

## SILICEOUS SAVIOUR OF SUGARCANE: SILICON ALLEVIATES NEGATIVE IMPACTS OF BELOWGROUND HERBIVORY UNDER ELEVATED ATMOSPHERIC CO<sub>2</sub>

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### Introduction

Atmospheric concentrations of carbon dioxide (CO<sub>2</sub>) have been gradually rising, and have risen at an average rate of 2.0 ± 0.1 ppm year<sup>-1</sup> during 2002–2011 and are expected to reach approximately 540–958 μmol mol<sup>-1</sup> by the year 2100 (Pachauri et al., 2014). In response to elevated atmospheric CO<sub>2</sub> concentrations (eCO<sub>2</sub>) many plants alter their nutritional value as the net carbon uptake of host plants increases resulting in an increase in the carbon to nitrogen ratio of the plant tissue (Robinson et al., 2012). As nitrogen is generally a limiting factor in insect diets many insects have a compensatory feeding response to an increase in the carbon to nitrogen ratio in attempts to acquire adequate nutrition (Simpson and Simpson, 1990). This increased feeding can potentially cause more damage to crop plants. As root-feeding insects are known to significantly reduce the yield of agricultural and forest systems (Hunter, 2001) it is important that novel control strategies that incorporate future climatic predictions are developed if they are to have long-term efficacy into the future. One plant trait that could contribute to such possible control strategies is plant silicon, which is known to play a role in defences against pathogens and herbivores (Epstein, 2009). While there is ample evidence that silicon has considerable negative impacts on aboveground herbivores (Massey and Hartley, 2009), almost no attention has

been paid to root-feeding herbivores. Considering that increasing atmospheric CO<sub>2</sub> concentrations are likely to increase the susceptibility of many plants to herbivorous insects, there is a need to investigate the role of silicon defences and their impacts on root-feeding insects under eCO<sub>2</sub>.

This research assesses the impacts of silicon soil supplementation on a belowground herbivore under elevated atmospheric CO<sub>2</sub> concentrations. Here we examine the responses of sugarcane (*Saccharum* sp. hybrid) and how these plant responses impact on greyback cane beetle larvae (*Dermolepida albobirtum* Waterhouse), also known as canegrubs.

### Methods

**Chambers.** Two glasshouse chambers, one maintained at ambient CO<sub>2</sub> of 400 μmol mol<sup>-1</sup> (aCO<sub>2</sub>) and the other at elevated CO<sub>2</sub> of 640 μmol mol<sup>-1</sup> (eCO<sub>2</sub>), were used. These chambers, (3 m × 5 m × 3 m; width×length×height) with UV transparent plexiglass (6 mm thick) walls and roof, were naturally lit throughout the experiment. Air temperature was regulated at 30°C (±4°C) and fell to 22°C (±4°C) at night. Humidity was controlled at 60% (±6%).

**Plant growth and treatments.** Thirty sugarcane (*Saccharum* species hybrid; Poaceae) plants were grown from single-eye cuttings from a cultivar commonly used within Australia, Q200. These were grown in 3.7 litre pots using gamma-irradiated 1:1 soil:potting mix (Richgro® All Purpose Potting Mix); the soil used was a sandy loam soil sourced from the Hawkesbury Forest Experiment, which is fully described in Barton et al. (2010). All pots had 2 g of Osmocote Controlled Release fertiliser added to ensure no nutrients were limiting to plant growth. Half of the plants in each chamber were watered every three days with 100 ml tap water (Si-), while the other half received 100 ml of 500 mgL<sup>-1</sup> soluble silica in the form of NaSiO<sub>3</sub>·9H<sub>2</sub>O (Si+). Throughout the experiment all plants received tap water as required. After 13 weeks all plants were removed from their pots and the plant material was placed in a 40°C oven for 48 hours, and then weighed; these dried samples were ground and used for analysis of carbon, nitrogen and silicon. One subsample of fresh root material was retained from each plant to be used in insect-feeding trials.

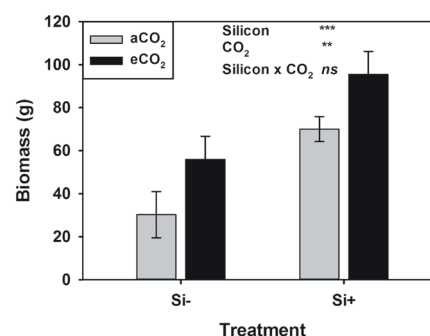
**Feeding trials.** To assess the impacts of silicon on the growth and root consumption by the canegrubs, we conducted feeding trials using an approach adapted from Massey and Hartley (2009). Individual third instar larvae were starved for 24 hours and weighed before being placed in a Petri dish (14 cm diameter) with a known mass of fresh sugarcane root material, taken from the harvested sugarcane plants that were grown under high and ambient silicon environments. Larvae were allowed to feed for 24 hours, after which time they were starved for a further 12 hours to ensure all frass had passed, before being reweighed. Values of water content, derived from root samples from the same plants, were used when converting fresh mass of roots to dry mass, to account for any evaporative water loss during of the experiment.

### Results and Discussion

Both elevated CO<sub>2</sub> and Si+ treatments had positive impacts on the overall growth of the sugarcane, which increased the overall biomass by 44.9% and 69.6% respectively.

#### Figure 1:

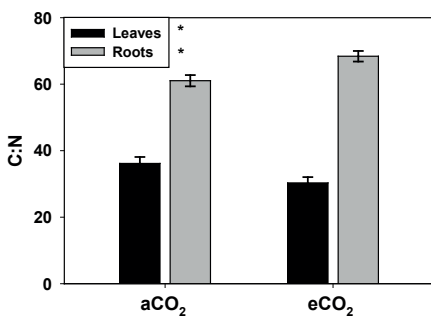
Effects of silicon supplementation (Si+) on the biomass (g) of sugarcane grown under ambient CO<sub>2</sub> (aCO<sub>2</sub>) or elevated CO<sub>2</sub> (eCO<sub>2</sub>). Mean values (±SE) shown. Significant terms indicated by \* (P < 0.05), \*\* (P < 0.01) and \*\*\* (P < 0.001).



The aboveground C:N decreased under eCO<sub>2</sub> treatment, while the root C:N increased under eCO<sub>2</sub> by 12% overall. This increase in the C:N ratio would indicate a decrease in the nutritional value of the roots as nitrogen concentrations decrease and insects are often limited by nitrogen in their herbivorous diets.

**Figure 2.**

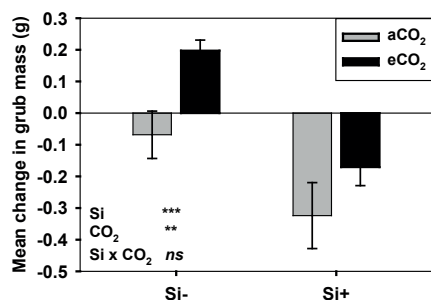
Carbon to nitrogen ratio of leaf and root tissue grown under ambient and elevated CO<sub>2</sub> concentrations. Mean values (±SE) shown. Significant terms indicated by \* (P < 0.05) to indicate significance of the main effect of the CO<sub>2</sub> treatments.



Elevated CO<sub>2</sub> caused the canegrubs to increase their change in mass overall compared with aCO<sub>2</sub>. The application of silicon significantly decreased the mass gain of the canegrubs compared with the controls. This is likely due to a decrease in the palatability and digestibility of the root material due to higher silicon concentrations, as well as an increase in root toughness (Massey and Hartley, 2009).

**Figure 3.**

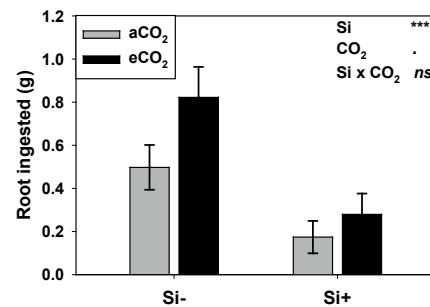
Effects of silicon supplementation (Si+) on the change in grub mass of canegrubs feeding on roots grown under ambient (aCO<sub>2</sub>) and elevated (eCO<sub>2</sub>) atmospheric CO<sub>2</sub> concentrations. Mean values (±SE) shown. Significant terms indicated by \* (P < 0.05), \*\* (P < 0.01) and \*\*\* (P < 0.001).



Canegrubs feeding on roots grown under eCO<sub>2</sub> consumed more root material compared with the canegrubs feeding on aCO<sub>2</sub> roots. This is likely to be a compensatory feeding response as the C:N of the roots increased under eCO<sub>2</sub> and therefore the canegrubs consumed more material in attempts to meet their nitrogen requirements.

**Figure 4.**

Effects of silicon supplementation (Si+) on the root consumption of sugarcane roots grown under ambient (aCO<sub>2</sub>) and elevated (eCO<sub>2</sub>) atmospheric CO<sub>2</sub> concentrations. Mean values (±SE) shown. Significant terms indicated by . (P < 0.1), \* (P < 0.05), \*\* (P < 0.01) and \*\*\* (P < 0.001).



The results from our study indicate that future atmospheric CO<sub>2</sub> concentrations may exacerbate the damage caused by root-herbivores to crops via increased herbivore performance and root consumption. However, our findings also indicate that the application of soil silicon can significantly decrease root-herbivore performance and consumption. Therefore to ensure crop protection under future atmospheric CO<sub>2</sub> concentrations the enhancement of plant silicon defences by silicon supplementation could be the focus for research towards food security in the future.

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