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Effect of elevated atmospheric carbon dioxide on the use of foliar application of *Bacillus thuringiensis*

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Abstract. Toxins from *Bacillus thuringiensis* have been used as pest management tools for more than 50 years. The effect of these toxins depends on the quantity *of Bacillus thuringiensis* (Bt) toxins ingested by susceptible insects. Food ingestion is affected by CO_2 concentration; plants grown in elevated CO_2 often have increased carbon/nitrogen ratios (C/N), resulting in greater leaf area consumption. Therefore, we hypothesized that elevated CO_2 would improve the efficacy of foliar applications of *B. thuringiensis*. Cotton plants were grown at either ambient (360–380 μl/l) or elevated CO_2 (900 μl/l). Groups of plants in both CO_2 treatments were exposed to low (30 mg/kg soil/week) or high (130 mg/kg soil/week) nitrogen (N) fertilization levels in a split plot design. The resulting plants were assessed for N and carbon (C) contents. Leaf disks from the same plants were dipped in a Bt solution and then fed to *Spodoptera exigua* (Hübner), an insect species of considerable economic importance. Elevated CO_2 significantly reduced total N, and increased the C/N. Nitrogen fertilization significantly affected consumption by early stadia larvae, larval weight gain, and relative growth rate (RGR). Interactions between CO_2 concentration and N fertilization level significantly impacted late stadia larval food consumption, and through differential Bt toxin intake, affected duration of larval stage and mortality to the adult stage. We conclude that the elevated atmospheric CO_2 concentrations expected in the next century will interact with commercial fertilization practices to enhance the efficacy of *B. thuringiensis* formulations applied topically to crops. The implications for improved control are discussed.

Key words: biological control, climate change, CO_2, *Bacillus thuringiensis* var. *aizawai*

Introduction

Bacillus thuringiensis (Bt) has been available for insect control since the mid 1930's (Tabashnik, 1997), and has been in widespread use for more than 30 years (Biever et al., 1994). This material is a critical component in many current pest management programs (Koppenhofer, 1997; Trumble, 1998; Tamez-Guerrara, 1999).

Because Bt toxins are activated in the insect gut, they must be ingested (van Frankenhuyzen, 1993). Some insects, such as *S. exigua* and *Trichoplusia*

ni (Hübner), may recover if sublethal amounts of Bt are ingested (Gharib and Wyman, 1991; Berdegué et al., 1996). An increased rate of ingestion therefore increases the potential for intoxication. In this way any factor that increases consumption could potentially increase the efficacy of foliar application of Bt. There is considerable evidence that increasing concentrations of CO_2 could cause such an effect. Working with lepidopterans that were fed on plants infected with Balansiae fungal endophytes, Marks and Lincoln (1996) found that increased feeding exacerbated the negative effects on both growth and survival.

According to ice core records from Antarctica and Greenland, the current average CO_2 level of 366 microliters per liter (μl/l) is the highest in at least the last 150,000 years (Bazzaz et al., 1992; Duplessy, 1992; Raynaud et al., 1993; Sowers et al., 1993). During the first half of the next century, the level of atmospheric CO_2 will continue to rise from the current 366 μl/l, to a new equilibrium level somewhere between 700 to 1,100 μl/l (Houghton et al., 1996; Mahlman, 1997; Fan et al., 1998; Joos et al., 1999). Predictions regarding the new equilibrium level depend on a variety of scenarios in the models, but some models have suggested future equilibrium levels as high as 1200 μl/l (Sarmiento et al., 1996; Sarmiento et al., 1998; Joos et al., 1999).

Among the probable effects of elevated atmospheric CO_2 are changes in plant nitrogen balance that will adversely affect host plant quality for many herbivorous insects (Coviella and Trumble, 1999). Plants grown in elevated CO_2 typically have lower N content per unit of plant tissue (Osbrink et al. 1987; Fajer et al., 1989; Bazzaz et al., 1992; Lincoln et al., 1993). This results in higher C/N (Polle, et al., 1997; but see Arnone et al., 1995). Insects that feed on plants with lower N per unit of plant tissue generally respond by increasing consumption, but may still suffer longer developmental times and higher mortality (Fajer et al., 1991; Wier et al., 1995; Traw et al., 1996). Other effects of elevated CO_2 on insect populations, resulting from an increase in mean temperatures and rainfall, are expected (Houghton et al, 1996; Coviella and Trumble, 1999). However, in this study we focus on the possible impact, and biological relevance, of elevated CO_2 levels on host plant mediation of food consumption by insects.

For this purpose, we hypothesized that topical applications of Bt toxins to plants grown in elevated CO_2 would result in increased intoxication in herbivore populations as compared to insects grown in ambient CO_2 concentrations. We also speculated that fertilization rates would interact with CO_2 concentrations to ameliorate these results.

Materials and methods

Six chambers were constructed inside a temperature-controlled greenhouse. The chambers were made of a PVC pipe frame covered with Teflon transparent film, and were 127 cm(w) × 122 cm(h) × 102 cm(d) with a volume of 1.58 m^3. A fan attached to each chamber (Dayton Shaded Pole Blowers, Dayton Electric MFG. Co., IL) allowed for 0.49 to 0.51 air exchanges/minute. Incoming air was filtered through charcoal filters before entering the chambers and exhausted outside the greenhouse directly from each of the chambers. Three of the chambers were selected randomly and maintained at ambient CO_2 (360–380 μl/l); the other three chambers were maintained at an elevated level of 900 μl/l. The upper CO_2 concentration was an intermediate value chosen from projections of future CO_2 atmospheric equilibrium levels (Houghton et al., 1996; Mahlman, 1997; Fan et al., 1998; Joos et al., 1999). CO_2 levels were monitored in all the chambers and controlled in the elevated CO_2 treatments. CO_2 was monitored with a non-dispersive Infrared Gas Analyzer (IRGA, Type GMP111, Vaisala OY, Helsinki, Finland). A rotatory pump (Q-Com, Inc., Irvine, California) collected air samples for thirty seconds from the chambers in sequence. Thus, each chamber was individually sampled every three minutes. These air samples were then directed to the IRGA. The CO_2 concentration was sent to a computer and, depending on the level in each particular chamber, a decision was made to open or close a solenoid valve for CO_2 injection in the elevated CO_2 chambers.

Each of the elevated CO_2 chambers has two separate lines for CO_2 gas injection. One line from the CO_2 tank had a needle valve that was set to inject gas continuously at a rate that raised the CO_2 level inside the elevated CO_2 chambers to a baseline of approximately 870 μl/l. A solenoid in the second line was opened when the CO_2 level in that particular chamber was 870 μl/l or below and it was closed when the CO_2 level was 900 μl/l or above. Both CO_2 lines injected the gas in the air current just before the blower at the base of the chambers, so the gas would be completely mixed with ambient air before entering the chamber. Four metal halide 1000-watt lamps provided additional light to maintain a 14L:10D photoperiod.

Cotton plants (*Gossypium hirsutum* L., Deltapine DP5415) were sown in twenty 3.0 liter pots in each of the environmental chambers. The plants received N fertilization at either a level of 130 mg N/kg soil/week (the ‘high’ nitrogen level) or 30 mg N/kg soil/week (the ‘low’ nitrogen level) (after Rogers et al., 1996). The experiment was arranged in a split plot design, with CO_2 concentration (ambient or elevated) as blocks, chambers as the experimental units (replicates), and two levels of nitrogen fertilization inside each chamber. Once the plants had the fourth leaf completely expanded at approximately day 45, leaves were taken as needed from plants from each

treatment and replicate. Each plant was used only once and discarded. The leaves were dipped in a LC_{25} solution of XenTaril' (Abbott Laboratories, North Chicago, IL) containing *Bacillus thuringiensis* var. *aizawai* (170 μg/ml solution).

Leaf discs of approximately 3.5 cm^2 were cut, and measured in a leaf area meter (Li-cor LI-3000, Li-Cor, Inc., Lincoln, NE). Known amounts of treated leaf material were then fed to the *S. exigua* larvae. The leaf disks were replaced when approximately 80% of the material had been consumed to avoid common measurement errors (Schmidt and Reese, 1986). The disks were then measured again to determine consumption.

All insects used in the bioassays were from a laboratory colony maintained at the Department of Entomology, University of California at Riverside. A total of 240 insects were examined in four treatments with three replicates each. For each replicate, twenty neonate larvae were reared individually on plant material in 30 ml plastic cups lined with agar to keep the plant material fresh (after Diawara et al., 1992). An additional 120 insects were fed non-Bt treated plant material, and used as controls.

From the same plants used for the insect bioassays, leaves were taken for analyses. Data were collected on total leaf carbon and total leaf nitrogen at the DANR Labs, U.C. Davis, using the combustion method (Tate, 1994) on a Carlo-Erba equipment, insect leaf consumption, larval weight at day 4 and day 8, days to pupa, pupal weight, days to adult, and mortality. The first control insect reached the prepupal stage on day 9, so the interval from day 0 through day 4 was considered the first half of the larval stage (younger larvae), and the interval between days 4 through day 8 to be the second half (older larvae). We then calculated larval weight gain and relative growth rates (mg biomass gained/mg larval biomass/day, Waldbauer 1968). When the errors were normally distributed, we performed ANOVA analyses (SuperANOVA, Abacus Concepts. Inc., Berkeley, CA). When the errors were binomially distributed, the Generalized Linear Interactive Model Program (GLIM 4, 1993 The Royal Statistical Society, London) was used. This program linearizes the binomial data using the logit link function and uses sample size to carry out a weighted analysis (Hails and Crawley, 1992; Crawley, 1993).

Results and discussion

Significant two-way $CO_2 \times N$ interactions were observed for plants for both total N content ($p < 0.001$) and C/N ratios ($p = 0.005$). Plants grown in elevated CO_2 had lower total N and higher C/N ratios per unit of plant tissue when compared with plants grown under ambient CO_2 (Figure 1). This result

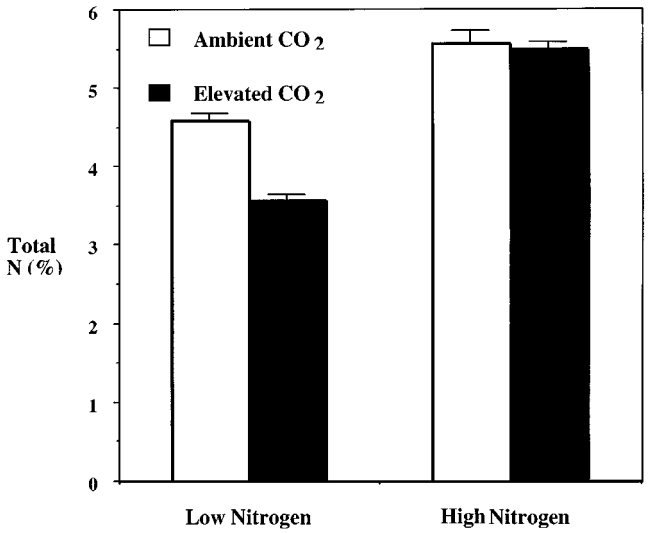

Figure 1. Significant two way $CO_2 \times N$ interaction for total plant nitrogen ($p < 0.001$). Plants receiving low N fertilization had significantly lower total N when grown in elevated CO_2. High N fertilization compensated for this effect and no significant difference was observed.

is consistent with the literature regarding CO_2 effects on plant physiology (Acock et al., 1985; Arp et al., 1991; Lindroth et al., 1993; Diaz et al., 1998; Kerslake et al., 1998). For plants grown with low N availability, the total N concentration decreased by nearly 25% when grown in elevated versus ambient CO_2. The difference was not significant when high levels of nitrogen were added to the soil.

Significant two-way $CO_2 \times N$ interactions were also observed in the treatment groups for several of the insect variables measured. Older larvae fed with plant material grown in elevated CO_2 with low N fertilization consumed significantly more ($p = 0.009$) than insects fed with plant material grown in ambient CO_2. Under low N fertilization, the difference in consumption was over 25% (5.159 cm^2 in ambient CO_2 vs. 6.468 cm^2 in elevated CO_2). No difference was observed with high N fertilization (Figure 2a). A significant $CO_2 \times N$ interaction ($p = 0.024$) also was evident in the duration of the larval stage (as days to pupation, Figure 2b); developmental times were longer (24 days vs. 21 days) when larvae were exposed to Bt treated plants grown with low N availability, and elevated CO_2. This represents a 12% increase in developmental time. No differences were observed in the control insects ($p = 0.096$). This result was consistent with the higher consumption rates observed when insects were fed plants with low N, which led to higher Bt toxin intake by these insects. Thus, not surprisingly, a significant CO_2

× N interaction was evident for larval mortality in these treatments (p = 0.032, Figure 2c); mortalities for low N and high CO_2treatments exceeded 60%, whereas the controls showed no difference (p = 0.47), with mortalities ranging from 5.5% to 12.2%. Total mortality through the adult stage (e.g., larval and pupal mortality) was also significantly different (p = 0.002); insects in the treatments suffered the highest mortality on low N plants. However, following the same pattern as with consumption, this higher mortality was not observed when N availability was high. No significant differences were observed in the control group (p = 0.675).

Some of the variables measured did not show significant two-way interactions and could be evaluated independently for N and CO_2 main effects. Consistent with the previous literature on the effects of CO_2, we found no main effect from CO_2 level alone on the insects (see Coviella and Trumble, 1999, and references therein). This is in agreement with CO_2 having an effect primarily through action on the N content of the plants. Nitrogen level alone had significant effects on RGR (p = 0.003). Insects fed on plants provided with high N had an RGR of 0.22 mg weight gain per day/g (s.e. = 0.002) compared with 0.21 mg weight gain per day/g (s.e. = 0.004) for insects fed on low N plants. Larval consumption was also affected. When larvae were young (neonates to 4 days), insects fed high N plants consumed significantly more (42.1% more; $p < 0.001$) than larvae fed plants with low N availability. This is consistent with reports of total N being correlated with soluble and amino N, which are known to be feeding stimulants (Minkenberg et al., 1989; Minkenberg et al., 1990; Bentz et al., 1995). However, this increased consumption led to higher Bt toxin intake, and after day 4, older larvae consumed less when feeding on plants in high N. This difference was presumably due to the known sub-lethal effects of Bt toxin on feeding behavior (Berdegué et al., 1996), since it was not observed in control insects fed on non-Bt treated plants. Despite a higher initial consumption, insects fed high N had overall lower consumption than insects under low N (p = 0.002). When measured from first instar to pupation, insects fed on plants grown with high N consumed less plant material than insects fed on plants grown with low N. Thus, it is imperative when evaluating these results to consider the effect of larval age and feeding behavior.

The data gathered is consistent with the hypothesis that the expected increase in CO_2 will lead to enhanced effects of Bt formulations in particular, and insecticides that depend on ingestion in general, through an increase in food consumption. This result should be maximized under conditions of low N availability for the plants, and minimized under conditions of very high N availability.

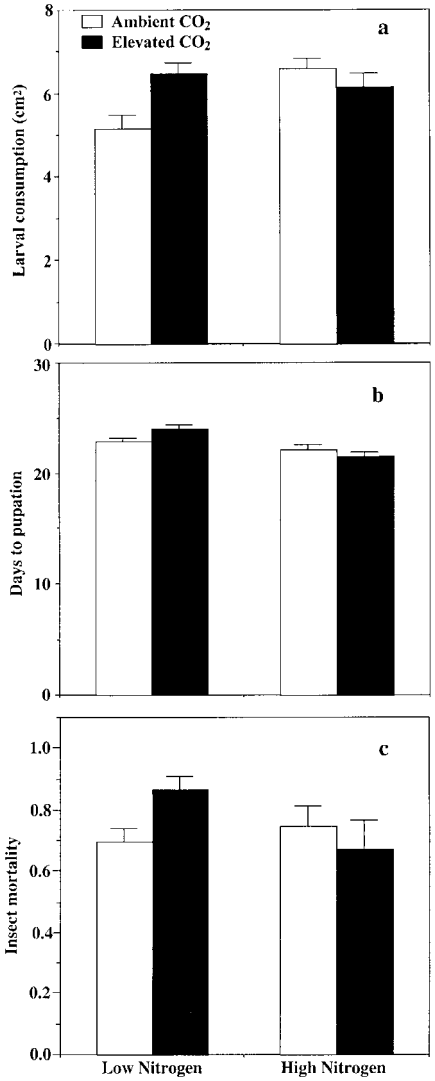

Figure 2. (a) Significant two way $CO_2 \times N$ interaction for larval consumption ($p = 0.009$). When fed plants grown in low N fertilization, insects consumed significantly more leaf material in elevated CO_2 than in ambient CO_2. No significant differences in consumption were observed in high N. (b) Significant two way $CO_2 \times N$ interaction on the duration of the larval stage ($p = 0.024$). In low N, insects fed plants grown in elevated CO_2 required significantly longer to develop to the pupal stage. No significant differences were observed in high N. Overall duration of larval stage for control insects was 16.74 days. (c) Significant two way $CO_2 \times N$ interaction for insect mortality ($p = 0.032$). Consistent with the effect of higher Bt toxin intake due to enhanced consumption, insects fed on low N plants had significantly higher mortality in elevated CO_2. No significant effect was observed in high N. Mortality for control insects was $\leqslant 0.10$.

An increase in food consumption has been previously shown for insects fed on plants grown under elevated CO_2 (Osbrink et al., 1987; Fajer et al., 1991; Wier et al., 1995; Traw et al., 1996). However, our results show that this increase is not only statistically significant, but is biologically relevant as well. As a consequence of the lower N content per unit of plant tissue in plants, the enhanced food consumption will make insects more susceptible to other substances present on or in the plants. Of particular interest is the probable increased effect of pathogens or insecticides that require ingestion. In addition, the efficacy and implementation of host plant resistance to herbivory likely will be affected. Secondary compounds not reduced by the interactions between the increase in CO_2 and fertilization level are likely to be more effective against herbivory under future elevated atmospheric CO_2 concentrations.

Despite more than half a century of continuous use of foliarly- applied Bt formulations, to date relatively few insect species have developed resistance in the field. For those populations where resistance has occurred, notably *Plutella xylostella* L. (Lepidoptera: Plutellidae), resistance may be conferred at a single locus (Tabashnik et al., 1997a). In addition, the single locus provided resistance to several related toxins, possibly speeding up the development of resistance (Tabashnik et al., 1997b; Wirth and Georghiou, 1997; Wirth et al., 1998). Finally, the development of resistance should be faster when insects are exposed to only one protein (Tabashnik et al., 1997a). Using *Culex* mosquitoes as a model for resistance development, Georghiou et al. (1997) found that resistance evolved most rapidly when insects were exposed to only one toxin (as in transgenic plants encoding for a single protein), and was minimized with exposure to a combination of 2–3 and 4 toxins, respectively. Thus, efforts should be made to conserve the activity of multiple-toxin foliar formulations at a time when increasing atmospheric CO_2 is making the foliar applications more efficacious.

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