

Consequences of simultaneous elevation of carbon dioxide and temperature for plant–herbivore interactions: a metaanalysis

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Abstract

The effects of elevated carbon dioxide on plant–herbivore interactions have been summarized in a number of narrative reviews and metaanalyses, while accompanying elevation of temperature has not received sufficient attention. The goal of our study is to search, by means of metaanalysis, for a general pattern in responses of herbivores, and plant characteristics important for herbivores, to simultaneous experimental increase of carbon dioxide and temperature (ECET) in comparison with both ambient conditions and responses to elevated CO₂ (EC) and temperature (ET) applied separately. Our database includes 42 papers describing studies of 31 plant species and seven herbivore species. Nitrogen concentration and C/N ratio in plants decreased under both EC and ECET treatments, whereas ET had no significant effect. Concentrations of nonstructural carbohydrates and phenolics increased in EC, decreased in ET and did not change in ECET treatments, whereas terpenes did not respond to EC but increased in both ET and ECET; leaf toughness increased in both EC and ECET. Responses of defensive secondary compounds to treatments differed between woody and green tissues as well as between gymnosperm and angiosperm plants. Insect herbivore performance was adversely affected by EC, favoured by ET, and not modified by ECET. Our analysis allowed to distinguish three types of relationships between CO₂ and temperature elevation: (1) responses to EC do not depend on temperature (nitrogen, C/N, leaf toughness, phenolics in angiosperm leaves), (2) responses to EC are mitigated by ET (sugars and starch, terpenes in needles of gymnosperms, insect performance) and (3) effects emerge only under ECET (nitrogen in gymnosperms, and phenolics and terpenes in woody tissues). This result indicates that conclusions of CO₂ elevation studies cannot be directly extrapolated to a more realistic climate change scenario. The predicted negative effects of CO₂ elevation on herbivores are likely to be mitigated by temperature increase.

Keywords: carbohydrates, carbon-based secondary compounds, climate change, CO₂, host plant quality, insect performance, metaanalysis, nitrogen, plant–herbivore interactions, temperature elevation

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Introduction

Climatic changes in past decades (reviewed by IPCC, 2001) have already caused detectable effects on different organisms and ecosystem processes (Hughes, 2000; Parmesan & Yohe, 2003), and increases of both atmospheric carbon dioxide and temperature are expected to further affect ecosystem structure and functions. Along

with direct effects on plant physiology and growth, modified interactions between herbivores and their host plants may have important consequences for forestry (Volney & Fleming, 2000; Logan *et al.*, 2003) and agriculture (Fuhrer, 2003; Scherm, 2004).

Studies of plant and ecosystem responses to a carbon dioxide-enriched atmosphere have become increasingly popular during the past decades, and the results of these experiments have been summarized in both narrative reviews (Ceulemans & Mousseau, 1994; Cannell & Thornley, 1998; Karnosky, 2003) and metaanalytical

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studies (Curtis & Wang, 1998; Wand *et al.*, 1999; Poorter & Navas, 2003). However, not only carbon dioxide concentrations but also globally averaged surface temperatures are expected to increase under all IPCC emissions scenarios during the 21st century. The projected concentration of CO₂ in the year 2100 ranges from 540 to 970 ppm, compared with about 280 ppm in the pre-industrial era and about 368 ppm in the year 2000, accompanied by an increase in the globally averaged surface temperature of 1.4–5.8 °C over the period from 1990–2100 (IPCC, 2001). Temperature is a very important factor affecting herbivore performance (reviewed by Bale *et al.*, 2002), and therefore, temperature elevation should not be overlooked when predicting the impacts of climate change on plant–herbivore interactions (Johns & Hughes, 2002; Newman, 2003).

Surprisingly few case studies have explored the effects of elevated concentrations of carbon dioxide and elevated temperature on herbivore performance in such a way that both individual and combined effects of these two factors could be evaluated. Several authors concluded that temperature did not influence plant and herbivore responses to CO₂ elevation (Buse *et al.*, 1998; Williams *et al.*, 2000), while others demonstrated strong interactive effects of CO₂ and temperature (Johns & Hughes, 2002; Veteli *et al.*, 2002; Johns *et al.*, 2003). Therefore, reliable prediction of the effects of climate change on plant–herbivore interactions requires generalization of somewhat contradictory results of case studies exploring responses of insect–plant systems to simultaneous elevation of carbon dioxide and temperature. In particular, this analysis is necessary to understand whether predictions made on the basis of numerous CO₂-enrichment experiments can be extrapolated to a more realistic situation, when an increase in CO₂ is accompanied by an increase in temperature.

The principal goal of our study is to search for a general pattern in responses of herbivores, and plant characteristics important for herbivores, to a simultaneous experimental increase of carbon dioxide and temperature in comparison with both ambient conditions and elevated CO₂ and temperature acting separately. This is done by means of metaanalysis, a technique that allows statistical exploration and combination of results from a number of publications (Gurvitich & Hedges, 2001).

Material and methods

Suitability criteria and selection of information

We limited our metaanalysis to studies that fit the following criteria: (i) the study was manipulative; (ii) ambient concentration of CO₂ in combination with

ambient temperature served as the control; (iii) study organisms were subjected to elevated CO₂ alone, elevated temperature alone and combination of elevated CO₂ with elevated temperature; and (iv) the study reported measurements of either insect herbivore performance or plant traits, both chemical and mechanical, which determine plant quality for herbivores.

We considered studies reporting concentrations (on the mass basis) of nitrogen, nonstructural carbohydrates (total, sugars and starch) and carbon-based secondary defensive compounds (phenolics and terpenoids) in all aboveground plant parts that are utilized by herbivores, both leaves (needles) and woody parts (stems, twigs, bark). Mechanical traits, associated with leaf toughness, were restricted to chlorophyll-containing plant parts; for studies lacking toughness data, leaf thickness or specific leaf weight or specific leaf area were included as they provide reasonable approximation of leaf toughness (Lincoln *et al.*, 1993).

In most studies, temperature elevation treatment followed ambient temperature fluctuations, but in some studies chambers had totally controlled temperature regimes. In these cases, the lowest temperature treatment served as the control in all studies except for Reddy *et al.* (1998b), where we selected a temperature of not 20 °C but 25 °C as a control because of an obvious growth retardation of the study plant (cotton) at the lowest temperature. If more than two temperature regimes had been studied (seven publications), we selected the treatment next to the control (5–6 °C temperature increase), thus eliminating treatments with extreme temperature elevations (10–20 °C). If some other environmental variables (such as nutrient and water supply or light availability) had been manipulated, we selected treatments where plants did not experience additional experimentally induced stress. If the results were given for several sampling dates, we used the last record, except for situations where the last record deviated from the feeding period of principal herbivores; in these situations we selected the record corresponding to the timing of maximum herbivore damage. If several needle age classes had been analysed (most frequently formed during the current and previous years), we considered previous-year needles because they are preferentially used by herbivores (Larsson & Tenow, 1980).

Database

In March of 2005, we conducted key word searches in the following databases: Agricola (CSA), Biological Abstracts (Biosis), Biosis Previews (Biosis), Pubmed (NLM) and Web of Science (ISI). The key words used were carbon dioxide (or CO₂) and temperature or

warming. All identified papers were screened for both their suitability (see the above criteria) and for additional references; some extra references were obtained from the review articles. In total, 120 + publications were been screened, and 42 of them (Tables 1 and 2) were accepted for the analysis.

To allow effect size calculation, the study needs to contain a mean value, some measure of variance (SE or SD) and sample size for each variable in either numerical or graphical form. If values had to be determined from a graph, figures were enlarged and manually digitized. If some information was lacking (absence of SE was the most frequent problem), the authors were requested to fill the gaps. In spite of the continuous effort, we were unable to obtain responses from authors of eight potentially suitable studies, and these papers (Cao *et al.*, 1994; Nijs & Impens, 1996; Cao & Tibbits, 1997b; Buse *et al.*, 1998; Fritschi *et al.*, 1999; Ro *et al.*, 2001; Thomas *et al.*, 2003; Wheeler *et al.*, 2004) were therefore excluded from the numerical analysis.

We divided the response variables into several groups: (1) total nitrogen and (2) C/N ratio (indices of plant nutritive quality), (3) water, (4) total nonstructural carbohydrates (TNC), (5) sugars, (6) starch, (7) pooled carbon-based secondary compounds (CBSC, measures of plant chemical defence), including individual compounds and groups of compounds, (8) phenolic compounds, (9) terpenoids, (10) mechanical leaf traits (leaf toughness, specific leaf weight or mass, specific leaf area, leaf thickness) and (11) direct measures of herbivore performance (RGR, survival, weight or mass, fecundity, duration of development) (Fig. 1). Consumption was not analysed because only two studies reported this character.

Exploration of the effects of methodology

To study the effects of methodology on plant responses (low number of studies reporting herbivore performance made a similar analysis impossible), we selected concentrations of nitrogen and phenolics, because they are often considered as indices of plant quality for herbivores and in the primary studies they were reported more frequently than other substances, allowing sufficient sample sizes for metaanalysis.

Majority of studies used greenhouses or chambers with an automatically controlled environment, sometimes under natural illumination. Only four studies were conducted by using open-top chambers (Norby *et al.*, 2000; Williams *et al.*, 2000, 2003) or semiclosed chambers (Snow *et al.*, 2003); we contrasted these two kinds of studies to check for the possible effect of experimental installation. We also compared responses with 1.5-fold vs. twofold increase in CO₂ concentrations, and to high (≥ 4 °C) vs. low (< 4 °C) temperature elevation.

The effect of treatment duration on woody plants was explored by comparing one-season (and shorter) treatments with multiyear experiments. Similar analysis had not been conducted for herbs, because for these plants the longevity of treatment was usually comparable with the length of the growth season. We also compared treatment effects between seedlings and cuttings. Comparison with mature trees was impossible because of the low number of measurements; saplings were absent in our database. Because a large fraction of the data (21.4% of nitrogen measurements and 50% of the measurements of phenolics conducted in leaves of woody angiosperms) was obtained by studying common birch, *Betula pendula* Roth., we contrasted this species with other woody angiosperms to check for possible taxonomic bias.

Finally, in order to check whether the use of data from pseudoreplicated primary studies (with only one chamber per treatment; see Hurlbert (1984) for a definition of pseudoreplication) had biased our conclusions, we (i) compared the effect sizes calculated for replicated and nonreplicated experiments and (ii) conducted each individual analysis twice – with and without pseudoreplicated studies. The second operation appeared redundant (data not shown); all the results reported below have been obtained by analysing the entire data set.

Metaanalysis

We have preferred the routine metaanalysis technique to factorial metaanalysis (Gurevitch *et al.*, 2000), because we were primarily interested in the magnitude of the combined effect, rather than in the interaction between the effects of elevated carbon dioxide and elevated temperature. Furthermore, responses to one of these two factors frequently were of different sign at different levels of another factor (see Results), thus hampering interpretation of the main effects in the factorial metaanalysis.

We used Hedge's *d* as a measure of the effect size, calculated as the difference between the means of the experimental and control data divided by the pooled standard deviation. For each study, elevated CO₂ alone (EC), elevated temperature alone (ET) and simultaneous elevation of CO₂ and temperature (ECET) were compared with the same control (ambient CO₂ + ambient temperature). The mean effect sizes for each treatment were calculated and compared using the MetaWin 2.0 statistical program (Rosenberg *et al.*, 2000). Separate metaanalyses were conducted for each class of response variables. The treatment was considered to have a statistically significant effect if the 95% CI of the mean effect size did not overlap zero (Gurevitch & Hedges, 2001). The effects of different treatments on the same variable and responses of different variables to the same treatment were compared by calculating between-class

Table 1 Plant species, class (A, angiospermae; G, gymnospermae), growth form (w, woody plants; h, herbaceous plants), plant parts, measured plant traits (N, total nitrogen; TNC, total nonstructural carbohydrates; SLW, specific leaf weight; SLA, specific leaf area) and references used in the metaanalysis

Plant species	Class	Form	Plant part	Plant traits	Reference
<i>Abutilon theophrasti</i>	A	h	Leaves, stem	N	Coleman & Bazzaz (1992)
<i>Acer rubrum</i>	A	w	Leaves	N, TNC, sugars, starch, phenolics	Williams <i>et al.</i> (2000)
<i>A. rubrum</i>	A	w	Leaves	N, TNC, sugars, starch, phenolics	Williams <i>et al.</i> (2003)
<i>A. rubrum</i>	A	w	Leaves	N, SLW	Norby <i>et al.</i> (2000)
<i>Acer saccharum</i>	A	w	Leaves	N, TNC, sugars, starch, phenolics	Williams <i>et al.</i> (2000)
<i>A. saccharum</i>	A	w	Leaves	N, SLW	Norby <i>et al.</i> (2000)
<i>Amaranthus retroflexus</i>	A	h	Leaves, stem	N	Coleman & Bazzaz (1992)
<i>Betula papyrifera</i>	A	w	Leaves	N, TNC, SLA	Tjoelker <i>et al.</i> (1999)
<i>B. papyrifera</i>	A	w	Leaves	Sugars, starch	Tjoelker <i>et al.</i> (1998)
<i>Betula pendula</i>	A	w	Leaves	N, water, phenolics	Kuokkanen <i>et al.</i> (2001)
<i>B. pendula</i>	A	w	Stem	N, water, phenolics	Kuokkanen <i>et al.</i> (2001)
<i>B. pendula</i>	A	w	Leaves	N, phenolics	Kuokkanen <i>et al.</i> (2003)
<i>B. pendula</i>	A	w	Leaves	N, starch, SLA	Kellomäki & Wang (2001)
<i>B. pendula</i>	A	w	Stem	Phenolics, terpenes	Kuokkanen <i>et al.</i> (2004)
<i>B. pendula</i>	A	w	Bark	Phenolics, terpenes	Mattson <i>et al.</i> (2004)
<i>Bouteloua gracilis</i>	A	h	Leaves	N, TNC, sugars, starch, SLW	Read <i>et al.</i> (1997)
<i>Dendranthema grandiflora</i>	A	h	Leaves	N, C/N, water	Chong <i>et al.</i> (2004)
<i>Echium plantagineum</i>	A	h	Leaves	N, C/N, leaf thickness	Johns & Hughes (2002)
<i>Eriophorum vaginatum</i>	A	h	Leaves	TNC	Tissue & Oechel (1987)
<i>Glycine max</i>	A	h	Seeds	Phenolic	Caldwell <i>et al.</i> (2005)
<i>G. max</i>	A	h	Leaves	N	Bunce & Ziska (1996)
<i>Gossipium hirsutum</i>	A	h	Leaves	Sucrose, starch	Reddy <i>et al.</i> (1998a)
<i>G. hirsutum</i>	A	h	Leaves	TNC, SLW	Reddy <i>et al.</i> (1998b)
<i>Lantana camara</i>	A	w	Leaves	N, C/N, leaf thickness, leaf toughness	Johns <i>et al.</i> (2003)
<i>Larix laricina</i>	G	w	Needles	N, TNC, SLA	Tjoelker <i>et al.</i> (1999)
<i>L. laricina</i>	G	w	Needles	Sugars, starch	Tjoelker <i>et al.</i> (1998)
<i>Lotus corniculatus</i>	A	h	Leaves	Sugars, starch, phenolics	Carter <i>et al.</i> (1999)
<i>L. corniculatus</i>	G	w	Stem	Sugars, starch, phenolics	Carter <i>et al.</i> (1999)
<i>Oryza sativa</i>	A	h	Leaves, culm	TNC, sugars	Rowland-Bamford <i>et al.</i> (1996)
<i>O. sativa</i>	G	w	Leaves	N	Baysa <i>et al.</i> (2003)
<i>O. sativa</i>	G	w	Leaves	Sugars, starch	Baysa <i>et al.</i> (1999)
<i>Pascopyrum smithii</i>	A	h	Leaves	N, TNC, sugars, starch, SLW	Read <i>et al.</i> (1997)
<i>Phalaris aquatica</i>	A	h	Leaves	N, TNC	Lilley <i>et al.</i> (2001)
<i>Phaseolus vulgaris</i>	A	h	Leaves	N	Prasad <i>et al.</i> (2004)
<i>Picea abies</i>	G	w	Needles	N, starch, SLA, phenolics, terpenes	Sallas <i>et al.</i> (2003)
<i>P. abies</i>	G	w	Stem	Terpenes	Sallas <i>et al.</i> (2003)
<i>Picea mariana</i>	G	w	Needles	N, TNC, SLA	Tjoelker <i>et al.</i> (1999)
<i>P. mariana</i>	G	w	Needles	Sugars, starch	Tjoelker <i>et al.</i> (1998)
<i>Pinus banksiana</i>	G	w	Needles	N, TNC, SLA	Tjoelker <i>et al.</i> (1999)
<i>P. banksiana</i>	G	w	Needles	Sugars, starch	Tjoelker <i>et al.</i> (1998)
<i>Pinus ponderosa</i>	G	w	Needles	SLA	Callaway <i>et al.</i> (1994)
<i>Pinus sylvestris</i>	G	w	Needles	Sugars, starch, SLA	Zha <i>et al.</i> (2001)
<i>P. sylvestris</i>	G	w	Needles	Sugars, starch, SLA	Zha <i>et al.</i> (2002)
<i>P. sylvestris</i>	G	w	Needles	N, starch, SLA, phenolics, terpenes	Sallas <i>et al.</i> (2003)
<i>P. sylvestris</i>	G	w	Stem	Terpenes	Sallas <i>et al.</i> (2003)
<i>Populus tremuloides</i>	A	w	Leaves	Sugars, starch	Tjoelker <i>et al.</i> (1998)
<i>P. tremuloides</i>	G	w	Leaves	N, TNC, SLA	Tjoelker <i>et al.</i> (1999)
<i>Pseudotsuga menziesii</i>	G	w	Needles	N, C/N, phenolics	Tingey <i>et al.</i> (2003)
<i>P. menziesii</i>	G	w	Stem	N, C/N, phenolics	Tingey <i>et al.</i> (2003)
<i>P. menziesii</i>	G	w	Needles	Terpenes	Snow <i>et al.</i> (2003)
<i>P. menziesii</i>	G	w	Needles	N	Lewis <i>et al.</i> (2004)
<i>P. menziesii</i>	G	w	Needles	C/N, sugars, SLW	Olszyk <i>et al.</i> (2003)

(conitnued)

Table 1. (Contd)

Plant species	Class	Form	Plant part	Plant traits	Reference
<i>P. menziesii</i>	G	w	Needles	N	Hobbie <i>et al.</i> (2001)
<i>P. menziesii</i>	G	w	Needles	Terpenes	Constable <i>et al.</i> (1999)
<i>Quercus robur</i>	A	w	Leaves	N, C/N, phenolics, leaf toughness	Dury <i>et al.</i> (1998)
<i>Salix myrsinifolia</i>	A	w	Leaves	N, water, SLW, phenolics	Veteli <i>et al.</i> (2002)
<i>Solanum tuberosum</i>	A	h	Leaves	N, starch, SLW	Cao & Tibbits (1997a)
<i>Trifolium subterraneum</i>	A	h	Leaves	N, TNC	Lilley <i>et al.</i> (2001)
<i>Triticum aestivum</i>	A	h	Shoot	N	Hakala (1998)
<i>T. aestivum</i>	A	h	Leaves	N	Delgado <i>et al.</i> (1994)

Table 2 Insect species, their host plants, feeding guild, performance characteristics and references used in the metaanalysis

Insect species	Host plant	Feeding guild	Performance indices	Reference
<i>Dialectica scariarilla</i> (Lepidoptera)	<i>Echium plantagineum</i>	Mining	Survival, duration of development, adult weight	Johns & Huhges (2002)
<i>Lymantria dispar</i> (Lepidoptera)	<i>Acer rubrum</i> , <i>A. saccharum</i>	Chewing	Weight gain	Williams <i>et al.</i> (2000)
<i>L. dispar</i>	<i>Acer rubrum</i>		Pupal weight, duration of development	Williams <i>et al.</i> (2003)
<i>Neodiprion sertifer</i> (Hymenoptera)	<i>Pinus sylvestris</i>	Chewing	RGR	Kozlov <i>et al.</i> (submitted)
<i>Ocotoma championi</i> (Coleoptera)	<i>Lantana camara</i>	Larva mining	Adult weight	Johns <i>et al.</i> (2003)
<i>Ocotoma scabripennis</i> (Coleoptera)	<i>Lantana camara</i>	Larva mining	Adult weight	Johns <i>et al.</i> (2003)
<i>Phenacoccus madeirensis</i> (Hemiptera)	<i>Dendranthema grandiflora</i>	Sucking	Survival, fecundity	Chong <i>et al.</i> (2004)
<i>Phratora vitellina</i> (Coleoptera)	<i>Salix myrsinifolia</i>	Chewing	RGR	Veteli <i>et al.</i> (2002)

homogeneity (Q_B) and testing it against the χ^2 distribution (Gurevitch & Hedges, 2001).

For some variables, we changed the sign of the effect size to be able to combine them with other variables within the same analysis. Among the insect performance indices, the sign was changed for developmental time, which decreases with an increase in plant quality, while other measures of performance (survival, relative growth rate, weight) increase. Similarly, we changed the sign of the effect size for specific leaf area, which increases when other plant physical traits (specific leaf weight, toughness, thickness) decrease.

When searching for sources of variation in response to treatments, we first contrasted angiosperms and gymnosperms, and within angiosperms compared annual herbs with woody plants. In woody plants, we compared, where possible, chlorophyll-containing and structural (woody) tissues.

Testing for publication bias

The bias that may have been caused by publication policy was explored by using the funnel plot technique for two most abundant classes of data: concentrations of nitrogen and phenolics. In case of no publication bias, the plot shape (effect size *vs.* sample size) should be like

a funnel because the studies with small sample sizes should include more variation than studies based on larger samples, and effect size should not correlate with sample size (Møller & Jennions, 2001). We also controlled the fail-safe numbers of the metaanalyses to observe how robust our conclusions were against the nonpublished results (Rosenthal, 1979). Rosenthal's fail-safe number demonstrates the number of insignificant unpublished studies that are required to turn the significant mean effect size into an insignificant one. In general, fail-safe numbers exceeding $5N$ (where N is the number of studies included in the metaanalysis) are considered as a proof that the analysis is robust against the unpublished results (Rosenthal, 1979; Møller & Jennions, 2001), although application of any fixed value to judge the results is not straightforward (Becker, 1994). Therefore, in our analysis we accepted that fail-safe numbers exceeding $3N$ indicate relatively robust results.

Results

Database

Our database included 41 papers (published from 1987 to January of 2005) and one submitted manuscript, describing results obtained by studying 31 plant species

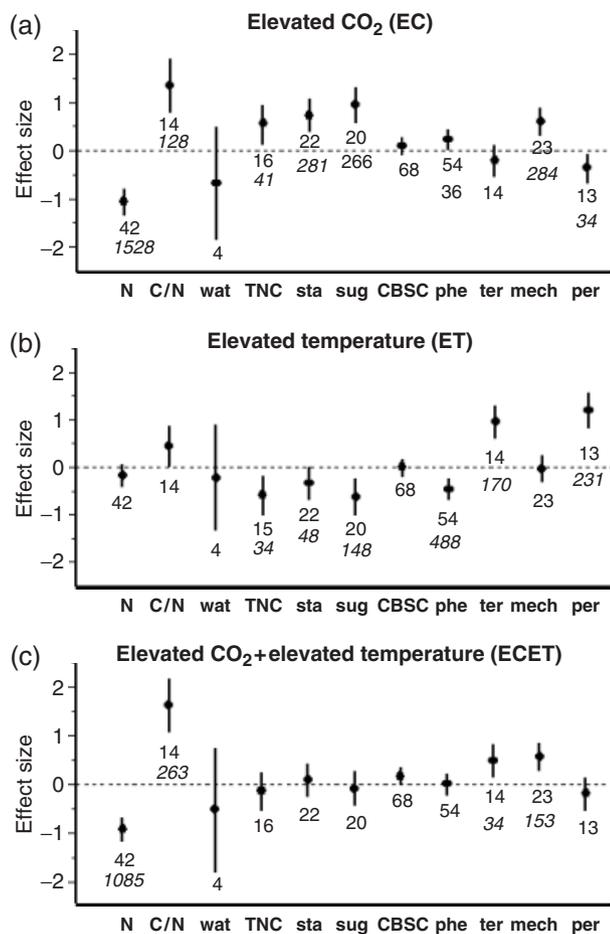


Fig. 1 Mean effect sizes (d), 95% confidence intervals and sample sizes for effects of CO₂ elevation (a), temperature elevation (b) and a combination of CO₂ and temperature elevation (c) on plant response variables and insect performance. Rosenthal's fail-safe numbers are given under sample size in italics when the effect is significant. N, nitrogen; C/N, carbon/nitrogen ratio; wat, water; TNC, total nonstructural carbohydrates; sta, starch; sug, soluble sugars; CBSC, carbon-based secondary compounds, all classes pooled; phe, phenolics; ter, terpenes; mech, mechanical traits; per, insect performance. The effect is significant if the 95% confidence interval does not overlap zero.

(211 measurements) (Table 1) and seven herbivore species (13 measurements) (Table 2). Plants included both gymnosperms (seven species) and angiosperms (eight woody and 16 herbaceous species); insects included Lepidoptera (two species), Coleoptera (three species), Hymenoptera (one species) and Hemiptera (one species), representing three feeding guilds (three species of chewers, two species of miners and one species of sap-feeders). CO₂ enrichment treatments generally used doubled or 1.5-fold elevated concentration of carbon dioxide compared with the ambient one. Temperature elevation varied from 2.5 °C to 15 °C, most frequently being in the range 2.5–6 °C.

None of the studies of herbivore performance allowed separation between direct and indirect effects. Eleven of 13 measurements were made when both host plant and herbivore were subjected to treatments. In the remaining two cases, only host plants were exposed to elevated temperature and carbon dioxide, while measurements of insect performance were conducted under ambient (non-modified) conditions.

Funnel plot analysis demonstrated an absence of correlation between the number of true replicates (chambers) and the effect size for either nitrogen ($r = 0.06$, $N = 129$, $P = 0.47$) or phenolics ($r = 0.06$, $N = 165$, $P = 0.48$), indicating the absence of publication bias.

Effects of methodology

The effect of all treatments did not differ between fully controlled (closed) and open-top chambers ($P > 0.10$). Doubling of CO₂ in the EC treatments caused a stronger effect than a 1.5-fold CO₂ increase in both nitrogen ($Q_B = 5.68$, $df = 1$, $P = 0.02$) and phenolics ($Q_B = 3.98$, $df = 1$, $P = 0.05$), while in ECET treatment the differences between the applied CO₂ concentrations were not significant (nitrogen: $Q_B = 0.32$, $df = 1$, $P = 0.77$; phenolics: $Q_B = 0.55$, $df = 1$, $P = 0.46$). The effects of temperature were independent of the magnitude of its elevation for both nitrogen and secondary compound concentrations in both ET and ECET treatments ($Q_B = 0.27$ – 2.16 , $df = 1$, $P = 0.14$ – 0.61).

Duration of woody plant exposure (one season vs. two to four seasons) did not affect nitrogen response in EC or ET treatments. In ECET treatment the decrease in nitrogen was weaker ($Q_B = 8.17$, $df = 1$, $P = 0.004$), although still significant, under multiseason exposure ($d = -0.74$, $N = 10$) than under short-term exposure ($d = -1.85$, $N = 14$). Changes in phenolic concentrations did not differ significantly between short and long exposures in all treatments ($Q_B = 0.53$ – 2.91 , $df = 1$, $P = 0.17$ – 0.47), although effect sizes tended to be larger in long-term experiments.

The decrease in nitrogen concentration was stronger in cuttings compared with seedlings ($Q_B = 6.07$, $df = 1$, $P = 0.01$) in EC treatment, but there were no differences in either ET or ECET treatments ($Q_B = 2.12$, $df = 1$, $P = 0.15$ and $Q_B = 1.76$, $df = 1$, $P = 0.18$, respectively). Changes in phenolics were similar in seedlings and cuttings of woody plants in all treatments ($Q_B = 0.51$ – 1.14 , $df = 1$, $P = 0.29$ – 0.62). The responses of foliar nitrogen or phenolics did not differ between *B. pendula* and other woody angiosperms in either of the treatments ($Q_B = 0.02$ – 1.05 , $df = 1$, $P = 0.20$ – 0.88), thus suggesting an absence of biases related to species-specific effects.

Effect sizes did not differ between replicated (two to four chambers per treatment) and pseudoreplicated studies (one chamber per treatment) in either of the treatments (nitrogen in woody angiosperms: $Q_B = 0.18-0.64$, $df = 1$, $P = 0.10-0.67$; phenolics: $Q_B = 0.01-1.56$, $df = 1$, $P = 0.21-0.93$), indicating that pseudoreplication detected in a large fraction of studies (102 of 224 measurements) did not influence our results.

Nitrogen and water

Nitrogen concentrations in the pooled sample of all plant groups and all tissues significantly decreased under both elevated CO_2 alone and in combination with elevated temperature, whereas temperature increase alone had no significant effect on this characteristic (Fig. 1a-c). Correspondingly, the C/N ratio was significantly increased by elevated CO_2 at both temperature regimes (Fig. 1a-c). Water concentration in plant leaves did not respond to any treatment (Fig. 1); however, this conclusion should be considered as tentative because of the small number of studies reporting this character.

Both woody and herbaceous angiosperms demonstrated a decrease in nitrogen concentrations in response to elevated CO_2 (Fig. 2a), although the nitrogen decrease in woody plants was stronger ($Q_B = 15.1$, $df = 1$, $P = 0.0001$) than in herbaceous plants. In contrast, in gymnosperms, the concentration of nitrogen did not respond to either EC or ET treatment but decreased in ECET treatment (Fig. 2). Thus, although angiosperms and gymnosperms responded differently to CO_2 enrichment alone ($Q_B = 12.3$, $df = 1$, $P = 0.0005$), in both groups nitrogen declined under combined elevation of CO_2 and temperature, and the difference between gymnosperms and angiosperms in ECET treatment became insignificant ($Q_B = 0.91$, $df = 1$, $P = 0.34$).

Non-structural carbohydrates

Concentrations of TNC, as well as of sugars and starch separately, demonstrated opposite responses to elevated CO_2 (increase: Fig. 1a) and elevated temperature (decrease: Fig. 1b), while acting simultaneously (ECET treatment), these factors had no significant effect on either group (Fig. 1c). No differences were found in the responses of non-structural carbohydrates to all treatments between woody and herbaceous angiosperms ($Q_B = 0.02-2.10$, $df = 1$, $P = 0.15-0.97$) or between angiosperm and gymnosperm woody plants ($Q_B = 0.08-2.61$, $df = 1$, $P = 0.10-0.78$). The only difference was found in the response of sugars in ECET treatment between angiosperm ($d = 0.55$, $N = 5$) and gymnosperm ($d = -0.43$, $N = 8$) woody plants ($Q_B = 4.75$, $df = 1$, $P = 0.03$).

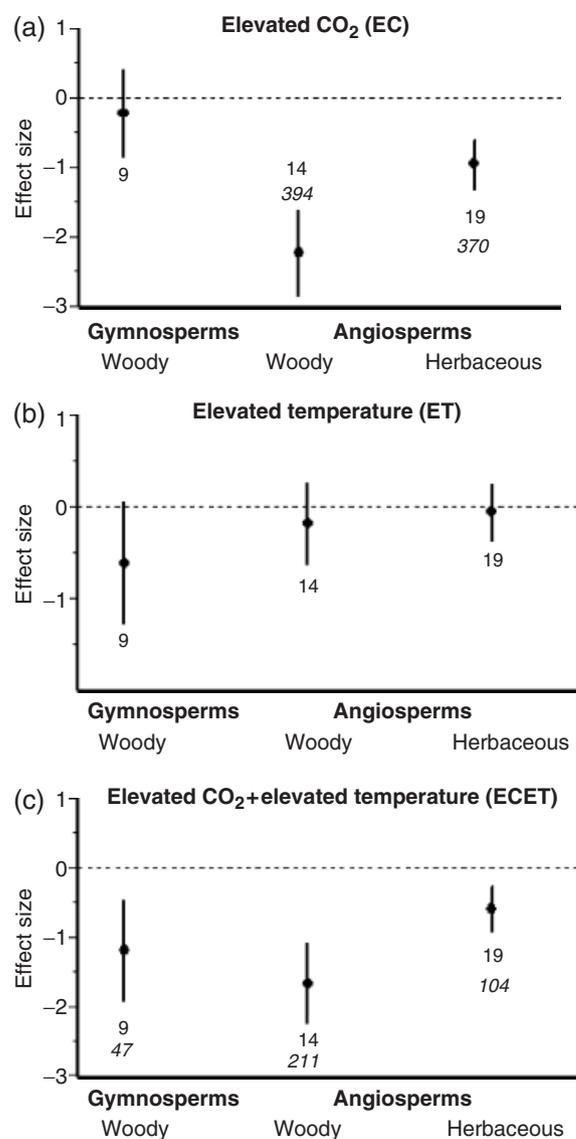


Fig. 2 Mean effect sizes (d), 95% confidence intervals and sample sizes for effects of CO_2 elevation (a), temperature elevation (b) and a combination of CO_2 and temperature elevation (c) on nitrogen concentration in different plant classes and growth forms. Rosenthal's fail-safe numbers are given under sample size in italics when the effect is significant. The effect is significant if the 95% confidence interval does not overlap zero.

CBSC

When all plant groups and tissues were pooled, CBSC did not show a significant response to either treatment (Fig. 1). In woody tissues, concentrations of secondary compounds increased in gymnosperms in response to elevated temperature, both alone (Fig. 3b) and in combination with elevated CO_2 (Fig. 3c), while angiosperms demonstrated no response (Fig. 3). Neither phenolics nor terpenes in woody tissues were significantly affected by CO_2 enrichment (Fig. 4a); in contrast,

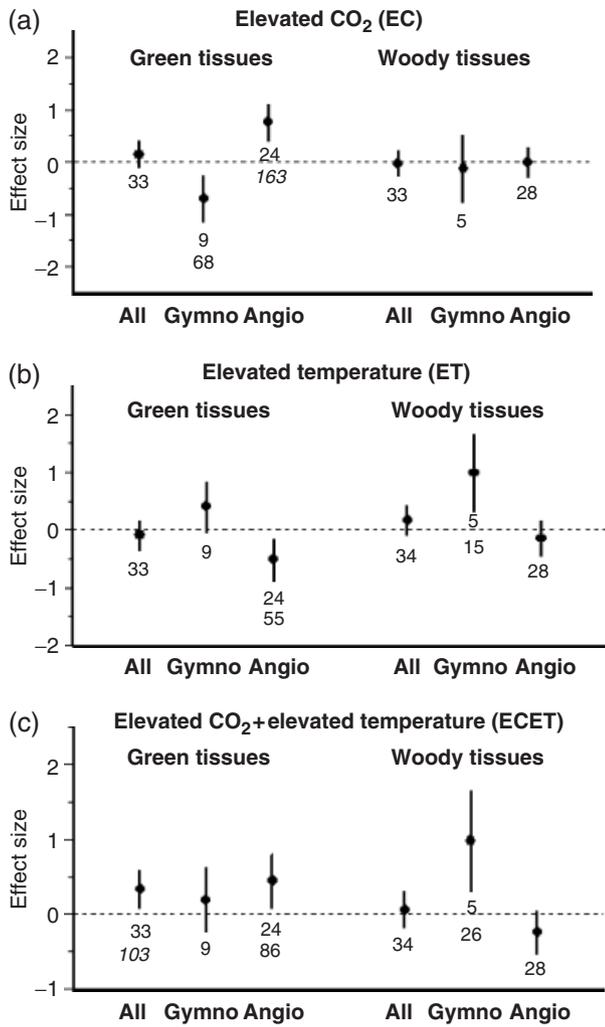


Fig. 3 Mean effect sizes (*d*), 95% confidence intervals and sample sizes for effects of CO₂ elevation (a), temperature elevation (b) and a combination of CO₂ and temperature elevation (c) on carbon-based secondary compounds (CBSC) in green (leaves and needles) and woody (stem, bark) tissues of woody plants. Rosenthal's fail-safe numbers are given under sample size in italics when the effect is significant. All, all woody plants pooled; gymno, gymnosperm woody plants; angio, angiosperm woody plants. The effect is significant if the 95% confidence interval does not overlap zero.

temperature elevation, both alone (Fig. 4b) and in combination with elevated CO₂ (Fig. 4c), caused a decrease in phenolics and an increase in terpenes.

In chlorophyll-containing tissues, CBSC increased only in ECET treatment (Fig. 3c). The absence of a response to CO₂ enrichment at ambient temperature resulted from opposite responses ($Q_B = 36.2$, $df = 1$, $P < 0.0001$) of gymnosperms and angiosperms (Fig. 3a). These two plant groups also responded to ET treatment differently ($Q_B = 15.6$, $df = 1$, $P < 0.0001$) (Fig. 3b), but a combination of elevated CO₂ with elevated

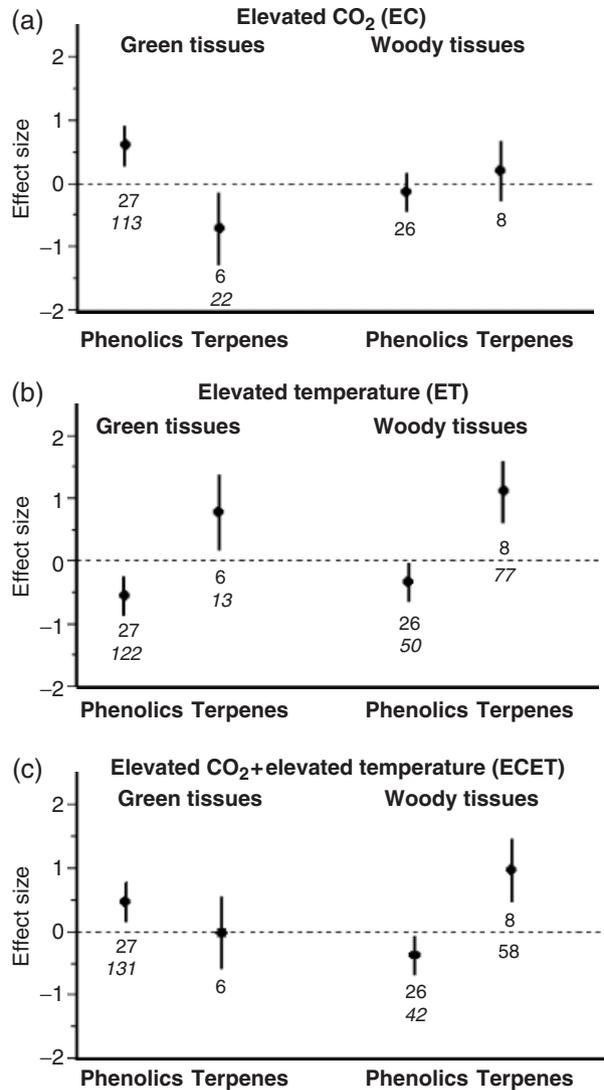


Fig. 4 Mean effect sizes (*d*), 95% confidence intervals and sample sizes for effects of CO₂ elevation (a), temperature elevation (b) and a combination of CO₂ and temperature elevation (c) on phenolic and terpenoid secondary compounds in green (leaves and needles) and woody (stem, bark) tissues of woody plants. Rosenthal's fail-safe numbers are given under sample size in italics when the effect is significant. The effect is significant if the 95% confidence interval does not overlap zero.

temperature similarly affected these plant classes ($Q_B = 0.70$, $df = 1$, $P = 0.40$), although the increase in CBSC concentrations was significant in angiosperms only (Fig. 3c).

Responses of both phenolics and terpenes to experimental manipulations differed between chlorophyll-containing and woody tissues. In green plant parts, elevation of CO₂ increased concentrations of phenolic compounds at both temperature regimes (Fig. 4a, c), while elevated temperature acting alone decreased

phenolic concentrations (Fig. 4b). In woody tissues, phenolics did not respond to EC treatment, but decreased under elevated temperature in both ET and ECET treatments. Concentrations of terpenes in green plant parts decreased in EC treatment, increased in ET treatment, but did not change in ECET treatment, whereas in woody tissues terpenes increased in both ET and ECET treatments, but did not respond to EC treatment (Fig. 4).

Mechanical leaf traits

Leaf characteristics related to toughness increased under elevated CO₂ at both ambient (EC) and elevated (ECET) temperatures. Elevation of temperature alone did not modify these characteristics (Fig. 1).

Herbivore performance

Herbivore performance characteristics were adversely affected by elevated CO₂ alone (Fig. 1a), and favoured by elevated temperature (Fig. 1b). Acting simultaneously, these factors did not modify herbivore performance (Fig. 1c). The exclusion of two studies in which effects were only mediated by plant quality did not change these results (data not shown).

Discussion

Data quality

Although the differences in methodology contributed to variation in the outcome of the primary studies, it seems unlikely that they may have biased the conclusions of metaanalysis, especially because most of the detected patterns differ between the treatments (EC, ET and ECET). This difference indicates the potential interaction between treatment and methodology; however, scarcity of data does not allow exploration of interactive effects in our database.

The absence of differences between the effects of relatively low ($\leq 4^\circ\text{C}$) and high ($>4^\circ\text{C}$) temperature elevation seems surprising at first glance. However, this result most likely is explained by the nonlinear response of plants to temperature: while small increases in temperature are usually beneficial, overheating leads to adverse effects (Levitt, 1980). Therefore, we question the relevance of studies involving extreme (up to 20 °C) temperature increases for exploration of possible effects of climate change. The existing experimental data do not allow exploration of dose-response regularities for realistic (minor) changes in either temperature or CO₂ concentrations, forcing us to disregard variation in temperature regimes and levels of CO₂ elevation

between experimental studies and contrast 'recent' and 'future' environmental conditions in our metaanalysis.

Although the statistical validity of the analyses can be seriously compromised by pseudoreplication, nonreplicated studies may still provide valuable information on the magnitude of the effects (Hurlbert, 1984). Our data set contained two kinds of studies: 20 with true replicates (chambers) and 22 with pseudoreplicates (plants within chamber, one chamber per treatment). Several earlier metaanalyses included primary studies that are classified as pseudoreplicated in our database, indicating no difference between these and replicated studies (e.g. data by Callaway *et al.* (1994) and Bunce & Ziska (1996) used by Wang & Curtis (2002); data by Tjoelker *et al.* (1998, 1999) used by Poorter & Navas, 2003). In our analysis, exclusion of pseudoreplicated studies did not change the conclusions, although it usually decreased the significance of the detected effects (assumedly because of an increase in CI with a decrease in sample size). However, this conclusion should not serve as an argument for conducting nonreplicated studies in the future: if we strictly follow the methodology of the metaanalysis (Gurevitch & Hedges, 2001), nonreplicated studies should receive much lower weight than properly replicated ones. The statistical approaches for using non-replicated studies in metaanalysis remain to be developed.

Plant quality for herbivores

The performance of insect herbivores is frequently linked to and explained by the chemical and mechanical quality of their host plants (Lincoln *et al.*, 1993; Peñuelas & Estiarte, 1998; Johns & Hughes, 2002), and the analysis of plant chemistry is often used to predict herbivore performance and plant damage under the changed environmental conditions (Coley, 1998; Keinänen *et al.*, 1999). However, predictions based exclusively on the data on host plants should be considered cautiously because of failure of several reviews in finding out consistent quantitative relationships between changes in insect performance and changes in host plant secondary chemistry (Bezemer & Jones, 1998; Nykänen & Koricheva, 2004). Therefore, in this study we validate the indirect information (i.e. changes in plant chemistry and structure) by direct measurements of herbivore performance.

The quality of plant tissues used as food by herbivorous insects generally depends on the concentrations of both essential nutrients and defensive secondary compounds. Insect performance usually increases with an increase in nitrogen concentration (Mattson, 1980; Scriber & Slansky, 1981). Starches may enhance the ability of insects to digest leaves (Lincoln *et al.*, 1993); however, as insect herbivores tend to be nitrogen rather than carbon limited (Mattson, 1980), the increase in starch

concentrations may reduce herbivore performance via dilution of foliar nitrogen (Lincoln *et al.*, 1993). More generally, the C/N ratio is often used as an index of plant nutritional quality (Fajer, 1989; Lincoln *et al.*, 1993). Increased water availability is beneficial for most herbivorous insects (Scriber & Slansky, 1981), because it aids in the digestion and assimilation of nutrients, particularly nitrogen (Scriber, 1984). In contrast, secondary compounds, such as phenolics and terpenes, adversely affect herbivore performance (Bennett & Wallsgrove, 1994). Also, changes in mechanical traits, for example an increase in leaf toughness, may considerably influence insect feeding behaviour and may adversely affect performance (Lincoln *et al.*, 1993 and references therein).

Effects of carbon dioxide at ambient temperatures

The large amount of experimental data on the effects of CO₂ on plant–herbivore relationships allowed a number of generalizations (summarized by Lincoln *et al.*, 1993; Watt *et al.*, 1995; Bezemer & Jones, 1998; Koricheva *et al.*, 1998; Peñuelas & Estiarte, 1998; Coviella & Trumble, 1999; Hunter, 2001). In particular, plants exposed to a CO₂-enriched environment demonstrate lower nitrogen concentrations, higher concentrations of non-structural carbohydrates and increased allocation to phenolic defensive compounds (Lincoln *et al.*, 1993; Watt *et al.*, 1995; Bezemer & Jones, 1998; Coviella & Trumble, 1999; Hunter, 2001). A decrease in water content, although detected in some case studies (Williams *et al.*, 2000; Kuokkanen *et al.*, 2001), was generally nonsignificant (Bezemer & Jones, 1998; Koricheva *et al.*, 1998). Leaf toughness and related physical traits, like specific leaf weight and thickness, increase (Lincoln *et al.*, 1993; Bezemer & Jones, 1998) because of enhanced allocation of carbon to structural carbohydrates. Herbivorous insects respond to these low-quality plants (grown in a CO₂-enriched atmosphere) by increasing consumption, and sometimes manage to compensate for reduced food quality (Williams *et al.*, 1994), but in general the fitness of insects is reduced (Lincoln *et al.*, 1993; Watt *et al.*, 1995; Bezemer & Jones, 1998; Coviella & Trumble, 1999; Hunter, 2001).

Responses of plants and herbivores to elevated CO₂ at ambient temperatures, detected by our study (Fig. 1a), are totally in line with all the conclusions mentioned above. This agreement demonstrates that studies used for our metaanalysis are representative for detecting general regularities in responses to the combined action of elevated CO₂ and temperature.

Effects of elevated temperature under ambient CO₂

While several general conclusions on the effects of elevated CO₂ on plant–herbivore interactions have

already been made (see above), to our knowledge, the effects of experimental temperature elevation on plant chemistry or plant–herbivore interactions have not yet been summarized. Case studies do not allow to draw the conclusions directly: for example, total phenolics in leaves of deciduous woody plants were reported either to increase (Kuokkanen *et al.*, 2001, 2003) or to decrease (Dury *et al.*, 1998; Veteli *et al.*, 2002) in treatments with elevated temperature. Our meta-analysis showed no effects of temperature increase on either nitrogen concentrations or C/N ratio or leaf mechanical characteristics (Figs 1b and 2b), while carbohydrates and phenolics decreased and terpenoids increased under the impact of elevated temperature. The latter effects were opposite to the effects of elevated CO₂ acting alone.

Bale *et al.* (2002) concluded that the direct effects of predicted temperature elevation on insect herbivore performance will be generally positive. Our metaanalysis also demonstrated that the performance of herbivores was significantly improved by temperature elevation (Fig. 1b). Indirect impacts of temperature (acting via changes in host plant quality) on herbivore performance have only rarely been studied (but see Veteli *et al.*, 2002), and therefore, the net effect is difficult to predict. However, our result suggests that indirect effects are not likely to counterbalance the direct positive effects, and the overall herbivore response to temperature increase will be positive.

Effects of simultaneous elevation of temperature and CO₂

Although a strong and consistent decrease in nitrogen and increase in C/N ratio in plants growing in a CO₂-enriched atmosphere is observed at both ambient and elevated temperatures, in other compounds temperature increase frequently affected the magnitude, and sometimes even changed the direction of the response to elevated carbon dioxide. Among CBSC, phenolics were increased by CO₂-enrichment only at ambient temperatures, while terpenes, not responding to CO₂ elevation at ambient temperatures, were increased by temperature elevation in both CO₂ regimes. The latter result agrees with several earlier studies demonstrating that phenolics and terpenes, which are produced by different biochemical pathways, respond to environmental factors differently (Peñuelas & Estiarte, 1998; Koricheva *et al.*, 1998).

Our analysis allows to divide all investigated characters into three groups on the basis of the comparison between EC and ECET treatments:

- (1) Responses to CO₂ do not depend on temperature (the effects of ECET are similar to the effects of EC):

nitrogen, C/N ratio and leaf toughness – all plant groups and tissues pooled; phenolics – in leaves of angiosperms.

- (2) Responses to CO₂ are mitigated by temperature (effect of ECET non-significant, in spite of significant EC effect): non-structural carbohydrates (total, sugars and starch) – all plant groups and tissues pooled; terpenes – in needles of gymnosperms; insect performance.
- (3) The effect of CO₂ emerges only under elevated temperature (effect of ECET significant, in spite of nonsignificant EC effect): nitrogen – in gymnosperms; phenolics and terpenes – in woody tissues.

This variety of relationships between elevated carbon dioxide and temperature indicates that only some conclusions of numerous CO₂-enrichment experiments conducted at ambient temperatures can be reliably extrapolated to the future environment.

Possible mechanisms behind CO₂-temperature interactions

An increase in both non-structural carbohydrates and phenolic compounds in a CO₂-enriched atmosphere (Fig. 1a) observed at ambient temperatures agrees perfectly with the predictions of carbon-nutrient balance (Bryant *et al.*, 1983), growth-differentiation balance (Herms & Mattson, 1992) and source-sink balance hypotheses (Peñuelas & Estiarte, 1998). At elevated temperature, however, an increase in non-structural carbohydrates or phenolics in response to CO₂ enrichment did not occur (Fig. 1c), possibly because the stimulating effect of elevated CO₂ on photosynthetic rates (Curtis & Wang, 1998), which generally determines carbohydrate availability, may be reduced by increased growth temperatures (Ziska & Bunce, 1994; Ziska, 2001). On the other hand, reduction in storage carbohydrates (starch, sugars) under elevated temperature may result from increased consumption of assimilates because of higher metabolism in sinks (Farrar & Williams, 1991). An increase in carbon sink strength may also lead to a decrease in plant tissue concentrations of carbon-based secondary compounds (Peñuelas & Estiarte, 1998). Thus, alleviation of CO₂-induced increase in carbon-based compounds by temperature revealed by our metaanalysis (Fig. 1c) is in agreement with the predictions of all three hypotheses mentioned above.

Terpenes respond to treatments in an opposite manner compared with other carbon-based compounds. The differences between responses of terpenes and phenolics to environmental changes found in numerous studies (summarized by Bezemer & Jones, 1998; Koricheva *et al.*, 1998) are usually explained by their different biosyn-

thetic origin. An increase in terpene concentrations at elevated temperatures may also be explained by an increase in terpene production to compensate for higher terpene emissions (Guenther *et al.*, 1993; Sallas *et al.*, 2003). However, this increase occurred mostly in woody tissues (Fig. 4) and may appear important only for bark beetles and other xylophagous insects.

Sources of variation in plant responses

Similar to other studies (Ceulemans & Mousseau, 1994; Curtis & Wang, 1998), we found considerable differences in the responses of gymnosperm and angiosperm plants to CO₂ elevation. In particular, phenolics in leaves of woody angiosperms and in needles of gymnosperms respond to CO₂ enrichment at ambient temperature differently. However, because of a similar response to temperature increase, the difference between plant classes disappears when both CO₂ and temperature are elevated. The increase in secondary compounds decreases plant quality for herbivores in both classes, but in gymnosperms this decrease will be much larger than predicted on the basis of CO₂-enrichment studies conducted at ambient temperature. Similarly, exposition to elevated CO₂ does not influence the concentration of nitrogen in gymnosperms, but the adverse effect became significant at elevated temperature. Thus, interactive effects of temperature and CO₂ significantly decrease the quality of gymnosperms for insect herbivores, whereas CO₂-induced adverse changes in the quality of angiosperms are less temperature dependent and can even be mitigated by elevated temperatures.

We also detected differential responses of secondary compounds in chlorophyll-containing and woody plant tissues. Neither phenolics nor terpenes in woody tissues appeared to be sensitive to CO₂ elevation at ambient temperatures, but because of a differential response to a temperature increase, they responded differently to a combined elevation of both factors: phenolics decreased, and terpenes increased considerably. Thus, the consequences of climate change for wood-feeding herbivores will depend on the main type of defensive compounds: if the defence of their host plants is determined by terpenes, the quality will decrease; if the main defensive compounds are phenolics, then the quality will increase. In contrast, for leaf-feeding herbivores host plant defences based on phenolics are expected to increase (leading to a decrease in quality), while defences based on terpenoids are not likely to change.

Gaps in knowledge and reliability of predictions

Studies exploring herbivore performance under simultaneous increase of both temperature and carbon

dioxide concentration [i.e. under the conditions that are believed to mimic the environment of about 2050–2080 (IPCC, 2001)] are surprisingly scarce. Papers reporting the effects of elevated carbon dioxide at ambient temperatures are much more frequent, but their conclusions are of limited value because, as demonstrated above, temperature may strongly modify plant responses to elevated carbon dioxide. Thus, in agreement with the repeatedly expressed opinion (Williams *et al.*, 2000; Johns & Hughes, 2002; Newman, 2003), additional studies involving manipulations of factors other than carbon dioxide are urgently needed to evaluate the consequences of the expected climate change for plant–herbivore interactions and to develop adaptation strategies and mitigation measures for pest control.

Most of our conclusions on the simultaneous effects of elevated CO₂ and temperature on insect herbivore performance are based on changes in plant chemistry. Although they often allow prediction of herbivore response, quantitative relationships between changes in insect performance and changes in host plant quality are not evident (Bezemer & Jones, 1998; Nykänen & Koricheva, 2004). Moreover, different guilds of herbivores respond to CO₂-mediated changes in host plant chemistry in different ways and responses vary from negative (in leaf chewing insects) to positive (in some phloem-feeders) (reviewed in Bezemer & Jones, 1998; Coviella & Trumble, 1999). Therefore, direct evaluation of the performance of herbivores, belonging to different feeding guilds, on plants subjected to elevation of both CO₂ and temperature is urgently needed and cannot be substituted by analysis of plant chemistry.

However, our metaanalysis shows that some predictions made on the basis of the detected responses of plant chemistry to CO₂ elevation may appear valid for future environmental conditions. For example, predictions made on the basis of nitrogen dilution effects, like an increase in food consumption (Fajer, 1989; Lincoln *et al.*, 1993), may remain valid in an environment with elevated temperature, although the duration of insect development will be reduced (Lindroth *et al.*, 1997; Johns & Hughes, 2002; Williams *et al.*, 2003). In contrast, predictions based on changes in concentrations of plant secondary metabolites are of a relatively low value, because the responses of both phenolics and terpenes to CO₂ are strongly modified by elevated temperature, and the responses of CBSC differ between angiosperm and gymnosperm plants. Mitigation of the negative effects of CO₂ enrichment by temperature is confirmed by direct measurements: herbivore performance was not decreased by the combined impact of elevated CO₂ and temperature. This alleviation of adverse effects of CO₂ on insect fitness is likely to also involve the impacts of natural enemies. For example, one of the

common responses of herbivores to elevated CO₂ – prolonged development – results in an increased exposure to natural enemies (Coviella & Trumble, 1999; Stiling *et al.*, 2003). Shortening of developmental time by elevated temperature will lead to further amelioration of negative CO₂ effects on herbivore fitness via the third trophic level.

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