3*clone	2	0.9	2	5.5 **	3.3 *	1.2	2.1
clone*chamber(CO_2*O_3)	8	1.2	8	1.9	1.6	0.8	1.0
insect species	2	27.4 ****	3	69.5 ****	192.0 ****	9.7 ***	19.4 ****
CO_2*insect	2	0.7	3	0.8	2.9 *	1.2	1.6
O_3*insect	2	1.3	3	0.5	0.1	0.2	2.0
CO_2*O_3*insect	2	3.9 *	3	0.2	0.1	1.2	0.8
insect*chamber(CO_2*O_3)	8	1.4	12	0.4	0.4	0.6	1.5
clone*insect	4	2.8 *	6	0.5	0.2	0.3	3.7 **
CO_2*clone*insect	4	2.8 *	6	0.5	1.4	1.7	0.6
O_3*clone*insect	4	1.3	6	1.3	1.2	1.2	0.1
CO_2*O_3*clone*insect	4	1.3	6	1.1	2.4 *	0.4	1.0
clone*insect*chamber(CO_2*O_3)	16	1.5	24	1.5	1.7 *	1.1	1.5
error	215		286				

The consumption rate (RCR) of large aspen tortrix was decreased 20 percent by elevated CO_2. Consumption rates of the other species were not affected (Figure 3, Table 1, significant CO_2*insect interaction). The unusually high consumption rates for large aspen tortrix are artefacts resulting because the image analyzer used to measure leaf area was unable to differentiate skeletonized foliage (characteristic of large aspen tortrix feeding) from completely consumed leaves. Hence, consumption was overestimated. This prevents direct comparisons with the other species, but will not affect relative comparisons of large aspen tortrix performance on the different CO_2 and ozone treatments. Elevated CO_2 had a minor but statistically significant effect on the ability of larvae to digest food, decreasing AD by only 5 percent (Table 1, Figure 4). The effect of elevated CO_2 on larval ability to convert digested food to biomass (ECD) was dependent on the aspen clone fed upon (Table 1, significant CO_2*clone interaction). Elevated CO_2 decreased ECD by 51 percent and 32 percent on clones 259 and 271, respectively, but ECD was increased 55 percent on clone 216 (Figure 5).

Ozone Effects on Larval Performance

Elevated ozone increased the growth rates of first instars of all three species (Table 1). Forest tent caterpillar larvae were affected most dramatically, with their growth rate increasing 10-fold on ozone exposed foliage. Elevated ozone increased the growth rates of gypsy moth and whitemarked tussock moth by 70 percent and 60 percent, respectively (Figure 6). The growth rate of fourth instars of all species was also increased by elevated ozone, with percent increases ranging from 27 percent for forest tent caterpillar to 59 percent for gypsy moth (Figure 7). Ozone had no effect on relative consumption rate (Table 1, Figure 8). However, elevated ozone decreased the ability of larvae to digest food (Figure 9), with the magnitude of the effect dependent on clone (Table 1, significant ozone*clone interaction). Elevated ozone decreased AD 29 percent on clone 259, 14 percent on clone 216, and 6 percent on clone 271. Ozone increased the ability of larvae of all four species to convert digested food to biomass (ECD) (Table 1), with the percent increases ranging from 35 percent for forest tent caterpillar to 230 percent for large aspen tortrix (Figure 10).

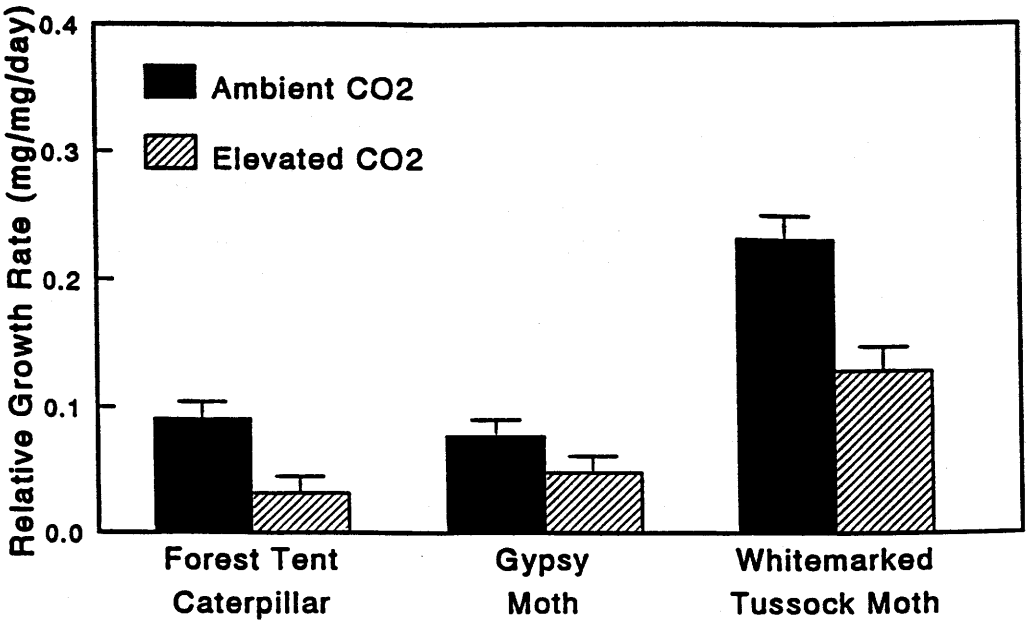

Figure 1. Effect of ambient and elevated CO2 on the relative growth rate (RGR) of first instar forest tent caterpillar, gypsy moth, and whitemarked tussock moth feeding on quaking aspen. Data are expressd as least square means ± one standard error.

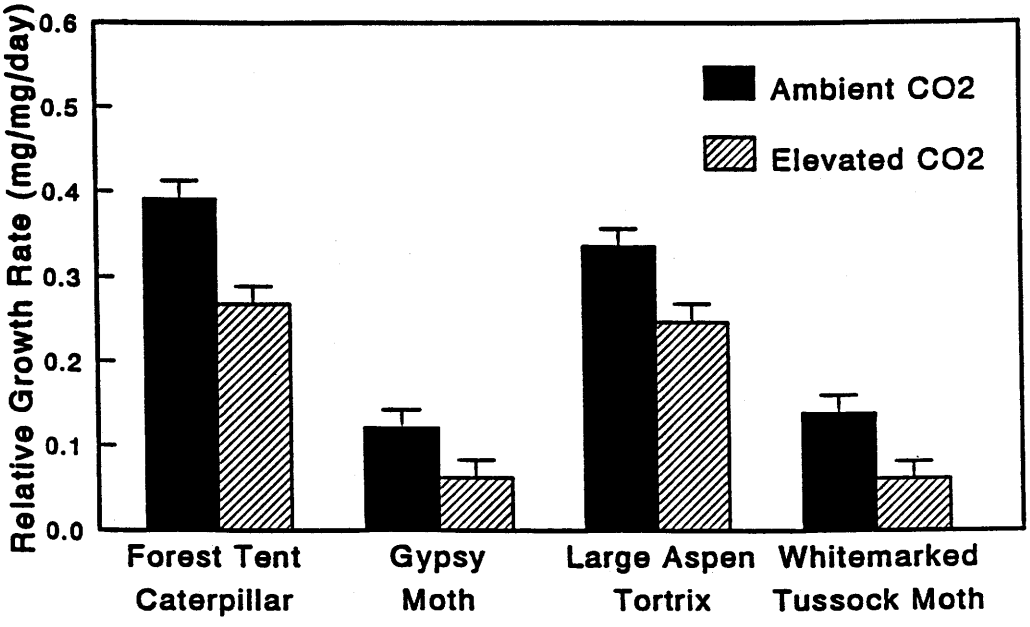

Figure 2. Effects of ambient and elevated CO2 on the relative growth rate (RGR) of fourth instar forest tent caterpillar, gypsy moth, large aspen tortrix, and whitemarked tussock moth, feeding on quaking aspen. Data are expressed as least square means ± one standard error.

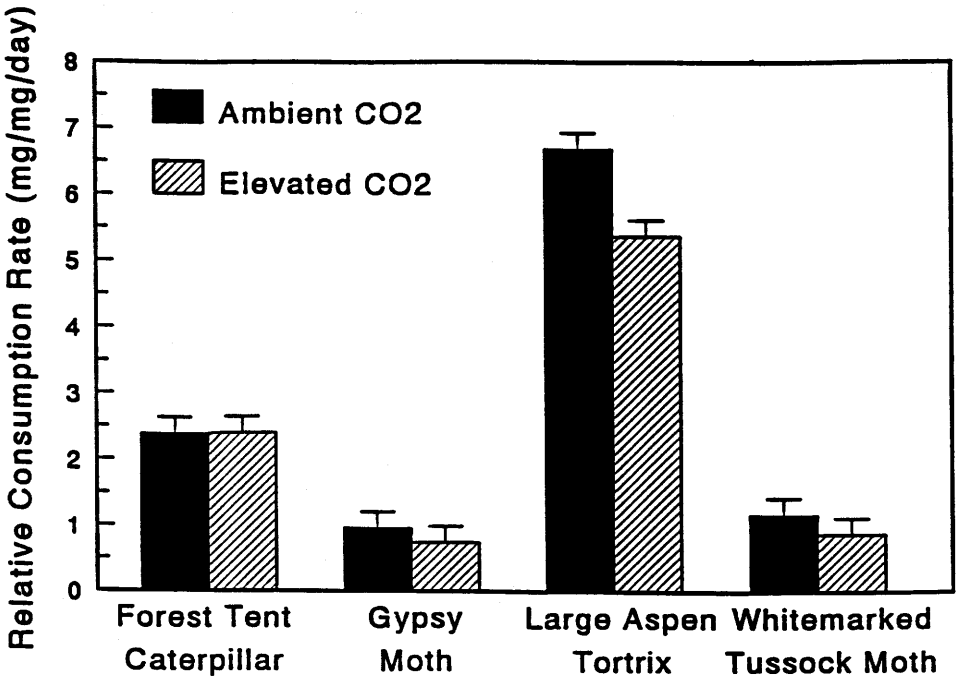

Figure 3. Effect of ambient and elevated CO2 on the relative consumption rate (RCR) of fourth instar forest tent caterpillar, gypsy moth, large aspen tortrix, and whitemarked tussock moth feeding on quaking aspen. Data are expressed as least square means ± one standard error.

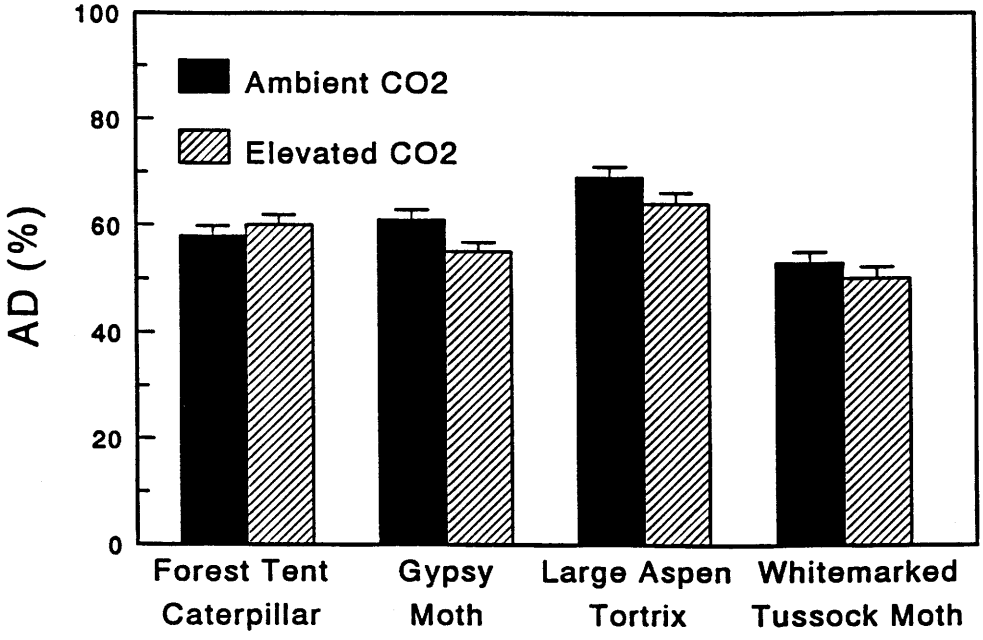

Figure 4. Effect of ambient and elevated CO2 on the percentage of consumed food digested (AD) by fourth instar forest tent caterpillar, gypsy moth, whitemarked tussock moth, and large aspen tortrix feeding on quaking aspen. Data expressed as least square means ± one standard error.

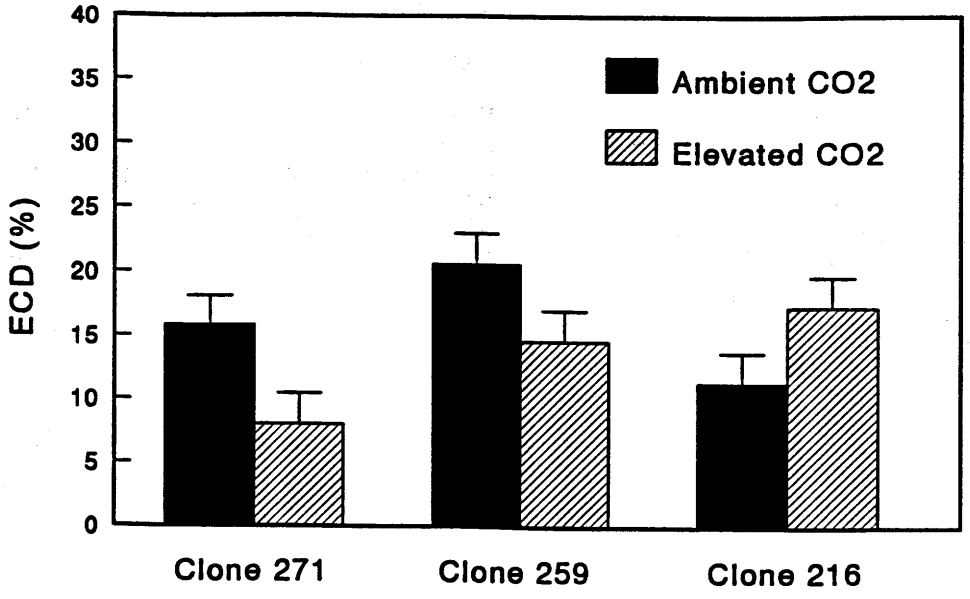

Figure 5. Effect of ambient and elevated CO2 on the persentage of digested food converted to biomass (ECD) by fourth instar forest tent caterpillar, gypsy moth, large aspen tortrix, and whitemarked tussock moth feeding on three clones of quaking aspen. Data are expressed as least square means of all insects combined ± one standard error.

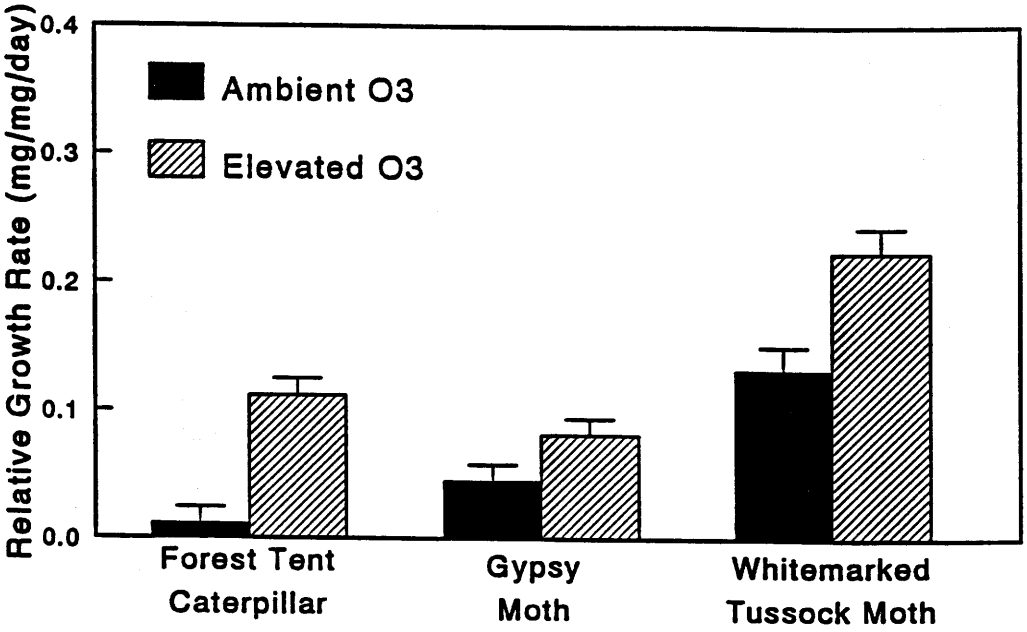

Figure 6. Effect of ambient and elevated ozone on the relative growth rate of first instar forest tent caterpillar, gypsy moth, and large aspen tortrix feeding on quaking aspen. Data are expressed as least square means ± one standard error.

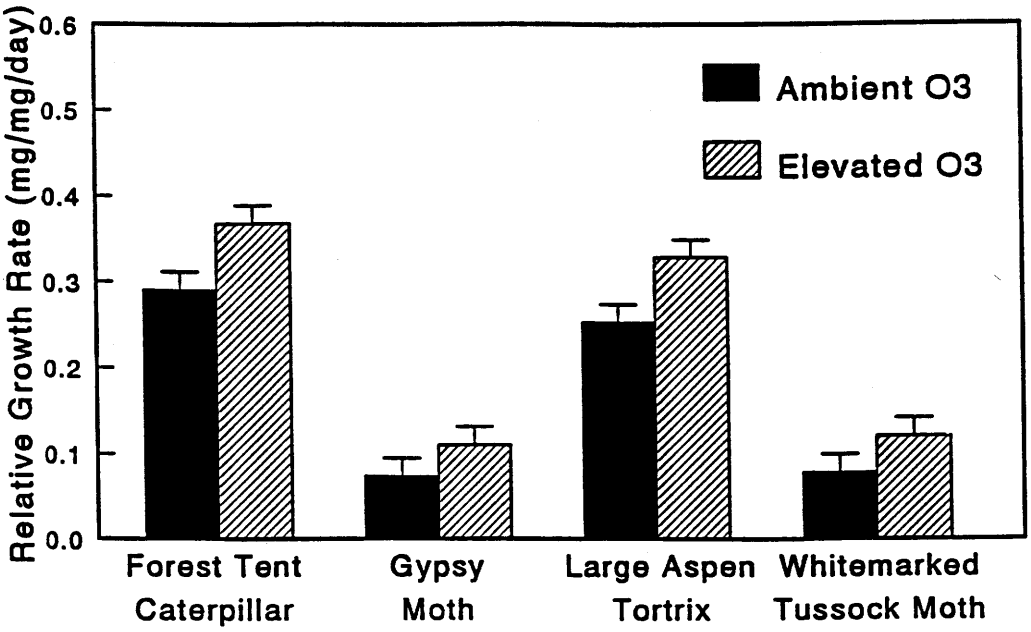

Figure 7. Effect of ambient and elevated ozone on the relative growth rate (RGR) of fourth instar forest tent caterpillar, gypsy moth, large aspen tortrix, and whitemarked tussock moth feeding on three clones of quaking aspen. Data are expressed as least square means ± one standard error.

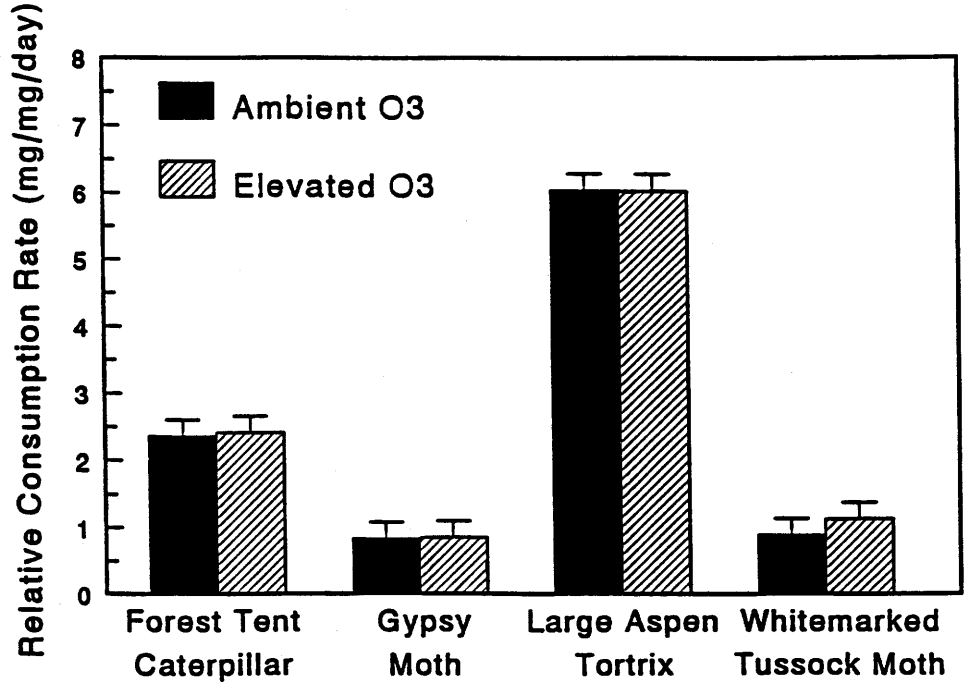

Figure 8. Effect of ambient and elevated ozone on the relative consumption rate (RCR) of fourth instar forest tent caterpillar, gypsy moth, whitemarked tussock moth, and large aspen tortrix feeding on quaking aspen. Data are expressed as least square means ± one standard error.

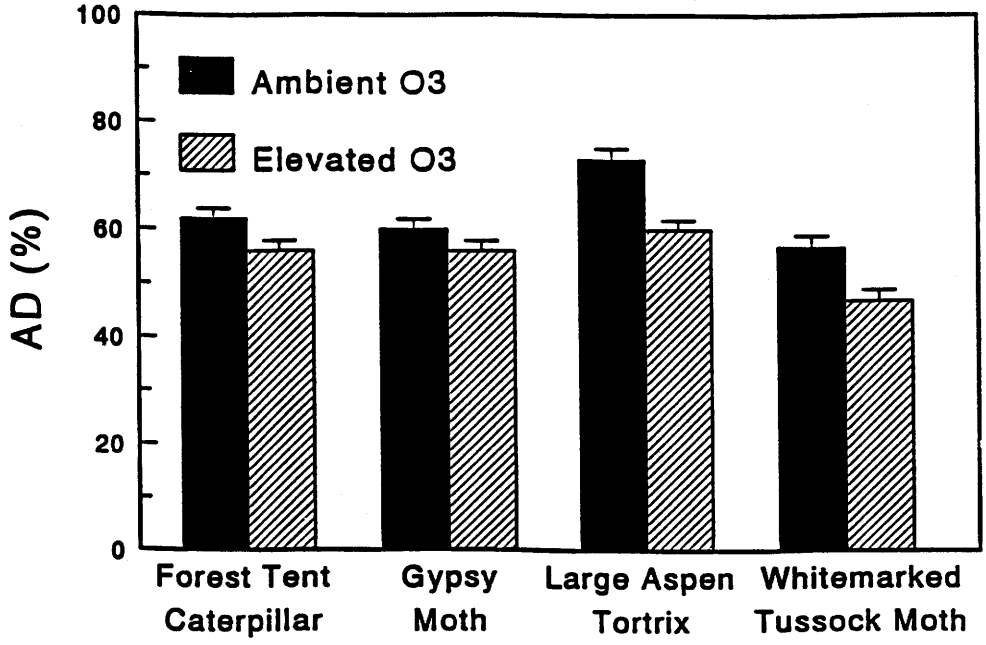

Figure 9. Effect of ambient and elevated ozone on the percentage of consumed food digested (AD) by fourth instar forest tent caterpillar, gypsy moth, large aspen tortrix, and whitemarked tussock moth feeding on three clones of quaking aspen. Data are expressed as least square means ± one standard error.

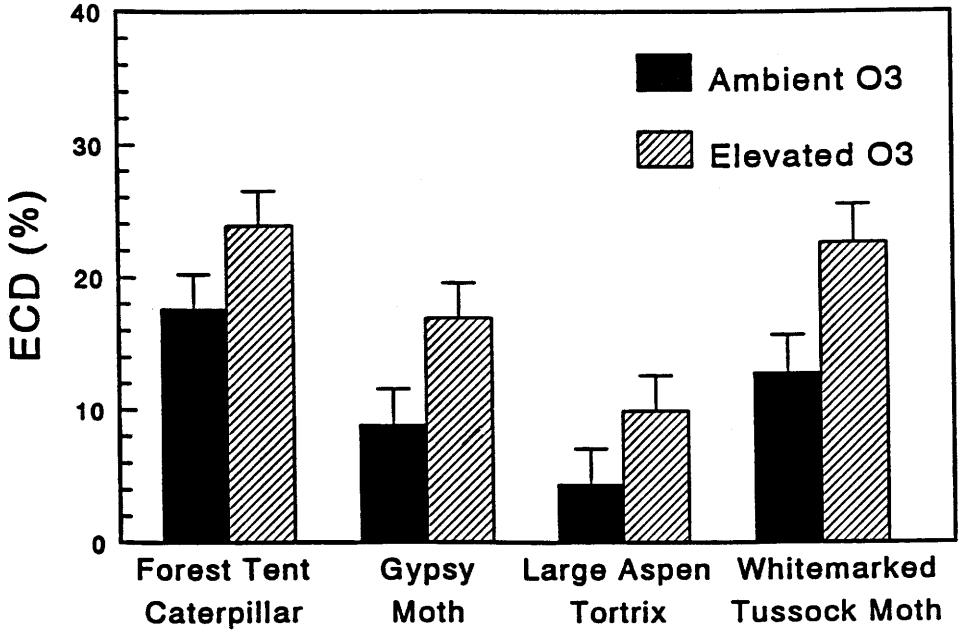

Figure 10. Effect of ambient and elevated ozone on the percentage of digested food converted to biomass (ECD) by fourth instar forest tent caerpillar, gypsy moth, whitemarked tussock moth, and large aspen tortrix feeding on quaking aspen. Data are expressed as least square means ± one standard error.

DISCUSSION

CO_2 Effects on Insect Performance

Most studies have found elevated CO_2 to decrease the nutritional quality of plants for insects, in some cases resulting in decreased growth and survival (Lincoln 1993, Lincoln and others 1993). In other cases, however, insects increased their consumption rates, compensating for decreased nutritional quality (Lincoln 1993, Lincoln and others 1993). Lindroth and others (1993) in the only published study of the effects of atmospheric CO_2 on forest insects found that consumption rates of gypsy moth and forest tent caterpillar increased substantially, but their growth rates declined.

In this experiment, elevated CO_2 decreased the growth rates of fourth instars of all species. However, the results of elevated CO_2 on the growth of first instars were dependent on the host genotype. Elevated CO_2 decreased the growth rate of first instars on two of the three clones tested. However, the growth rate of first instar forest tent caterpillar increased dramatically on clone 216, and the growth of the other two species were not affected on this clone.

Previous studies have found that insects frequently increase consumption rates to compensate for elevated CO_2-induced reductions in foliage quality (Lindroth and others 1993, Lincoln and others 1993). In this experiment, however, elevated CO_2 decreased the consumption rate of large aspen tortrix and had no effect on the other species. The small reduction in the ability of larvae to digest food caused by elevated CO_2 was not enough to account for the dramatic decreases observed in growth rate. Rather, decreased growth was due to decreased ability of larvae to convert digested food to biomass (ECD), and in the case of large aspen tortrix, decreased consumption rate. However, the effect varied by clone. Elevated CO_2 decreased the ECD of larvae feeding on clones 259 and 271, but increased the ECD of larvae feeding on 216 (the same clone on which first instar forest tent caterpillar growth rate increased).

Lindroth and others (1993) found that elevated CO_2 decreased the ECD of gypsy moth and forest tent caterpillar on aspen. Decreased ECD is consistent with increased concentrations of toxins in the diet. In a companion study using the same plants, we did see a corresponding increase in the phenolic glycosides tremulacin and salicortin in response to elevated CO_2 (see abstract by Nitao et al.). Tremulacin in particular has been shown previously to decrease growth and survival of gypsy moth and forest tent caterpillar (Lindroth 1991). Tremulacin concentrations were lowest in clone 216, the clone on which ECD and first instar forest tent caterpillar growth rate were increased. However, affects of CO_2 on other secondary metabolites and nutrients also may have affected growth. Elevated CO_2 almost universally decreases foliar N concentrations (Lincoln and others 1993), which frequently limits insect growth (Mattson 1980). Analyses of condensed tannins and N are currently underway.

Ozone Effects on Insect Performance

To our knowledge, this study represents the first report on the effects of ozone on the growth and nutritional physiology (as opposed to feeding preferences) of Lepidoptera larvae feeding on woody plants. The growth rates (RGR) of first and fourth instars of all species fed foliage exposed to elevated ozone were increased. Growth rates were increased because elevated ozone increased the ability of larvae to convert digested food to biomass (ECD), which overcompensated for the smaller negative effect of elevated ozone on their ability to digest foliage. Ozone had on effects on consumption rates. The enhanced quality of foliage exposed to elevated ozone may be due to the observed decrease in foliar concentrations of tremulacin (see abstract by Nitao et al.).

No Interactions Between CO_2 and Ozone

To our knowledge, this is the first study that simultaneously tested the effects of atmospheric ozone and CO_2, and there were no interactions between them. The ozone and CO_2 treatment levels used in this experiment had roughly equal and opposing effects on insect performance, with elevated ozone decreasing and elevated CO_2 enhancing aspen resistance to the four species.

SUMMARY AND CONCLUSIONS

The effects of elevated atmospheric CO_2 and ozone on the resistance of three aspen clones to four species of Lepidoptera larvae were tested. Elevated ozone generally increased, and elevated CO_2 generally decreased insect growth. However, elevated CO_2 increased the growth rates of first instar forest tent caterpillar feeding on aspen clone 216. Most previous studies have found elevated CO_2 to increase insect consumption rates. However, in this study, elevated CO_2 decreased the consumption of large aspen tortrix and had no effect on the other species. Ozone also had no effect on consumption. Effects on insect growth occurred primarily because of treatment effects on the ability of larvae to convert digested food to biomass, but in the case of elevated CO_2, the effect was clone dependent. Elevated CO_2, while having an overall negative effect on ECD, increased the ECD of larvae feeding on clone 216. Effects of CO_2 and ozone on insect performance corresponded with ozone and CO_2 effects on foliar concentrations of the toxic phenolic glycoside tremulacin. There were no interactions between the ozone and CO_2 treatments.

ACKNOWLEDGMENTS

Funding for this study was provided by the USDA Forest Service Northern Global Change Program. We thank Matthew P. Ayres and John R. Spence for critical reviews of this manuscript. We are especially grateful to Bob McCron and the Insect Production Unit at the Forest Pest Management Institute, Canadian Forest Service, Sault Ste. Marie, Ontario. Their efforts in synchronizing the production of first and fourth instars of four species of insects made the experiment possible.

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