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Elevated CO_2 levels and herbivore damage alter host plant preferences

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Interactions between the moth *Spodoptera littoralis* and two of its host plants, alfalfa (*Medicago sativa*) and cotton (*Gossypium hirsutum*) were examined, using plants grown under ambient (350 ppm) and elevated (700 ppm) CO_2 conditions. To determine strength and effects of herbivore-induced responses assays were performed with both undamaged (control) and herbivore damaged plants. CO_2 and damage effects on larval host plant preferences were determined through dual-choice bioassays. In addition, larvae were reared from hatching to pupation on experimental foliage to examine effects on larval growth and development.

When undamaged plants were used *S. littoralis* larvae in consumed more cotton than alfalfa, and CO_2 enrichment caused a reduction in the preference for cotton. With damaged plants larvae consumed equal amounts of the two plant species (ambient CO_2 conditions), but CO_2 enrichment strongly shifted preferences towards cotton, which was then consumed three times more than alfalfa. Complementary assays showed that elevated CO_2 levels had no effect on the herbivore-induced responses of cotton, whereas those of alfalfa were significantly increased. Larval growth was highest for larvae fed undamaged cotton irrespectively of CO_2 level, and lowest for larvae on damaged alfalfa from the high CO_2 treatment. Development time increased on damaged cotton irrespectively of CO_2 treatment, and on damaged alfalfa in the elevated CO_2 treatment.

These results demonstrate that elevated CO_2 levels can cause insect herbivores to alter host plant preferences, and that effects on herbivore-induced responses may be a key mechanism behind these processes. Furthermore, since the insects were shown to avoid foliage that reduced their physiological performance, our data suggest that behavioural host plant shifts result in partial escape from negative consequences of feeding on high CO_2 foliage. Thus, CO_2 enrichment can alter both physiology and behaviour of important insect herbivores, which in turn may to impact plant biodiversity.

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The global increase of atmospheric CO_2 is likely to impact ecological systems. CO_2 is a key resource for plants and CO_2 enrichment in general cause increased growth and photosynthetic rate (Bazzaz et al. 1990, Bazzaz and Miao 1993, Ceulemans and Mousseau 1994, Curtis 1996), as well as altered chemical composition of foliage, including both primary and secondary metabolites (Lincoln et al. 1993, Lindroth 1996, Poorter et al. 1997, Koricheva et al. 1998, Peñuelas and Estiarte 1998, Agrell et al. 2000). However, plant responses to elevated

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CO_2 levels show enormous interspecific variation. (Bazzaz et al. 1990, Lindroth et al. 1993, Bezemer and Jones 1998, Peñuelas and Estiarte 1998). Such variation in plant CO_2 sensitivity is likely to alter competitive balance between species and cause ecosystem changes (Peñuelas and Estiarte 1998, Saxe et al. 1998, Körner 2000).

In this process insect herbivores play an important role. CO_2 induced changes in foliar levels of nutrients and secondary substances represent altered food plant quality, which affects performance of associated herbivores (Fajer et al. 1989, Lindroth 1996, Coviella and Trumble 1999, Agrell et al. 2000, Goverde and Erhardt 2003). CO_2 enrichment can thus have significant impact on insect populations (Coviella and Trumble 1999), which in turn would affect plant populations. Behavioural responses, especially altered host plant preferences, are central in this process because of effects on plant competitive balance and biodiversity (Arnone et al. 1995, Díaz et al. 1998, Körner 2000). Only a few studies have attempted to examine the influence of CO_2 on host plant preferences, in general detecting only limited effects (Arnone et al. 1995, Traw et al. 1996, Lederberger et al. 1997, 1998, Díaz et al. 1998, Peters et al. 2000, but see Goverde and Erhardt 2003), and the need for further research within this area has repeatedly been pointed out (Lincoln et al. 1993, Körner 1996, 2000, Bezemer and Jones 1998). Changes in herbivore host plant preferences in an altered atmospheric environment also play an important role for predictions about herbivore consumption. Insects fed CO_2 enriched foliage often exhibit increased consumption rate, presumably as a response to reduced food quality (compensatory consumption, Fajer et al. 1989, Roth and Lindroth 1995, Lindroth 1996, Bezemer and Jones 1998, Agrell et al. 2000). However, an alternative response, i.e. selection of an alternative food source, has not been possible in most feeding experiments (Körner 2000). Available data actually suggest that elevated CO_2 levels do not increase consumption if herbivores have several food plants available (Peters et al. 2000).

Despite the strong association between CO_2, plants and insects a factor seldom accounted for in CO_2 research is herbivore damage. This is surprising considering that CO_2 effects might be largest in the presence of stresses, such as herbivory (Kruger et al. 1998), and that many plant species show strong responses to herbivory (Karban and Baldwin 1997). Interactive effects of CO_2 and herbivore damage are likely, since herbivore damaged plants, similar to CO_2 enriched ones, commonly exhibit altered levels of important secondary substances (reviewed by Karban and Myers 1989, Karban and Baldwin 1997). For example, increased availability of CO_2 may, through enhancement of photosynthesis (Ceulemans and Mousseau 1994), help defoliated plants to restore altered source:sink balance after an herbivore attack (Trumble et al. 1993, Kruger et al. 1998). Furthermore, considering the known variation in plant responses to CO_2 enrichment, it would be surprising if high CO_2 levels did not also differently affect herbivore induced responses of plants. However, studies so far have almost exclusively focussed on CO_2 effects on undamaged plants, thereby examining only constitutive levels of secondary substances, and only a few have included herbivore damage as an experimental variable. These have examined interactions between deciduous trees and defoliating larvae, and all have reported responses to herbivore attack to be limited and little affected by CO_2 availability (Lindroth and Kinney 1998, Roth et al. 1998, Agrell et al. 1999), presumably because trees are in general limited by factors other than carbon availability (Körner 2003).

In this study we examined effects of CO_2 enrichment and herbivore damage on interactions between alfalfa (*Medicago sativa* L.), cotton (*Gossypium hirsutum* L.), and the Egyptian cotton leafworm (*Spodoptera littoralis* Boisduval). These three species, co-occurring in e.g. southern Europe, Africa and the Near East, are widely used model organisms in ecological research, wherefore extensive background data are available. Both alfalfa and cotton respond to increased CO_2 with increased growth, better water use efficiency, and altered foliar chemistry (Reddy et al. 1997, Sgherri et al. 1998, 2000, Heagle et al. 1999, Skinner et al. 1999, Booker et al. 2000). Furthermore, both plant species show herbivore-induced responses. Cotton responds to herbivore damage primarily by a substantial increase in levels of terpenoid aldehydes, which effectively deter insect herbivores (Karban 1985, Alborn et al. 1996, McAuslane et al. 1997, Anderson et al. 2001). The herbivore-induced response of alfalfa was only recently discovered and includes increased foliar levels of saponins and flavonoids (Agrell et al. 2003). *S. littoralis* is a generalist defoliator and a major pest on both alfalfa and cotton (Brown and Dewhurst 1975). Herbivore induced responses of both alfalfa and cotton have strong impact on behaviour and performance of *S. littoralis* larvae (McAuslane and Alborn 2000, Agrell et al. 2003). To determine CO_2 effects on herbivore induced responses of alfalfa and cotton and effects on *S. littoralis* performance, plants were grown in ambient or elevated CO_2 levels, and were either undamaged or damaged by *S. littoralis*. Assays with *S. littoralis* larvae included feeding preference tests to examine treatment effects on larval host plant selection, but we also reared larvae on experimental foliage from hatching to pupation to determine effects on development time, growth, pupal mass and survival.

The overall aims of this study were to determine:

1) Separate and interactive effects of CO_2 enrichment and herbivore damage on performance of *S. littoralis* larvae.
2) How elevated levels of CO_2 alone or in combination with herbivore damage alter *S. littoralis* host plant preferences.
3) If CO_2 enrichment, herbivore damage and available host plants influence consumption patterns of *S. littoralis*.

Methods

Plants

Alfalfa (var. Julus) and cotton (var. Delta Pineland 90) plants were grown individually in 1.5 l plastic pots. At sowing each pot received 2 g slow release fertilizer (Bayer Osmocote). Plants were grown in climate chambers (initially 80 alfalfa and 80 cotton plants in each chamber), with light, temperature and humidity levels similar to those in southern Europe and the Near East during spring/early summer. The plants received a daily horizontal irradiation of 5 $kWh \times m^{-2} \times day^{-1}$, evenly distributed over the day (620 $\mu mol \times m^{-2} \times s^{-1}$, with a L16: D8 photoperiod, Osram Powerstar, HQI-T, 400 W/D, Daylight). Temperatures were 24°C during day and 20°C during night, and relative humidity 70%. The study was performed in four identical climate chambers ($3 \times 2.5 \times 2$ m), each being used once with ambient CO_2 levels (355 ± 6 ppm), and once with elevated CO_2 levels (700 ± 2 ppm, i.e. twice ambient conditions). Five weeks after sowing, the plants within each chamber were randomly assigned to become either damaged or undamaged (control) plants. To obtain insect damaged plants, two 3rd instar *S. littoralis* were placed in plastic bags enclosing one stem (alfalfa) or the second true leaf (cotton). The larvae were left to feed for approximately 24 h, removing 5–10% of the leaf biomass, after which the bags and larvae were removed. Undamaged (control) plants of both alfalfa and cotton had a stem/leaf enclosed by an empty plastic bag, thereby controlling for possible direct effects of the plastic bags. Damaged and control cotton plants were kept together in the chambers until harvested or used in bioassays.

Insects

Spodoptera littoralis larvae were obtained from a laboratory culture, which have been supplemented with wild material yearly for the last seven years. Larvae were reared on a semi-synthetic diet (Hinks and Byers 1976), and individuals used in food preference assays thus had no previous experience of either alfalfa or cotton. The larvae were kept at 25°C, 70% relative humidity, and a L16: D8 photoperiod.

Food plant preference assays

Two-choice food preference assays with 4th instar *S. littoralis* larvae were performed during the 7th week after sowing. The larva was either given a choice between alfalfa and cotton (interspecific assays), or between a control and an insect damaged plant of the same species (intraspecific assays). From alfalfa plants we cut off the distal parts (8–10 cm) of stems. Only undamaged stems were used, both from control and damaged alfalfa plants. From cotton plants the youngest expanding top leaf with a width exceeding 3 cm was used in the food preference assays. Damaged plants were used seven days after herbivore damage, since previous studies under similar conditions have shown that both alfalfa and cotton have a defence peak occurring around one week after being attacked by insect herbivores (McAuslane et al. 1997, Anderson et al. 2001, Agrell et al. 2003). The fact that the plant material was detached was unlikely to influence the foliar chemistry during the assays, since neither herbivore nor clipping damage responses are detectable until after a few days (Agrell et al. 2003, Agrell and P. Anderson, unpubl.).

At the start of a bioassay each alfalfa stem and cotton leaf was weighed to the closest 10^{-4} grto obtain the wet mass. To keep the plant material fresh throughout the duration of the experiment the cut ends of stems and/or leaf petioles were inserted into 1 ml glass vials filled with tap water and sealed with parafilm. Stems/leaves were then placed horizontally at opposite ends of a transparent plastic box ($24 \times 18 \times 7$ cm). The bioassay was initiated by weighing one *S. littoralis* larva to the closest mg and then placing it at the middle of the box, which was then closed with a ventilated lid. The larva was left to feed for 24 h and was then removed. The stems and leaves were visually inspected for feeding damage, completely dried at 65°C and weighed to the nearest 10^{-4} g. Interspecific assays included control alfalfa vs control cotton and damaged alfalfa vs damaged cotton, and were performed both with plants from ambient and from elevated CO_2 treatments. Intraspecific assays were performed to determine species-specific CO_2 effects on the induced defence. These included, from each CO_2 treatment, control alfalfa vs damaged alfalfa and control cotton vs damaged cotton. Tests were replicated 12 to 16 times for each plant and treatment combination.

Larval food preferences were determined from the relative dry mass consumption of the two food plants presented in the assay, i.e. as % of total consumption during the assay. Plant dry mass (DM) at the beginning of the food preference assay was estimated from plant

fresh mass (FM) by using species and treatment specific DM/FM relationships. These were obtained by harvesting 15 additional alfalfa stems and cotton leaves for each treatment combination, and after weighing, drying and re-weighing the plant material, a regression between DM and FM could be calculated. The relationships between DM and FM, which turned out to be very robust ($r > 0.88$ in all cases), were thus used to calculate plant DM at the start of the experiment, and hence the consumed biomass. For minute changes in biomass the use of a DM/FM relationship is less accurate so when the leaf area consumed was < 2 mm^2, DM was arbitrarily set to 1 mg. In cases were no damage was observed, biomass consumed was set to zero. For each food preference assay we also calculated total consumption (of the two plants available) to determine relative consumption rate, i.e. total mg dry mass consumed $\times$ mg dry mass larvae$^{-1} \times$ day^{-1}. To estimate the initial dry mass of larvae at the onset of the assays we calculated proportional dry mass of 20 larvae of the same size as those used in the bioassays.

Rearing experiments

To examine the effects of CO_2 level and herbivore damage on herbivore performance we reared *S. littoralis* larvae on foliage from experimental plants. Larvae were reared from hatching to pupation on either alfalfa or cotton. The plants were either from control or damaged plants, and grown in either ambient or elevated CO_2 levels, i.e. a total of eight different diets. Rearing experiments were initiated six weeks after sowing. Ten newly hatched larvae without prior feeding experience were placed in a transparent plastic box, identical to the ones used in food preference assays. Plant material placed in the box (outer parts of alfalfa stems or young, expanding cotton leaves) was kept fresh by inserting the cut ends into 20 ml plastic vials sealed with parafilm. All plant material was replaced every second day or more often if necessary. In assays where larvae were fed herbivore damaged alfalfa or cotton, plants were used for feeding larvae between day six and eight after damage. On day four after hatching the number of larvae was randomly reduced to five. The larvae were from then on counted every second day, and starting on day 10 they were also weighed. On day 15 boxes were supplied with 50 ml of moist soil to facilitate pupation, and were then checked daily for pupating individuals to determine time from hatching to initiation of pupation. After pupation was completed the sex of each pupa was recorded, before it was dried (65°C) to determine dry mass. The rearing boxes with larvae were kept in the climate chambers throughout the experiment. Three replicates were performed per plant species, damage treatment and chamber, resulting in a total of 96 rearing assays.

Statistical analyses

Statistical analyses were performed with SPSS 11.5 (SPSS Inc. 2002). To avoid pseudo-replication only the mean value for each experimental chamber was used in the analyses (thus $n = 4$ for ambient CO_2 and $n = 4$ for elevated CO_2). Potential differences between chambers were examined by initially including "chamber" as a random factor in all analyses. No significant effects of chamber were detected ($p > 0.20$ in all cases), so in order to facilitate data presentation this variable was excluded from the final analyses. To correct for heterogeneity of variances, data computed as proportions were transformed (arcsine-square root) prior to statistical analyses. Food plant preferences, determined as relative consumption of each plant type (i.e.% of total consumption during the assay), were analysed with paired t-test for each set of two-choice assays. Consumption rate during these assays were analysed with two-way ANOVA, with either CO_2 and damage treatment (interspecific assays), or with CO_2 level and plant species as independent variables (intraspecific assays). Data from rearing experiments were analysed with repeated measures ANOVA with CO_2, plant species and damage treatment as independent variables (larval fresh masses during the rearing period), or as a corresponding three-way ANOVA (development time from hatching to pupation, and pupal dry mass). We found no indications of any sexual dimorphism in this study, so data for males and females were pooled. To facilitate presentation of ANOVA analyses only main and interactive effects with P values less than 0.10 are provided.

Results

Larval food preferences and consumption

In general *S. littoralis* larvae showed a preference for cotton over alfalfa, although both CO_2 and damage treatments were found to significantly influence food preferences. In tests with control (undamaged) plants from ambient CO_2 conditions larvae consumed about twice as much cotton as they consumed alfalfa (paired t-test, $n = 8$ [4 pairs], $t = 9.12$, $p = 0.003$, Fig. 1a). When using control plants grown in elevated CO_2 environments the preference for cotton was reduced and only tended to be significant (paired t-test, $n = 8$ [4 pairs], $t = 2.78$, $p = 0.068$, Fig. 1a). A different pattern was found with plants damaged seven days prior to the experiments. With damaged plants grown in ambient CO_2 levels larvae consumed equal amounts of alfalfa and cotton (paired t-test, $n = 8$, $t = 0.68$, $p = 0.544$, Fig. 1b), whereas if the plants had been grown in elevated CO_2 environments, larvae instead showed a strong preference for damaged cotton over damaged alfalfa (paired t-test, $n = 8$, $t = 7.90$, $p = 0.004$, Fig. 1b).

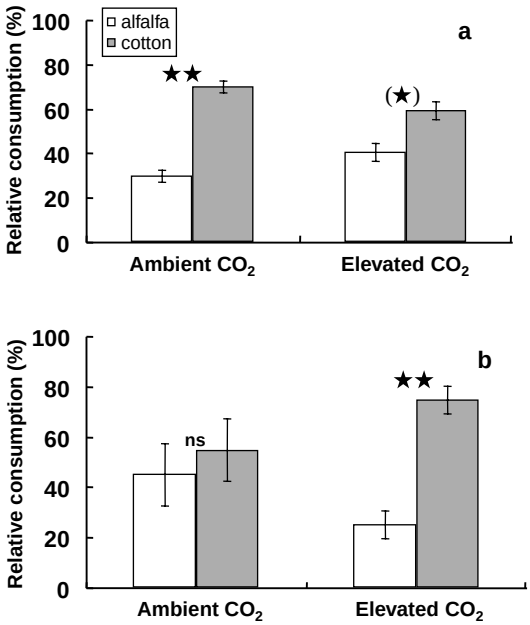

Fig. 1. Relative consumption (% of total dry mass consumed) of alfalfa and cotton in two-choice interspecific assays with 4th instar *S. littoralis* larvae. Assays were conducted separately for plants grown in ambient (355 ppm) and elevated (700 ppm) CO_2 environments. Data are presented for (a) control (undamaged) plants and (b) insect damaged plants, i.e. plants exposed to *S. littoralis* feeding for 24 h seven days prior to the experiment. Vertical bars represent SE. ** = $p < 0.01$, (*) = $0.05 < p < 0.10$, ns = $p > 0.10$.

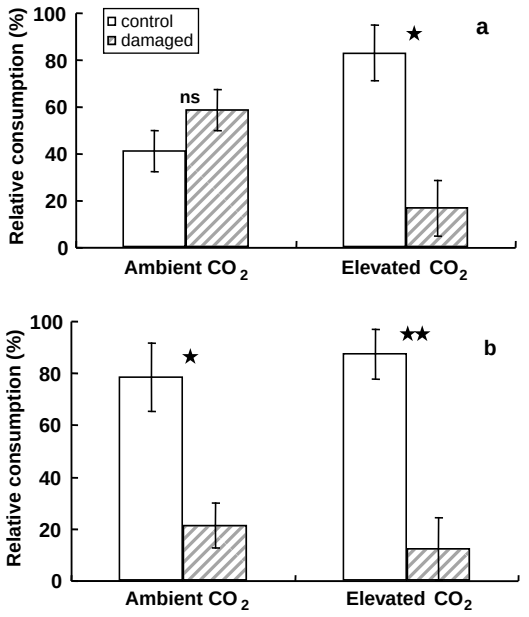

Fig. 2. Relative consumption (% of total dry mass consumed) of control (undamaged) and insect damaged alfalfa (a) and cotton (b) plants in two-choice intraspecific assays with 4th instar *S. littoralis* larvae. Assays were conducted separately for plants grown in ambient (355 ppm) and elevated (700 ppm) CO_2 environments. ** = $p < 0.01$, * = $p < 0.05$, ns = $p > 0.10$.

To determine effects of CO_2 on the induced defence of the two plant species, we performed within species assays with control vs damaged plants. For alfalfa grown in ambient CO_2 levels, insect damage had little effect on larval preferences, since similar amounts of control and damaged plants were consumed (paired t-test, $n = 8$, $t = 1.76$, $p = 0.183$, Fig. 2a). However, with alfalfa from the elevated CO_2 treatment larvae consumed significantly less of damaged compared with control plants (paired t-test, $n = 8$, $t = 4.78$, $p = 0.017$, Fig. 2a). No corresponding CO_2 effect on the induced defence of cotton could be detected: larval consumption was approximately five times greater of control than of damaged plants irrespectively of CO_2 treatment (paired t-tests, $n = 8$: $t = 3.76$, $p = 0.032$ in ambient CO_2, and $t = 6.82$, $p = 0.006$ in elevated CO_2, Fig. 2b).

Treatment effects on larval consumption (calculated as mg dry plant mass consumed per mg dry mass larvae and day) depended on the food plant species available. During trials with alfalfa vs cotton consumption was similar irrespectively if plants were damaged or not, and unaffected by CO_2 treatment ($X \pm SE$, control plants-ambient CO_2: 0.44 ± 0.08 mg $\times$ mg^{-1} $\times$ day^{-1}; control plants-elevated CO_2: 0.51 ± 0.05 mg $\times$ mg^{-1} $\times$ day^{-1}; damaged plants-ambient CO_2: 0.60 ± 0.06 mg $\times$ mg^{-1} $\times$ day^{-1}; damaged plants-elevated CO_2: 0.50 ± 0.04 mg $\times$ mg^{-1} $\times$ day^{-1}, two way ANOVA: $p > 0.10$ for CO_2, damage, and $CO_2 \times$ damage effect). Consumption during the intraspecific trials, i.e. with control vs damaged plants of either alfalfa or cotton, was more variable. In alfalfa assays larval consumption was higher when CO_2 enriched plants were used, than when trials were performed with plants from ambient CO_2 treatments (0.74 ± 0.04 and 0.40 ± 0.07 mg $\times$ mg^{-1} $\times$ day^{-1}, respectively). In cotton trials no corresponding CO_2 effect could be detected, and larvae consumed similar amounts in trials with plants from ambient (0.45 ± 0.09 mg $\times$ mg^{-1} $\times$ day^{-1}) and elevated (0.42 ± 0.12 mg $\times$ mg^{-1} $\times$ day^{-1}) CO_2 conditions. The strong CO_2 effect on larval consumption in alfalfa trials, and lack of such an effect in cotton trials resulted in a significant $CO_2 \times$ plant species interaction (two way ANOVA: $F_{1,12} = 6.38$, $p = 0.027$), whereas independent effects of CO_2 and plant species only tended to be significant (CO_2 effect: $F_{1,12} = 4.42$, $p = 0.057$; diet effect $F_{1,12} = 3.74$, $p = 0.077$).

Larval growth and development

Rearing larvae from hatching to pupation on control and damaged alfalfa and cotton, grown in either ambient or elevated CO_2 conditions, demonstrated strong treatment effects on larval physiological performance (Fig. 3). Larval growth (as determined by larval fresh mass) was on average highest for larvae feeding on

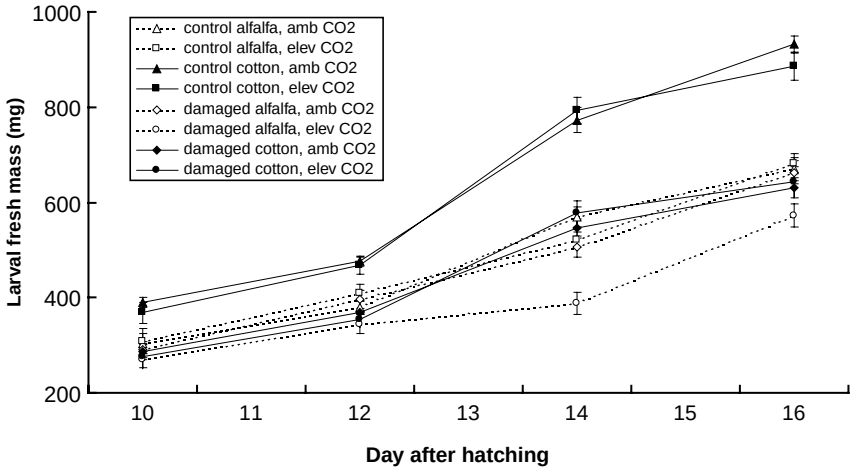

Fig. 3. Larval mass of *S. littoralis* larvae raised from hatching to pupation on alfalfa or cotton grown in either ambient (355 ppm) or elevated (700 ppm) CO_2 levels. Plants fed to larvae were either control (undamaged) or damaged, i.e. had been exposed to *S. littoralis* feeding for 24 h seven days before being fed to the larvae. Data are presented for day 10–16 after hatching. Vertical bars represent SE.

cotton, on control plants, and on plants from ambient CO_2 conditions (repeated measure ANOVA, effect of plant species: $F_{1,24}=100.5$, $p<0.001$; effect of damage: $F_{1,24}=129.3$, $p<0.001$; effect of CO_2: $F_{1,24}=4.38$, $p=0.047$). However, growth was more negatively affected by previous insect damage for larvae feeding on cotton than for those feeding on alfalfa (species × damage: $F_{1,24}=46.2$, $p<0.001$). Furthermore, interactive effects of CO_2 level, species and damage significantly influenced larval growth (CO_2 × species × damage: $F_{1,24}=4.97$, $p=0.035$). This demonstrates that for larvae fed cotton the negative impact of damage on larval growth was independent of CO_2 level, whereas for larvae fed alfalfa growth was reduced on damaged plants only in the elevated CO_2 treatment (Fig. 3). Thus, larval growth was strongly affected in three treatment combinations: larvae on diets of undamaged cotton from both ambient and elevated CO_2 grew better, and larvae fed damaged alfalfa from elevated CO_2 grew worse than did the average larvae in any other treatment.

Development time (from hatching to start of pupation) was also affected by the experimental treatments (Fig. 4a). On average larvae on damaged plants increased development time by 1.5 days (three way ANOVA, effect of damage: $F_{1,24}=117.1$, $p<0.001$). This effect differed significantly between treatment combinations, though, so that damage effects were greater for larvae fed cotton (species × damage: $F_{1,24}=15.14$, $p=0.001$), and when the diet consisted of CO_2 enriched plants (CO_2 × damage: $F_{1,24}=13.70$, $p=0.001$). Finally, similar to treatment effects on larval growth, development time was found to increase on damaged cotton irrespectively of CO_2 treatment, whereas on alfalfa this effect only existed in the elevated CO_2 treatment (CO_2 × species × damage: $F_{1,24}=5.19$, $p=0.032$, Fig. 4a).

Treatment effects on pupal dry mass largely followed the same pattern (Fig. 4b), although CO_2 enrichment had a limited impact. Larvae reared on cotton showed the overall highest pupal masses (effect of plant species: $F_{1,24}=100.1$, $p<0.001$), whereas larvae fed damaged plants showed reduced pupal masses compared with those reared on control plants (effect of damage: $F_{1,24}=51.0$, $p<0.001$). However, pupal mass reductions due to damage were significantly greater for cotton fed larvae, than for larvae fed alfalfa (species × damage: $F_{1,24}=21.0$, $p<0.001$, Fig. 4b).

Survival during rearing assays was overall high (91–99%) and very similar between treatments ($p>0.40$ in all cases), so detailed data are not presented.

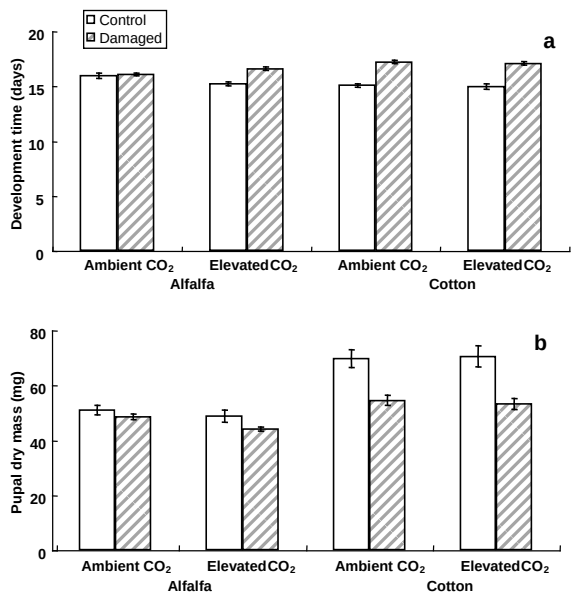

Fig. 4. Development time (a) and pupal dry mass (b) for larvae raised on control (undamaged) and insect damaged alfalfa and cotton grown in either ambient (355 ppm) or elevated (700 ppm) CO_2 environments. Damaged plants had been exposed to *S. littoralis* feeding for 24 h seven days before being fed to the larvae. Vertical bars represent SE.

Discussion

The results of this study demonstrate that interactive effects CO_2 enrichment and herbivore damage can alter host plant preferences of important herbivores. Altered host plant preferences were here due to that herbivore-induced responses of the plant species were differently affected by increased CO_2 levels. Two main conclusions can be drawn from our results. The first is the importance of taking herbivore damage into account when examining and making predictions about environmentally induced effects on host plant preferences, which is well illustrated by the fact that we found CO_2 effects to be totally opposite when undamaged and damaged plants were used. The other is that interactions between generalist herbivores and their different host plant species can be significantly modified by previous herbivory. However, this study was performed in an artificial environment, where several potentially important ecological variables are excluded, e.g. presence of predators/parasites and fluctuating abiotic conditions. Direct interpretations regarding effects in a natural environment must thus be made with caution.

CO_2 and damage effects on host plant choice

CO_2 and damage induced changes in the plants that strongly affected performance of *S. littoralis* larvae. Regarding host plant preferences, tests with undamaged plants from ambient CO_2 conditions showed that larvae of *S. littoralis* have a general preference for cotton over alfalfa. An increase in CO_2 availability had relatively little effect on the overall pattern as long as undamaged plants were used (Fig. 1a). Previous herbivore damage strongly affected larval preferences, though. Under ambient CO_2 conditions, damage caused larvae to consume similar amounts of cotton and alfalfa, i.e. resulted in that the general preference for cotton was lost (Fig. 1a, 1b). Obviously the comparably strong induced defence of cotton (Alborn et al. 1996, McAuslane et al. 1997, Anderson et al. 2001) shifted preferences towards alfalfa. Also, with damaged plants the influence of CO_2 on host plant preferences became evident: elevated CO_2 levels shifted larval preferences away from damaged alfalfa and back towards damaged cotton (Fig. 1b). This CO_2 effect was due to that alfalfa showed a stronger response to herbivore damage in elevated than in ambient CO_2 environments, whereas the response of cotton was similar in both treatments (Fig. 2).

This study thus demonstrates that changes in the atmospheric environment, through effects on plants, can significantly alter host selection behaviour of important herbivores. Examples of such CO_2 effects are rare, since most previous studies reported negligible effects on host plant preferences (Arnone et al. 1995, Traw et al. 1996, Lederberger et al. 1997, 1998, Díaz et al. 1998). We know of only a few indications of a CO_2 induced change in herbivore preferences between plant species. One is a study on a generalist herbivore slug (*Deroceras reticulatum*), which tended to increase preference for legumes over non-legumes in response to CO_2 enrichment (Peters et al. 2000). Also, elevated CO_2 levels have been shown to alter preferences of *Coenonympha pamphilus* larvae for different grass species (Goverde and Erhardt 2003), and cause Forest tent caterpillars (*Malacosoma disstria*) to make a partial switch from birch to aspen (Agrell et al. 2005). It is interesting that in the present study CO_2 enrichment only had a significant impact on host plant preferences when herbivore damage was included as an experimental variable. This suggests that the constitutive defence of the two plant species responded similarly to CO_2 enrichment, whereas CO_2 effects on the induced defence differed.

Consumption during host preference assays with both alfalfa and cotton available was unaffected by CO_2 and damage treatments, despite the fact that both CO_2 and damage significantly altered foliage quality and reduced larval performance. In preference assays with only one species available (control vs damaged), on the other hand, larvae showed increased consumption on alfalfa compared with cotton, especially if CO_2 enriched alfalfa was used. Although consumption estimates from 24 h tests are crude, these data show that insect herbivores can respond to CO_2 enrichment with compensatory consumption (Fajer et al. 1989, Roth and Lindroth 1995, Lindroth 1996, Bezemer and Jones 1998, Agrell et al. 2000), but that this effect may be buffered if larvae can escape reduced food quality by feeding on an alternative, presumably less affected host species. We agree with Körner (2000) and Peters et al. (2000) that predictions about consumption in altered environments need to take potential host plant shifts into account.

Physiological effects on larvae fed CO_2- enriched and damaged foliage

Data on larval performance corresponded well with observed treatment effects on host plant preferences. Larval growth and pupal mass was highest for larvae fed undamaged cotton, and lowest on damaged alfalfa grown in high CO_2. Survival was little affected, but data on development time further supported that CO_2 effects on the induced defence differed between the two plant species. Herbivore damage increased development time on cotton irrespectively of CO_2 treatment, whereas with alfalfa this negative effect on larval performance was observed only with CO_2 enriched plants. Thus, the combination of data on behavioural and physiological performance show that generalist insect herbivores can

detect and respond to variations in host plant quality caused by environmental changes, and adjust their host plant preferences accordingly.

What then caused larvae to show altered performance (and host plant preferences) on CO_2 enriched and/or damaged plants? The strong negative response towards damaged cotton was most likely due to high levels of terpenoid aldehydes, which increase dramatically in cotton plants after an herbivore attack (Alborn et al. 1996, McAuslane et al. 1997). In a companion study, where plants were grown under similar conditions as in the present study, levels of terpenoid aldehydes doubled in damaged cotton (Agrell et al. 2004). Interestingly, CO_2 enrichment had no impact on cotton secondary compounds, but reduced nitrogen content in undamaged plants. In contrast, secondary compounds of alfalfa were affected by both CO_2 and herbivore damage (Agrell et al. 2004). Especially levels of total saponins and the flavonoid free apigenin, which act as defences against generalist herbivores (Nozzolillo et al. 1997, Oleszek et al. 1999, Simmonds 2001), reached very high levels in damaged, CO_2 enriched alfalfa, which also showed reduced nitrogen content (Agrell et al. 2004). Thus the CO_2 induced reduction in preference for cotton observed when undamaged plants were used was probably due to a combination of reduced nitrogen levels in cotton and relatively low levels of secondary compounds in alfalfa. In contrast, with damaged plants CO_2 enrichment shifted preferences away from alfalfa and towards cotton most likely because levels of saponins and flavonoids in alfalfa were boosted up and nitrogen content was reduced.

Ecosystem consequences of interactions between CO_2, plants and herbivores

Ecosystem changes in a CO_2 enriched world may occur through interactive effects of altered competitive balance between plant species and changing impact of important herbivorous insect species (Lindroth et al. 1993, Saxe et al. 1998). Interactions with herbivorous insects thus play an important role in these processes, since plants which benefit more from increasing CO_2 levels are likely to suffer less from insect attacks, compared with plants that do not have the capacity to grow faster and/or boost up defence systems in response to CO_2 enrichment. There are three primary ways that would lead to altered herbivore impact on plant species in a changing environment. One is that insect herbivores simply do worse on its host species, which would result in reduced population density and less impact on the plants. Second, herbivorous insects may counter reduced food quality of CO_2 enriched plants by increasing consumption, as has been observed in several studies (Fajer et al. 1989, Roth and Lindroth 1995, Lindroth 1996, Bezemer and Jones 1998, Agrell et al. 2000). Third, insects may switch from one host plant to another, thereby altering their relative impact on potential host species. Our data show that elevated CO_2 levels, here in combination with herbivore damage, can alter physiological performance of important insect herbivores. However, to what extent reductions in larval growth, pupal mass, and in the end reproduction will cause declining insect populations will largely depend on if the insects counter altered host plant quality with behavioural adjustments, e.g. partial or complete host plant shifts. This would not mean that insect impact on the competitive balance between plants is reduced. On the contrary, behavioural responses are likely to result in a more rapid redistribution of insects among potential host plant species, compared with if host plant shifts came about primarily through the process of natural selection. Exactly how these processes may operate under natural conditions is not known and further studies in the field are required. Nevertheless, this study demonstrates that insect herbivores can respond to environmentally induced changes in food quality, and thus the potential for such processes to play an important role in shaping future ecosystems.

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