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RAPID COMMUNICATION

Changes in Nutritional Value of Cyanogenic *Trifolium repens* Grown at Elevated Atmospheric CO_2

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Abstract Global food security in a changing climate depends on both the nutritive value of staple crops as well as their yields. Here, we examined the direct effect of atmospheric carbon dioxide on the toxicity of the important pasture crop, *Trifolium repens* L. (clover). Shoots of *T. repens* contain cyanogenic glycosides that break down to release toxic hydrogen cyanide when damaged. The ability of animals to tolerate cyanogenic compounds is dependent, in part, on their overall protein intake. We grew *T. repens* communities at ambient and approximately twice-ambient CO_2 in a controlled environment greenhouse experiment. We found that the ratio of total cyanogenic glycosides to total protein ratio was nearly two times higher in leaves of *T. repens* grown at elevated CO_2. This study highlights the importance of assessing the nutritive value of this and other plants in response to rising CO_2 so that steps can be taken to address any adverse consequences for herbivores.

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Introduction

Two major problems facing the world today are climate change and population growth. Despite the large body of research on the effect of elevated CO_2 on primary productivity, few studies consider the overall nutritional value of plants. Typically, leaf nitrogen and protein concentrations are 10–20% lower in plants grown at twice-ambient CO_2 (Ainsworth and Long 2005; Taub et al. 2008). In addition, concentrations of carbon-based defense compounds such as phenolics are often higher (Lincoln et al. 1993), again decreasing the nutritive value.

Less is known about the effect of elevated CO_2 on cyanogenic glycosides. Cyanogenic glycosides are constitutive defense compounds found in about 5% of all plants (Jones 1998; Gleadow and Woodrow 2002). When plant tissue containing cyanogenic glycosides is crushed or chewed, the glycosides are mixed with endogenous β-glucosidases, and toxic hydrogen cyanide (HCN) is released. Given the high proportion of crop plants that are cyanogenic (c. 60%, Jones 1998), it is vital to know whether or not such plants will become more cyanogenic in the future and to compare that with predicted decreases in leaf protein levels.

Trifolium repens (white clover) is an important component of pastures in many parts of the world. It contains two cyanogenic glycosides, mostly linamarin (α-hydroxyisobutyronitrile-β-D-glucopyranoside) and a smaller proportion of lotaustralin (2-hydroxy-2-methylbutyronitrile-beta-D-glucopyranoside). To our knowledge, only one elevated CO_2 study of *T. repens* has included an analysis of the

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endogenous cyanogenic glycosides (Frehner et al. 1997). Those results were inconclusive, as there was a lot of seasonal variation, and the workers did not measure leaf nitrogen. Gleadow et al. (1998), in a study of cyanogenic *Eucalyptus cladocalyx*, found that cyanogenic glycoside concentration per mass did not increase in leaves of seedlings grown at elevated CO_2, but there was a 40% decrease in leaf protein, increasing the overall leaf toxicity. The aim of this study, therefore, was to quantify the protein and cyanogenic glycoside concentrations in *T. repens* grown under ambient and twice-ambient CO_2 in order to predict the ability of pastures to support grazing animals in the future.

Methods and Materials

This experiment was part of a larger study described in detail by Edwards et al. (2006). Briefly, *Trifolium repens* cv. Haifa L. was grown in 36 plastic containers (525×370×450 mm) filled with steam-treated sand in temperature-controlled, naturally lit greenhouses (25°C/20°C; day/night) for 15 months. Each container was inoculated with *Rhizobium leguminosarum* bv. Trifolii strain ANU843. Greenhouses were supplied with air containing ambient (360 ppm) or twice-ambient (700 ppm) CO_2. Pots were watered daily and fertilized three times per week with 500 ml Rorison's solution, with the N components adjusted to contain low or high NO_3 (0 or 4 mM nitrate) (N=6). Cyanogenic glycosides were measured as evolved HCN on subsamples of freeze-dried leaf material, expressed as mg CN $g^{-1}{}_{DW}$ (Woodrow et al. 2002). Remaining leaves were oven dried and then analyzed for total leaf nitrogen by using an EA 1110 CHN-O Carlo-Erba Elemental Analyzer (Carbo-Erba instruments, Milan, Italy) and total protein with the Lowry method. Data were analyzed with Minitab15.

Results and Discussion

This is the first study on the impact of elevated CO_2 on nutritional quality in cyanogenic pasture plants. We found that leaf nitrogen was c. 20% lower in foliage of *T. repens* grown at elevated CO_2 ($P<0.05$; Fig. 1a), and protein concentration was c. 25% lower ($P<0.01$; Fig. 1b). These findings are consistent with other studies of many other C3 plants, and usually those reflect some degree of down-regulation in photosynthesis (Ainsworth and Long 2005). Such acclimation is usually more pronounced when growth rates are limited by nitrogen supply (Gleadow et al. 1998; Ainsworth and Long 2005). In our study, there was no significant difference in the response to elevated CO_2 between the two N treatments, probably because *T. repens*

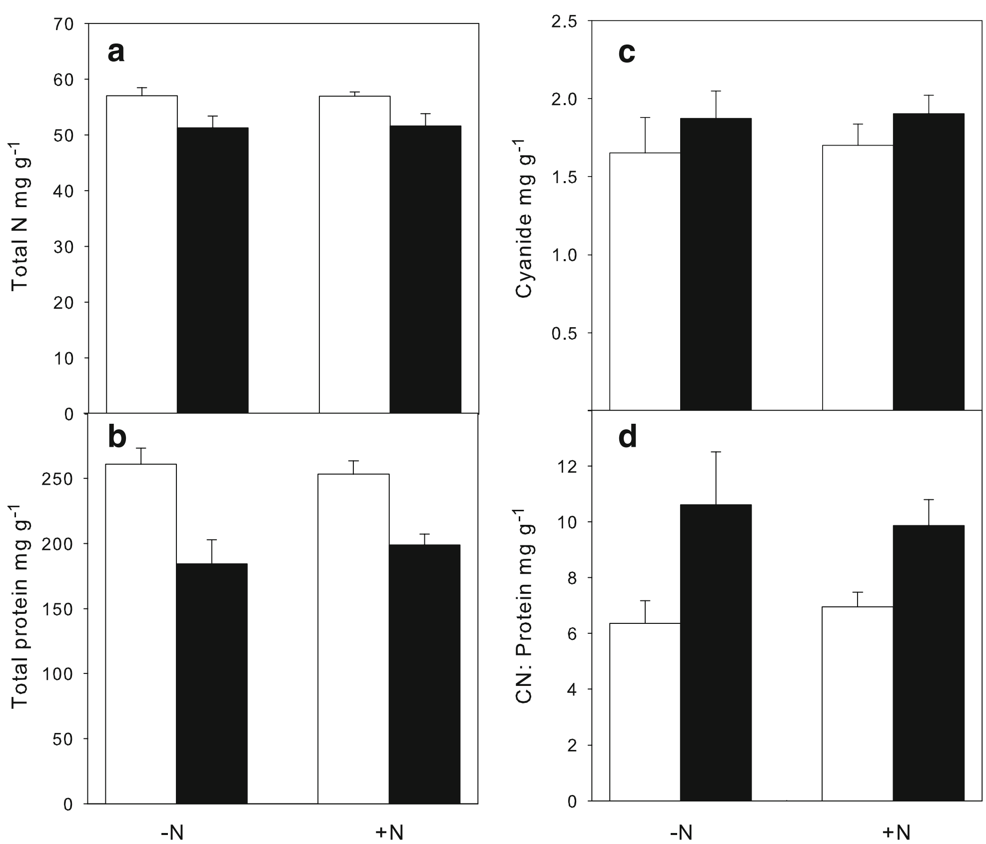

Fig. 1 Chemical composition of *Trifolium repens* grown at ambient (*open*) and elevated CO_2 (*closed*) and supplied with nutrient solutions containing nitrate (+N) or without nitrate (-N). **a** Total leaf nitrogen; **b** total protein; **c** cyanogenic glycosides (measured as evolved cyanide); **d** ratio of evolved cyanide to total leaf protein. All data are expressed on a dry weight basis

supplements its N requirements by fixing atmospheric N through symbiotic associations with *Rhizobium* bacterium (Edwards et al. 2006).

There have been, to our knowledge, only three published studies on the effect of atmospheric CO_2 on cyanogenic glycosides. We found here that total cyanogenic glycoside concentration in *T. repen*s was not significantly different in plants grown at ambient and twice-ambient CO_2 (Fig. 1c), consistent with these earlier studies (Frehner et al. 1997; Gleadow et al. 1998; Bazin et al. 2002). By contrast, we did not detect a significant impact of the nitrate supply on cyanogenic glycoside concentration as has been observed by others (Gleadow et al. 1998), reflecting the consistent leaf N concentrations across treatments.

Animals have the ability to convert cyanide to the less toxic thiocyanate, but the capacity to do this is dependent on the rate of ingestion and the availability of sulfur-rich proteins (Westley 1988; Gleadow and Woodrow 2002). We calculated the cyanide-to-protein ratio as an index of the nutritional quality of *T. repens* grown at elevated CO_2. The amount of cyanide relative to protein increased by 40% in plants supplied with NO_3 and by 30% in plants not given additional NO_3 (Fig. 1d; $P<0.001$), although the NO_3 effect alone was not statistically significant. Forage with greater than 0.8 mg CN $g^{-1}{}_{DW}$ generally is considered to be toxic to cattle under field conditions (Westley 1988), about half the value reported here (overall mean=1.7 mg CN $g^{-1}{}_{DW}$). Typically, *T. repens* occurs in mixed pastures, and animals would be unlikely to ingest toxic quantities. However, if cyanogenic glycoside concentration increases relative to the protein in the future, then the overall nutritional value of the pasture would be substantially less.

Protein content of food crops such as wheat and rice are predicted to contain to 15–20% less protein by the end of this century (Taub et al. 2008). Lower protein content of leaves of pasture plants is also of concern as this would mean that grazing animals would need to increase their consumption in order to maintain their current protein intake. Insect studies have shown that animals compensate for the lower protein content of plants grown at elevated CO_2 by eating more (Lincoln et al. 1993). If this is also true of grazing mammals, then they would ingest more cyanogenic glycosides along with the rest of the plants in mixed pastures. Other species in the pasture are likely to have higher concentrations of antifeedants such as phenolics as well, which are known to interfere with protein uptake (Lincoln et al. 1993; Taub et al. 2008). If these results hold true for other cultivars, it is possible that pastures rich in *T. repens* could become unsuitable for livestock if atmospheric CO_2 continues to increase. These results demonstrate the importance of testing the effect of climate change on the nutritive value of crop plants as well as yield.

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