Atmospheric Co2 and Soil Water Availability: Consequences for Tree-Insect Interactions

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Atmospheric CO_2 and soil water availability: consequences for tree–insect interactions

Sherry Roth, Evan P. McDonald, and Richard L. Lindroth

Abstract: The consequences of elevated CO_2 for interactions between trees and associated insects will be influenced by the availability of other plant resources. We investigated the effects of CO_2 and water availability on phytochemistry of quaking aspen (*Populus tremuloides* Michx.) and sugar maple (*Acer saccharum* Marsh.) and the associated performance of the forest tent caterpillar (*Malacosoma disstria* Hbn.). Seedlings were grown under ambient or elevated CO_2 concentrations and under well-watered or drought conditions. We measured rates of gas exchange and subjected foliage to phytochemical assays. Bioassays were conducted to quantify larval performance on foliage from the various treatments. In general, elevated CO_2 increased photosynthetic rates and had no effect on stomatal conductance, while drought reduced both parameters. Foliar nitrogen levels declined and secondary metabolite concentrations increased under enriched CO_2, but starch and sugar levels were unaffected. All phytochemicals measured, with the exception of simple sugars, declined or did not change in response to drought. CO_2- and drought-mediated changes in phytochemistry reduced forest tent caterpillar growth and food processing efficiencies, but the patterns were host-species specific. This work demonstrates that CO_2 effects on forest trees will be mediated by the availability of water and that the direction and magnitude of responses will depend on the tree species involved, which will, in turn, affect patterns of host use by herbivorous insects.

Résumé : Les conséquences de l'augmentation du CO_2 sur les interactions entre les arbres et les insectes qui y sont associés devraient être influencées par la disponibilité des autres ressources nécessaires aux plantes. Nous avons étudié les effets du CO_2 et de la disponibilité en eau sur la phytochimie du peuplier faux-tremble (*Populus tremuloides* Michx.) et de l'érable à sucre (*Acer saccharum* Marsh.) ainsi que la performance de la livrée des forêts (*Malacosoma distria* Hbn.) en lien avec les divers traitements. Les semis furent cultivés à des concentrations ambiantes ou élevées de CO_2 associées à des conditions d'irrigation abondante ou de sécheresse. Nous avons mesuré le taux d'échange gazeux et soumis le feuillage à des tests phytochimiques. Des bioessais furent réalisés pour quantifier la performance des larves sur le feuillage des plants soumis aux différents traitements. En général, une élévation du CO_2 a provoqué une augmentation du taux de photosynthèse et n'avait pas d'effet sur la conductance stomatale alors que la sécheresse a réduit ces deux paramètres. Le niveau d'azote foliaire a diminué et la concentration des métabolites secondaires a augmenté dans un environnement enrichi en CO_2 mais les niveaux d'amidon et de sucres n'étaient pas affectés. Tous les composés chimiques mesurés, à l'exception des sucres simples, ont diminué ou sont demeurés stables en réaction à la sécheresse. Les changements phytochimiques causés par le CO_2 et la sécheresse ont réduit la croissance de la livrée des forêts et son efficacité à digérer la nourriture mais les comportements étaient spécifiques à chaque essence hôte. Cette étude démontre que les effets du CO_2 sur les arbres en forêt sont influencés par la disponibilité en eau et que la direction et l'importance de ces réactions dépendent de l'espèce hôte, ce qui en retour se répercutera sur le patron d'utilisation de l'hôte sur lequel se développent les insectes herbivores.
[Traduit par la Rédaction]

Introduction

Rising levels of atmospheric CO_2 are expected to affect forest communities directly, by enhancing productivity, and also indirectly, via changes in chemical composition of foliage. Such changes may have significant impacts on herbivore performance and population dynamics (Bazzaz 1990; Lincoln et al. 1993; Lindroth 1996*a*). The degree to which plant–herbivore interactions are altered by enriched CO_2 conditions, however, will likely depend on other environmental factors, such as temperature, light, nutrients, and water (Bazzaz 1990). Of these, water is particularly important for several reasons. First, patterns of tree species distribution and forest composition are strongly influenced by the availability of water (Hinckley et al. 1981). In addition, insect performance is highly correlated with foliar water content, and, in fact, water may be one of the most limiting nutrients for tree-feeding herbivores (Scriber and Feeny 1979; Schroeder 1986). Finally, drought stress may play an important role in initiating insect outbreaks (Mattson and Haack 1987*a*, 1987*b*). Research on the interactive effects of atmospheric CO_2 and water availability on tree–insect interactions is, therefore, a critical step towards a more complete understanding of how forest communities will be altered by rising levels of atmospheric CO_2.

To date, several studies have explored the independent effects of CO_2 and drought on tree physiology, phytochemistry, and herbivore performance. While elevated CO_2 tends to increase photosynthetic rates and reduce stomatal conductance, drought typically reduces both parameters (Hsiao 1973; Eamus and Jarvis 1989). These physiological changes translate into shifts in the C:N ratio of foliage. Under enriched CO_2 conditions, levels of carbon-based compounds (carbohydrates and (or) secondary metabolites) typically increase, while levels

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of nitrogen typically decline (Lindroth et al. 1993; Roth and Lindroth 1994, 1995; Watt et al. 1995). In response to persistent drought, leaf water potentials decline continuously (Hinckley et al. 1978), and partitioning of nonstructural carbohydrate may be altered as trees osmoregulate by converting starch into simple sugars (Kramer 1983; Mattson and Haack 1987*a*). Nitrogen metabolism often increases during drought, resulting in protein hydrolysis and accumulations of amino acids in foliage (Kramer 1983). Secondary metabolism may also be affected by drought stress; moderate drought may limit tree growth more than photosynthesis, resulting in an increase in carbon-based secondary metabolites (Horner 1990; Ayres 1993).

Phytophagous insects respond to the phytochemical changes induced by enriched CO_2 and drought in a variety of ways. Under elevated CO_2, reductions in foliar nitrogen, together with increases in deleterious secondary compounds (e.g., phenolic glycosides or tannins), often prolong development and reduce growth of phytophagous insects (Lincoln 1993; Lindroth et al. 1993; Roth and Lindroth 1994, 1995). During drought, increased levels of foliar nitrogen and sugars may stimulate insect feeding (Mattson and Haack 1987*b*), while low leaf water content and (or) increased concentrations of secondary metabolites may prolong development and reduce growth rates (Scriber 1977; Martin and Van't Hof 1988; Ayres 1993).

Despite our understanding of the independent effects of CO_2 and drought on tree physiology, phytochemistry, and insect performance, we know very little about how the patterns described above will be altered by the interactive effects of CO_2 and water. Because elevated CO_2 conditions stimulate dry matter production and reduce transpiration of drought-stressed trees, water use efficiencies are often improved, leading to amelioration of drought stress (Tolley and Strain 1984; Mooney et al. 1991). Indeed, significant CO_2 stimulations of tree growth can occur under moderate water stress (Morison and Gifford 1984; Tolley and Strain 1984; Conroy et al. 1986). However, we do not know whether such an ameliorating effect occurs with respect to phytochemistry and, consequently, insect performance.

The overall purpose of this study was to assess how CO_2 and drought interact to alter tree–insect interactions. Our specific objectives were to (1) examine the direct and interactive effects of CO_2 and drought on the physiology and phytochemistry of quaking aspen (*Populus tremuloides* Michx.) and sugar maple (*Acer saccharum* Marsh.) and (2) evaluate the effects of CO_2- and drought-mediated changes in tree chemistry on the performance of the forest tent caterpillar (*Malacosoma disstria* Hbn.).

Quaking aspen and sugar maple are both widely distributed throughout the Great Lakes region and are important components of forest ecosystems. These species also differ markedly in terms of life history patterns, with aspen being a fast-growing, early successional species and maple a relatively slow-growing, late successional species. While the physiology and phytochemistry of both species respond to changes in CO_2 and water availability (Ellsworth and Reich 1992; Lindroth et al. 1993; Greitner et al. 1994), the magnitude and direction of some of the responses differ between the species. Aspen and maple are both suitable hosts for the forest tent caterpillar, which is polyphagous throughout the northeastern and northcentral United States.

Materials and methods

Experimental design

This experiment was conducted in controlled environmental rooms at the University of Wisconsin Biotron. We used a split split plot design for this experiment, with CO_2 level (350 and 700 ppm) as the whole plot; three rooms at each CO_2 level provided true replication at the whole plot level. Each room was divided into two subplots, corresponding to water treatment (control or drought); three large planters per water treatment provided replication at this level. Within each planter, the two tree species represented sub-subplots.

Experimental setup

We obtained 2-year-old sugar maple seedlings from the Wisconsin Department of Natural Resources (Boscobel, Wis.) and grew quaking aspen (full siblings) from seed 1 year prior to the experiment (seed source: University of Minnesota Northcentral Experiment Station, Grand Rapids, Minn.). Dormant seedlings were planted in plastic-lined plywood planters (90 × 80 × 30 cm), perforated at the bottom for drainage, containing 200 L of a 1:1:1:1 topsoil–peat–sand–Perlite mixture. This soil mixture gave the most uniform dry-down of several mixtures evaluated during a preliminary study. Osmocote® fertilizer (19:6:12 N–P–K, 3- to 4-month release rate) was added to the soil mix to achieve a moderate level of fertilization (3.5 $g \cdot L^{-1}$). Within each planter, 7 aspen and 13 maple seedlings were arranged in an alternating, uniform pattern and were watered twice daily with an autoirrigation system. Twenty-five days after planting, water was withheld from three of the six planters per room to begin the drought treatment (day 0), which progressed for the remainder of the experiment.

The benefits of using planters instead of individual pots for a drought study are numerous. First, plants within a planter should experience more uniform soil moisture conditions than plants in separate pots. Second, the onset of drought in large soil volumes is gradual and more realistically recreates natural drought conditions (Hinckley et al. 1978) than do pot dry-down studies, which often have rapid onset of extreme drought stress and require rewatering during the experiment. The gradual onset of drought allows plants time to respond morphologically and physiologically in a more natural manner. Third, large soil volumes minimize root restriction, and interplanting provides some degree of the complexity and heterogeneity of a natural community.

Rooms were maintained at 15 h light : 9 h dark, with a photosynthetic photon flux density (PPFD) of 566 ± 4 (SD) $\mu mol \cdot m^{-2} \cdot s^{-1}$. The spectral quality of sunlight was approximated by providing incandescent lighting during the first and last hour of each photoperiod. Light and dark temperatures and humidity were 25 and 19°C and 40 and 40%, respectively. Throughout the experiment, relative humidity and temperature were altered slightly, as needed, to maintain steady dry-down conditions. Daily predawn water potential measurements enabled us to track the onset of water stress within the planters. Our foliage sampling and insect feeding trials began when aspen trees reached a moderate stress level (predawn water potential of approximately –1.0 MPa, day 20; Fig. 1).

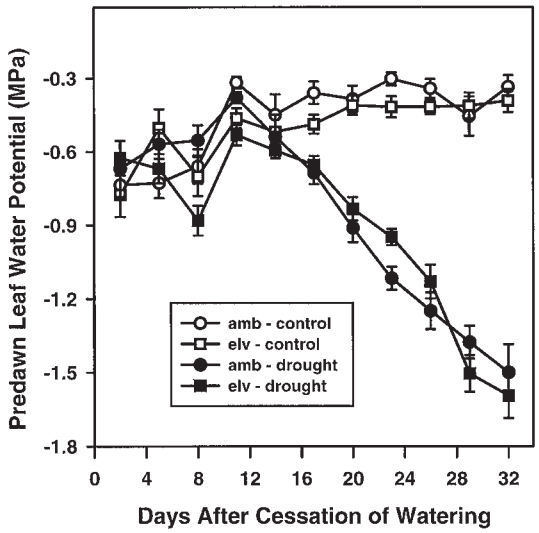
Tree physiology

Beginning on the first day of drought (day 0), we randomly subsampled trees from each treatment for predawn leaf water potential measurements. One hour prior to dawn, we excised individual leaves at the petiole for measurements with a pressure bomb (Soilmoisture Corp., Santa Barbara, Calif.). We sampled one aspen and one maple seedling from each water treatment per chamber per day, such that all nine planters in each CO_2 × water treatment were sampled over a 3-day period. We were thus able to obtain a 3-day mean leaf water potential for each CO_2 × water treatment. Because maple seedlings exuded large amounts of phloem sap which prevented reliable measurements using the pressure bomb, we present water potential data for aspen only.

Table 1. Larval nutritional indices (Waldbauer 1968) (all weights are expressed in milligrams dry weight).

RGR: relative growth rate = biomass gained·initial insect weight^{-1}·day^{-1}
RCR: relative consumption rate = food ingested·initial insect weight^{-1}·day^{-1}
AD: approximate digestibility = [(food ingested – feces)/food ingested] × 100
ECD: efficiency of conversion of digested food = [biomass gained/(food ingested – feces)] × 100
ECI: efficiency of conversion of ingested food = (biomass gained/food ingested) × 100

Fig. 1. Water potential measurements of well-watered (control) and droughted quaking aspen seedlings grown in ambient (amb) or elevated (elv) CO_2 atmospheres. Day 0 = first day of drought treatment. Each point represents a 3-day average of three samples taken per day.



To determine the degree of dry-down among treatments, we measured soil dryness using a soil tensiometer (Soilmoisture Corp., Santa Barbara, Calif.). On day 18, soil tension readings were taken from the center of each planter 15–20 cm from the soil surface.

A portable gas exchange system (LI-COR 6400, Lincoln, Nebr.) was used to measure leaf gas exchange at the beginning of the insect feeding trials (day 20, see below). To obtain measures of in situ photosynthetic rates, a climate-controlled leaf cuvette reproduced chamber light intensity, temperature, and CO_2 levels. During the middle of the photoperiod on days 19–21, we measured gas exchange rates for one aspen and one maple tree per planter, for a total of nine trees per CO_2 × water × species treatment.

Foliar chemistry

To assess the effects of CO_2 and drought on leaf chemistry, foliage was collected during the insect bioassays (see below), flash frozen in liquid nitrogen, freeze-dried, ground, and stored at –20°C until analyzed. Samples were collected from the same trees as those used for insect bioassays (n = 108) and were analyzed for primary and secondary compounds likely to be altered by elevated CO_2 and (or) drought.

We used a modified Kjeldahl procedure followed by Nesslerization to quantify foliar nitrogen (Lang 1958; Parkinson and Allen 1975). This assay is insensitive to NO_3^-, a relatively small fraction of total tissue nitrogen. Levels of soluble plant protein were measured with the Bradford assay (Bradford 1976; Jones et al. 1989) using bovine serum albumin as the standard. Samples were analyzed for total nonstructural carbohydrates (starch and simple sugars) by the method of Schoeneberger et al. (unpublished method). Condensed tannins were measured in both species by the method of Porter et al. (1986). We used purified aspen tannin as the standard and therefore emphasize that between-species comparisons should be interpreted with appropriate caution. For maple samples only, gallotannins and ellagitannins were quantified with gallic acid and ellagic acid as the standards, respectively (Inoue and Hagerman 1988; Wilson and Hagerman 1990). Aspen does not contain measurable levels of these hydrolyzable tannins (Lindroth et al. 1993). Finally, we used high-performance thin-layer chromatography (HPTLC) to measure levels of the aspen phenolic glycosides salicortin and tremulacin (see Lindroth et al. 1993 for detailed methods).

Insect bioassays

Forest tent caterpillar egg bands were obtained from Forestry Canada (Fredericton, N.B.) and placed in a Percival growth chamber at 25°C, 15 h light : 9 h dark. Upon hatching, larvae were reared on foliage collected from local, field-grown aspen or maple. When the trees reached a moderate stress level (day 20), insects entering the molt to the fourth larval stadium were used in feeding bioassays. The use of the penultimate instar is advantageous because the stadium has easily observed, well-defined beginning and endpoints, and high consumption rates allow for good assessments of food utilization efficiencies. Newly molted fourth-instar larvae were weighed and caged individually onto a single aspen or maple leaf. We measured the area of each leaf with a leaf area meter (CID, Vancouver, Wash.) and obtained measures of specific leaf mass from each tree to quantify consumption rates. Larvae fed until the end of the fourth stadium, with cages moved to new leaves, as necessary, to replenish the food supply. At the end of the stadium, larvae, frass, and all remaining leaf material in the cages were collected, dried, and weighed. Standard Waldbauer (1968) nutritional indices were calculated to provide measures of insect performance (e.g., growth; Table 1). On aspen, we assayed one insect on each of two trees per planter per room for a total of 72 feeding trials. Because gas exchange rates of maple are much lower than those of aspen, we anticipated a later onset of moderate stress in maple, and therefore, feeding trials on maple were delayed slightly, relative to feeding trials on aspen. Consequently, we had sufficient larvae for assaying only one maple tree per planter (n = 36 total). The remaining trees in each planter were used for other experiments, to be reported elsewhere.

Statistics

We used analysis of variance (ANOVA, PROC GLM; SAS Institute Inc. 1989) for statistical analysis of data on tree physiology and foliar chemistry. The split split plot model was

$$Y_{ijkm} = \mu + C_i + E_{ij} + W_k + (CW)_{ik} + e_{ijk} + S_m + (CS)_{im} + (WS)_{km} + (CWS)_{ikm} + \varepsilon_{ijkm}$$

where Y_{ijkm} represents the average response over all trees of species m in CO_2 level i, room j, and water treatment k. Fixed effects included CO_2 level (C_i), water treatment (W_k), species (S_m), and each of the various interaction terms ($(CW)_{ik}$, $(CS)_{im}$, $(WS)_{km}$, and $(CWS)_{ikm}$). Random effects included the whole plot error (E_{ij}), subplot error (e_{ijk}), and sub-subplot error (ε_{ijkm}). F-tests were conducted for C_i with E_{ij} as the error term ($F_{[1,4]}$), for W_k and $(CW)_{ik}$ with e_{ijk} as the error term ($F_{[1,4]}$ for both), and for S_m, $(CS)_{im}$, $(WS)_{km}$, and $(CWS)_{ikm}$ with ε_{ijkm} as the error term ($F_{[1,8]}$ for each). Means for each CO_2 × water × species combination were calculated (PROC MEANS; SAS Institute Inc. 1989) prior to ANOVA. The standard errors we report are for the treatment means Y_{ikm} (n = 3 rooms). Because hydrolyzable tannins

occur only in maple, we used a simplified version of this model (i.e., split plot only) to perform *F*-tests on C_i, W_k, and $(CW)_{ik}$, using the error terms described above. A similar split plot model was used for the analysis of phenolic glycosides, which occur only in aspen.

We also used the model described above to analyze results from our insect bioassays. The analysis of growth and consumption parameters in insect performance bioassays has been debated (Raubenheimer and Simpson 1992; Horton and Redak 1993). Historically, growth and consumption measures have been presented in the relative form (i.e., relative growth rate (RGR) and relative consumption rate (RCR)) to correct for variation in insect weight at the start of the experiment. Raubenheimer and Simpson (1992), however, have recently argued that performing a covariate analysis on absolute measures of growth and consumption, using initial insect weight as a covariate, is more appropriate.

The split split plot design of this experiment precluded a straightforward covariate analysis of insect performance, and the modified version that we used previously for split plot designs (Roth and Lindroth 1994) could not be used because of imbalance in our design (fewer bioassays on maple than aspen). For these reasons, we analyzed growth and performance data in terms of standard ratio variables, using ANOVA on the model described above (PROC GLM; SAS Institute Inc. 1989).

Results

Tree physiology

Water potential measurements did not differ among treatments until 17 days after the onset of drought (Fig. 1). After day 17, we observed a significant decline in water potential in the drought treatments, while the water potential in the control treatments remained relatively constant. In addition, soil tension measurements revealed a significant drought-mediated increase in the degree to which water adhered to soil particles ($P < 0.001$). Means (±1 SE) for the ambient CO_2 – control, ambient CO_2 – drought, elevated CO_2 – control, and elevated CO_2 – drought treatments were 0.622 (0.137), 23.33 (1.39), 0.722 (0.147), and 21.11 (0.444) kPa, respectively. Given the large soil volumes and water holding capacity of the soil, the onset of water stress in the drought planters was gradual.

With respect to gas exchange parameters (Fig. 2), photosynthetic rates increased under elevated CO_2 and declined during drought, but the magnitude of response was significantly greater for aspen than for maple ($CO_2 \times$ spp and $H_2O \times$ spp effects). Stomatal conductance was significantly reduced by drought, and the decrease was slightly smaller in elevated than in ambient CO_2 (80 vs. 86%, respectively; $CO_2 \times H_2O$ effect) and greater in aspen than in maple ($H_2O \times$ spp effect). For both gas exchange parameters, the species differed significantly, with aspen having at least double the values of maple for any given treatment (spp effect).

Foliar chemistry

CO_2 and water availability affected primary metabolites both directly and interactively, and the responses were species specific (Fig. 3). With respect to foliar water content, we observed a significant but slight (2.5%) decline in foliar water content under elevated CO_2. In terms of drought effects, foliage from drought treatments contained 10% less water than foliage from control treatments. The water content of maple was 17% less than that of aspen.

Nitrogen levels declined on average 14% in response to elevated CO_2, with aspen and maple responding similarly. The species responded differently, however, to drought stress, as nitrogen levels declined in aspen but not in maple ($H_2O \times$ spp effect). Overall, maple contained 40% less nitrogen than did aspen.

Aspen and maple differed in terms of the effects of CO_2 and water on protein concentrations. For aspen, protein levels were influenced by an interaction between CO_2 and water; drought caused protein concentrations to decline, but the magnitude of the decline was less under elevated than ambient CO_2 (12 vs. 28%, respectively). In contrast, maple protein levels were unaffected by either elevated CO_2 or drought. Similar to the pattern observed for nitrogen, maple contained half as much protein as aspen.

For both aspen and maple, starch levels were unaffected by CO_2 treatment but declined significantly under drought stress. Maple exhibited a greater reduction in starch concentrations under drought than did aspen (68 vs. 40% respectively; $H_2O \times$ spp effect). Starch levels also varied between species, with maple containing three times more starch than aspen.

With respect to hexose, the two species responded to elevated CO_2 and drought very differently. For aspen, elevated CO_2 resulted in an overall increase in hexose concentrations, while drought caused hexose levels to decline. Maple responded in the opposite manner, with levels declining 39% under elevated CO_2 but doubling under drought conditions. Levels of hexose in both species, however, were influenced by an interaction between CO_2 and water such that drought-mediated changes in hexose concentrations were substantially

Fig. 2. Photosynthetic rates and stomatal conductance measurements for well-watered (unhatched bars) or droughted (hatched bars) quaking aspen and sugar maple seedlings grown under ambient (white bars) or elevated (shaded bars) CO_2 conditions. Vertical lines represent 1 SE. spp, species effect; amb, ambient CO_2; elv, elevated CO_2.

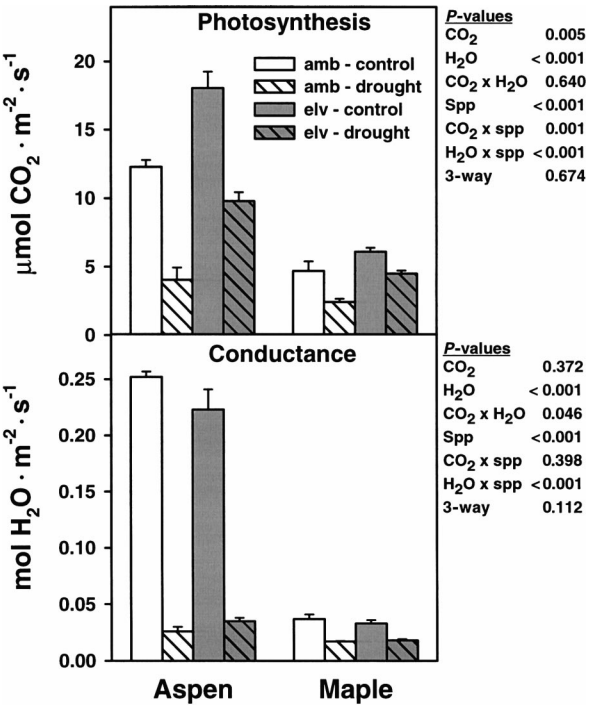
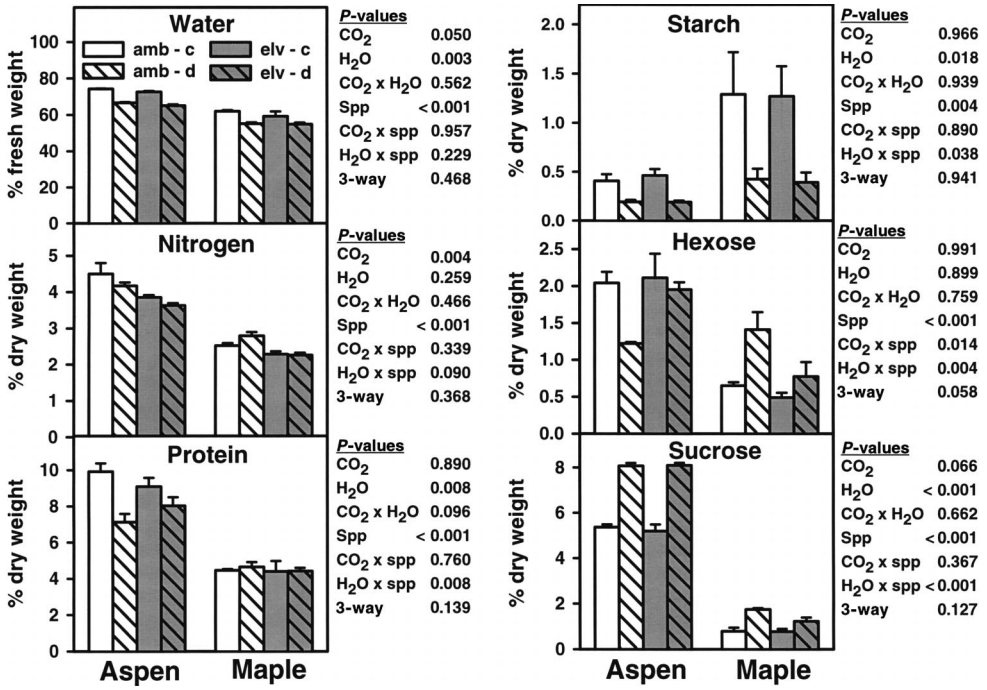


Fig. 3. Concentrations of primary metabolites in foliage of well-watered (unhatched bars) or droughted (hatched bars) quaking aspen and sugar maple seedlings grown under ambient (white bars) or elevated (shaded bars) CO_2 conditions. Vertical lines represent 1 SE. spp, species effect; amb, ambient CO_2; elv, elevated CO_2; c, control; d, drought.



smaller under elevated CO_2 than under ambient CO_2, regardless of the species or the direction of the change (three-way interaction). Overall, aspen contained twice as much hexose as maple.

Sucrose levels were in general affected less by CO_2 than by water treatment, with levels changing little in high CO_2 but increasing significantly under drought stress for both species. As was the case for starch, maple responded more strongly to drought than did aspen in terms of changes in concentrations of sucrose (increases of 89 vs. 53%, respectively; $H_2O \times$ spp effect). Similar to the pattern observed for hexose, aspen contained more sucrose than did maple.

Concentrations of secondary metabolites in both aspen and maple were affected by CO_2 and drought independently but not interactively (Fig. 4). For both species, condensed tannin levels increased under elevated CO_2 but were not altered by drought stress. In addition, maple responded more strongly to enriched CO_2 than did aspen ($CO_2 \times$ spp effect).

Aspen phenolic glycosides were affected by CO_2 treatment, with concentrations of salicortin and tremulacin increasing 45 and 69%, respectively, in enriched CO_2 environments. Drought also altered phenolic glycoside levels, resulting in 21 and 14% declines in salicortin and tremulacin, respectively.

With respect to hydrolyzable tannins, which are limited to maple, gallotannin levels nearly tripled under elevated CO_2, while ellagitannin levels rose 55%. Drought had no effect on the production of either of these tannins.

Insect bioassays

Forest tent caterpillar performance varied between host species and in response to CO_2- and drought-mediated changes in tree chemistry (Fig. 5). We found no effects of CO_2 or drought on stadium duration (data not shown). RGR declined on average 37% under elevated CO_2 and 36% under drought stress. These responses were influenced, however, by host species (three-way interaction). For example, drought caused a 45% decline in growth under ambient CO_2 but only a 4% decline under elevated CO_2 for aspen-fed larvae. In contrast, this mitigation of drought effects by elevated CO_2 did not occur for larvae feeding on maple; drought reduced growth similarly in ambient and elevated CO_2. Overall, growth rate on maple was reduced 65% relative to aspen, irrespective of CO_2 level or water treatment.

Larval consumption was also influenced by a complex interaction of CO_2, H_2O, and species (i.e., no significant main effects). Drought-induced changes in consumption differed between the two tree species; aspen-fed larvae increased consumption under drought stress, while maple-fed larvae reduced consumption (i.e., $H_2O \times$ spp effect). The extent to which CO_2 level modified these drought responses depended on the species (i.e., three-way interaction). On aspen, larval consumption rates increased under drought stress, regardless of CO_2 level (27 and 21%, respectively, in ambient and elevated CO_2). In contrast, larval consumption on maple declined under drought conditions, and this effect was more severe in enriched CO_2 environments (reductions of 7 vs. 42% in ambient versus elevated CO_2). In general, consumption rates on maple were slightly less than on aspen. Patterns for total larval consumption (data not shown) in general mirrored those for RCR.

The efficiency of conversion of ingested food (ECI) is the mathematical product of approximate digestibility (AD) and conversion efficiency of digested food (ECD) (see Table 1). As such, ECI is a measure of how efficiently an insect digests food and converts the products to body mass. ECI was significantly affected by host species, CO_2 level, water treatment, and the interactions of these factors. Insects fed high CO_2 or

droughted foliage of either species exhibited reduced ECIs, and the decline in ECI on droughted foliage was substantially less under elevated than ambient CO_2 ($CO_2 \times H_2O$ effect). Although this pattern of response was observed for insects feeding on both aspen and maple, maple-fed insects, in general, responded less strongly to CO_2- or H_2O-mediated changes in foliar quality ($CO_2 \times$ spp, $H_2O \times$ spp, and three-way effects). Overall, aspen-fed larvae converted ingested food to body mass three times more efficiently than did maple-fed larvae. These effects of CO_2 and water on ECI were likely mediated by shifts in larval ECDs rather than ADs. Approximate digestibility did not differ significantly between CO_2 or water treatments, while changes in ECDs (data not shown) were similar to the changes observed for ECIs.

Discussion

Tree physiology

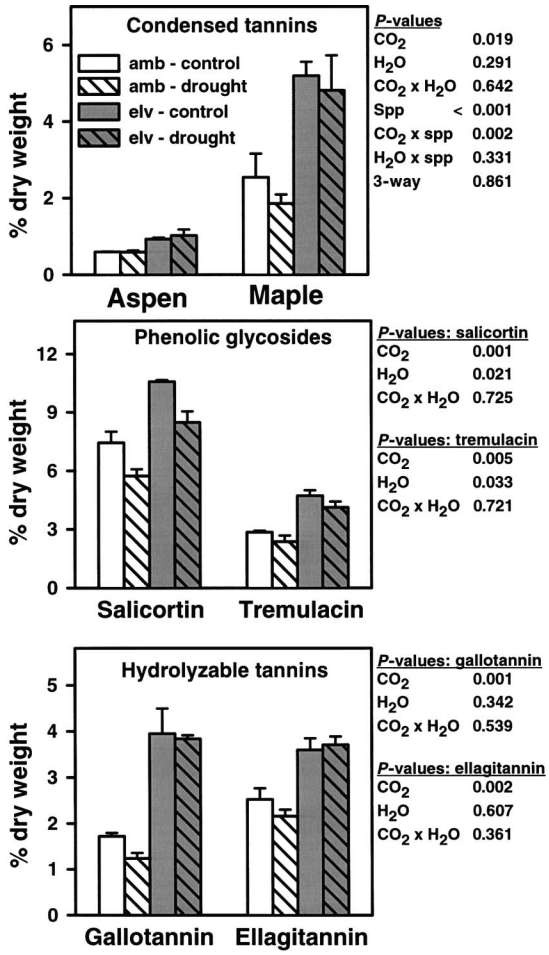
CO_2 and drought effects

In terms of gas exchange, elevated CO_2 increased photosynthetic rates for both aspen and maple, as expected. This stimulation of photosynthesis could be due to a steeper CO_2 diffusion gradient alone or in conjunction with an increase in photosynthetic capacity due to physiological adjustment. Our results are in accordance with Ceulemans and Mousseau's (1994) estimation that the average enhancement of photosynthesis is 61% among broad-leaved trees grown under an approximate doubling of ambient CO_2 conditions. Although several studies have documented the down-regulation of photosynthesis in response to long-term exposure to elevated CO_2 (see Ceulemans and Mousseau 1994), we cannot determine whether or not down-regulation occurred in this study. The fact that photosynthetic rates of both tree species remained stimulated by elevated CO_2 throughout this study suggests that if indeed photosynthetic down-regulation did occur, the magnitude is much smaller than that observed in previous studies (Ceulemans and Mousseau 1994). Perhaps the relatively unrestricted root growth in the large soil volumes of the planters maintained root sink strength and thereby reduced the accumulation of carbohydrates that trigger down-regulation of photosynthesis (Herold 1980).

CO_2 had no significant effect on stomatal conductance. Although the stomatal conductance of broad-leaved trees often declines 10–60% in enriched CO_2 (Eamus and Jarvis 1989), several studies have failed to detect significant CO_2 effects on stomatal conductance (Conroy et al. 1988; Bunce 1992; Gunderson et al. 1993). Tschaplinski et al. (1995) suggested that CO_2-mediated declines in stomatal conductance are more likely observed when initial rates are high. Thus, the lack of a CO_2 effect on conductance may have been due to the relatively low conductance of both species under ambient conditions.

Both photosynthetic rates and stomatal conductance were significantly reduced by drought, as expected. Ni and Pallardy (1991) and Ellsworth and Reich (1992) documented declines in photosynthesis and stomatal conductance in sugar maple during drought, and the magnitude of the effects was similar to those observed in our study. Similarly, Rhodenbaugh and Pallardy (1993) showed that photosynthesis and stomatal conductance are sensitive to even moderate declines in water potential among *Populus* species, and our data are in accordance with their findings.

Fig. 4. Concentrations of secondary metabolites in foliage of well-watered (unhatched bars) or droughted (hatched bars) quaking aspen and sugar maple seedlings grown under ambient (white bars) or elevated (shaded bars) CO_2 conditions. Vertical lines represent 1 SE. spp, species effect; amb, ambient CO_2; elv, elevated CO_2. Phenolic glycoside data are for aspen only; gallotannin and ellagitannin data are for maple only.



A common response to both elevated CO_2 and drought is improved water use efficiency (WUE), expressed as the ratio of photosynthesis to transpiration. In this study, WUE improved under enriched CO_2 conditions, due to the stimulation of photosynthetic rates. In addition, WUE doubled in response to drought, despite declines in both photosynthesis and transpiration rates, because the decline in transpiration was larger than the decline in photosynthesis. Under water-limiting conditions, changes in WUE may have an ecological impact on a plant's ability to survive or compete. It should be noted that, in this study, soil water conserved by individual plants due to improved WUE was available to other plants within each planter, as is the case in natural communities. For this reason, it is difficult to assess the degree to which improved WUE benefits an individual or aids competitors. Moreover, an increase in WUE when water is not limiting would appear to have little ecological significance in terms of species interactions.

CO_2 × drought interactions

In terms of interactive effects of CO_2 and drought, we observed statistically significant effects only in relation to stomatal conductance. The magnitude of the differences, however, was fairly slight and of little biological significance. In contrast, elevated CO_2 appeared to ameliorate the drought-induced reductions in photosynthesis; averaged across species, drought reduced photosynthetic rates by 58% in ambient CO_2 but only 36% in elevated CO_2. Although these differences were not statistically significant, integrated over the course of the drought, the differences in photosynthetic rates between CO_2 treatments could result in significant differences in carbon gain. Depending on allocation patterns, differences in carbon gain could strongly influence phytochemical responses.

Foliar chemistry

CO_2 effects

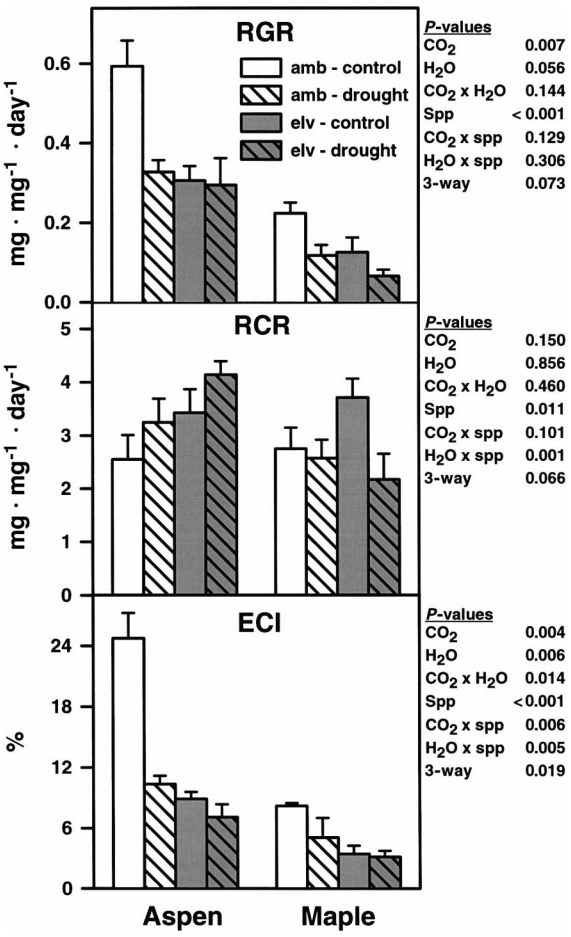
As predicted, we observed an increase in the C:N ratio of foliage grown in enriched CO_2 environments. Foliar nitrogen levels typically decline 5–25% under elevated CO_2 conditions (Fajer 1989; Johnson and Lincoln 1990; Lindroth 1996*b*), and the overall decline of 14% observed in this experiment is consistent with other studies. The results of our protein analyses are also in accordance with earlier studies showing little to no CO_2-mediated change in protein concentrations (Roth and Lindroth 1994, 1995). The lack of similarity between nitrogen and protein responses is puzzling, but may be due to failure of the protein assay to quantify insoluble proteins, levels of which may be sensitive to elevated CO_2.

In terms of carbon-based primary metabolites, soluble sugars typically exhibit variable responses to CO_2 enrichment, depending on the tree species and the type of sugar considered (Lindroth 1996*b*). In our study, for example, hexose levels increased under elevated CO_2 in aspen but declined in maple, while sucrose levels remained unchanged in both species. We observed no CO_2-mediated increase in foliar starch concentrations, in contrast with several previous studies (Lindroth et al. 1993; Roth and Lindroth 1994, 1995; Kinney et al. 1997). This study differed from previous ones, however, in that seedlings were grown together in large planters instead of individually in pots. Less root restriction may have enabled roots to serve as a carbohydrate sink to a greater extent than in previous studies, minimizing starch accumulation in leaves. With respect to carbon-based secondary metabolites, concentrations increased under elevated CO_2 for both species, similar to results observed in previous studies (Lindroth et al. 1993; Roth and Lindroth 1994, 1995; Kinney et al. 1997).

Drought effects

Drought stress caused primary metabolites to change as expected. In response to drought, rates of protein hydrolysis typically increase, resulting in a shift of plant nitrogen from protein to amino acids, which accumulate in the foliage and serve as osmotica (Kramer 1983). Furthermore, overall rates of nitrogen metabolism may increase (Kramer 1983). Declines in nitrogen and protein levels were observed for aspen, while levels in maple were unresponsive to drought stress. In addition to changes in plant protein, breakdown of starch to soluble sugars is a common response to drought stress (Kramer 1983; Mattson and Haack 1987*b*). Our results suggest that both aspen and maple responded in this manner, since levels of starch declined and sugars (sucrose for aspen, hexose for maple) increased in the drought treatments.

Fig. 5. Performance of fourth-instar forest tent caterpillars fed foliage from well-watered (unhatched bars) or droughted (hatched bars) quaking aspen or sugar maple seedlings grown under ambient (white bars) or elevated (shaded bars) CO_2 conditions. Vertical lines represent 1 SE. spp, species effect; amb, ambient CO_2; elv, elevated CO_2; RGR, relative growth rate; RCR, relative consumption rate; ECI, conversion efficiency of ingested food.



Our expectation that secondary metabolites would increase under drought stress was not supported. None of the three types of tannins measured showed significant responses to drought, opposite to the predicted trend. Levels of phenolic glycosides actually declined in the drought treatments, similar to the responses observed by Kruger and Manion (1994). This lack of an increase in secondary metabolites may be attributed to a number of factors. First, drought may have suppressed activity of enzymes required for phenolic biosynthesis (Gershenzon 1984), preventing an increase in production. Also, drought may have retarded photosynthesis more than nutrient uptake, leading ultimately to reductions in secondary metabolism (Ayres 1993).

Species differences

Aspen and maple differed markedly in terms of their physiological responses to elevated CO_2 and drought. These differences

likely reflect variation in growth rates and life history patterns between the two species. Aspen is an early successional species with a high growth rate and, thus, relatively high rates of gas exchange when compared with maple, a slow-growing, late successional species. These inherent differences in growth rates may allow greater plasticity for aspen in response to changes in resources such as CO_2 or water. In our study, the magnitude of physiological responses to CO_2 and drought was greater, overall, in aspen than in maple. With respect to drought in particular, aspen exhibited greater leaf area reductions (personal observations) and more highly improved WUEs than did maple, representing a large change in total water use per plant. Aspen also maintained higher photosynthetic rates than maple, providing additional carbon during moderate drought stress.

The inherent plasticity of aspen, in terms of physiological responses to changes in resources, may translate into more pronounced phytochemical changes in aspen, relative to maple. In response to elevated CO_2, the species responded similarly in terms of changes in nitrogen and secondary compounds, and neither species demonstrated a CO_2-mediated change in starch concentrations. The species were affected very differently, however, by drought stress. Aspen responded to drought stress more strongly in terms of changes in protein and nitrogen than did maple, while both species exhibited substantial reductions in foliar starch and increases in sugars under drought. This physiological and phytochemical variation in response to CO_2 and drought between aspen and maple may have important implications for herbivorous insects.

Insect performance

The effects of CO_2- and drought-mediated changes in tree chemistry on forest tent caterpillars varied substantially between the species. To facilitate interpretation, we will discuss results separately for insects feeding on aspen versus maple.

Aspen hosts

Aspen-fed larvae exhibited reductions in growth and food processing efficiencies under elevated CO_2, despite a 30% increase in consumption rate. This pattern is typical for insects feeding on high-CO_2 foliage (Lindroth 1996*b*). Although increased consumption may compensate for the low nitrogen content of foliage, increased exposure to toxic secondary metabolites often results in reduced larval growth (Lincoln 1993, Lindroth 1996*a*).

Similar to elevated CO_2, drought negatively affected insect performance. Despite their increased consumption rates, larvae grew more slowly and processed food less efficiently when fed droughted foliage. According to Mattson and Haack (1987*b*), increased concentrations of osmolytes such as sugars can stimulate herbivore feeding, which may have been the case here. Decreases in growth rates were probably due to the fact that droughted foliage contained 10% less water than did control foliage. Scriber (1977) showed that 15–20% reductions in foliar water content negatively affected growth of *Hyalophora cecropia*, primarily due to impaired nitrogen utilization and assimilation efficiencies on the low-water treatments. In addition, low diet water content can also result in elevated larval respiration rates (Van't Hof and Martin 1989), and some insects may supplement their body water with metabolic water from respiration to compensate for low water content of the diet (Scriber 1977). Changes in either nitrogen utilization efficiencies or respiration rates may reduce growth rates via changes in food conversion efficiencies. In this study, drought-mediated declines in ECIs suggest that, indeed, a greater proportion of assimilated energy was metabolized, but we were unable to assess whether this was due to a decreased nitrogen utilization efficiency or an elevated respiration rate.

The reduction in larval performance on drought versus control treatments was greater in ambient than in elevated CO_2 ($CO_2 \times H_2O$ interaction). Overall, however, growth rates were reduced to the same extent by the effects of elevated CO_2 and drought alone or in combination, relative to the ambient CO_2 – control treatment, thus indicating that the effects of CO_2- and drought-mediated changes in tree chemistry were not additive. These nonadditive effects may be partially explained by complex interactions among protein, water, and phenolic glycoside levels.

Maple hosts

Like insects fed aspen, maple-fed larvae suffered reductions in performance under elevated CO_2. Our results are in accordance with those of Lindroth et al. (1993) who documented reductions in larval mass of forest tent caterpillars fed high-CO_2 maple foliage. The observed declines in growth rates under elevated CO_2 in this study may be attributed to the decline in foliar nitrogen, together with substantial increases in all three types of tannins.

Similar to results observed for larvae fed aspen, forest tent caterpillar growth declined on droughted maple foliage, reflecting changes in consumption rates and ECIs. Although neither tannin nor protein levels were affected by drought stress, water content of foliage declined 10%. This decline may have contributed to the reductions in ECIs, and thus growth, because of impaired nitrogen use efficiencies, as described for aspen-fed larvae. Consumption rates of maple-fed larvae either did not change or declined under drought conditions, in contrast with the increased consumption observed for larvae fed aspen. This difference may be attributed to the fact that nitrogen and protein levels declined under drought for aspen but remained unchanged in maple.

In contrast with the phenomenon observed for aspen-fed larvae, elevated CO_2 and drought together reduced larval growth more than did either factor alone. This is likely due to an additive effect of higher tannin concentrations and lower water content in the elevated CO_2 – drought treatment. In addition, consumption was reduced by drought more under elevated than ambient CO_2, which may have contributed to the poor larval growth in this treatment.

Conclusions and implications

This research demonstrates that both CO_2 environment and water availability can strongly influence physiology and foliar chemistry of deciduous trees. Moreover, the magnitude and direction of these responses may be affected by interactions between the two resources and may differ among tree species. The overall stronger response of aspen to drought stress and the partial amelioration of this stress under elevated CO_2 contrasts sharply with the response of maple, indicating that interactions of aspen with associated insect fauna may change less than will interactions between insects and maple under future climate conditions. Although this study examined responses of

seedlings only, future studies using larger, open-top chambers and (or) FACE (free air carbon enrichment) technology should help us determine the degree to which our results are applicable to established forest stands. We also recognize that the frequency and magnitude of drought conditions themselves will likely be affected by temperature changes associated with global warming. Consequently, we emphasize the need for further research exploring how drought in combination with changes in CO_2 and temperature will modify the types of responses observed in this study.

Our results for CO_2- and drought-mediated effects on phytochemistry have implications for the distribution and abundance of herbivores such as the forest tent caterpillar. Under current CO_2 conditions, the effects of drought on forest tent caterpillar performance are not influenced by host plant species. This study suggests, however, that herbivory levels and patterns of host use could shift during drought under future elevated CO_2 conditions, where drought would have less of an effect on aspen than on maple. To determine whether the effects on fourth-instar forest tent caterpillars observed in this study will translate into altered survival or fecundity, future studies should examine responses over an entire generation rather than a single stadium.

Finally, our results are not consistent with Mattson and Haack (1987*a*, 1987*b*), who suggested that insect performance will be enhanced by drought-mediated changes in phytochemistry. In this study, forest tent caterpillar growth was reduced in drought treatments relative to the control, and elevated CO_2 did not significantly ameliorate the observed growth reductions. However, other factors associated with droughts, such as changes in temperature or natural enemy efficacy, undoubtedly interact with foliar chemical changes to initiate outbreaks, as suggested by Mattson and Haack (1987*a*, 1987*b*).

In conclusion, the numerous interactive effects observed in this study reinforce the need for research examining multiple factors and species. We must continue to examine variation among tree species in response to CO_2 and water availability and acknowledge that intraspecific variation may also influence the patterns observed. In addition, we advocate the use of a greater variety of insect feeding guilds in future work, as most work to date has been limited to lepidopteran species. Doing so will greatly enhance our understanding of the ways in which elevated CO_2, in combination with other abiotic forces, will influence interactions between trees and insects in forest communities.

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