Soybean flavonoid profiles altered by CO_2, O_3, and herbivory

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Abstract

Japanese beetles (*Popillia japonica* Newman) live longer on soybean plants grown at projected future levels of elevated CO_2. Increased longevity could reflect changes in foliar flavonoid content, secondary compounds that increase in other plant species when grown under elevated atmospheres. Flavonoids in beetle-damaged and undamaged soybean leaves on two dates (6/30/05 and 7/19/05) were quantified by HPLC. All compounds were present in significantly higher concentrations in foliage grown under elevated O_3 conditions. Two flavonols (quercetin glycosides) increased in foliage grown under elevated CO_2 conditions, whereas concentrations of two isoflavonoids (daidzein and genistein) decreased. Beetle damage resulted in increased amounts of quercetin, genistein, and daidzein glycosides. In that flavonoids have been associated with longevity enhancement, the observed changes in flavonoid concentrations may account for the increased longevity of beetles under elevated CO_2.

Figure 1a

Figure 1b

Figure 1 – a, the SoyFACE field site in central Illinois. b, mesh cage for excluding or containing beetles.

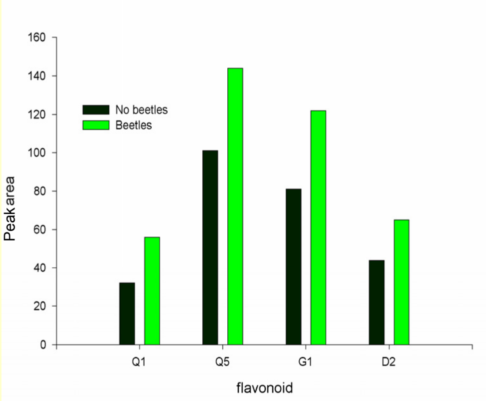

Figure 2 - Flavonoid profiles for significantly different undamaged and beetle-damaged plants, regardless of date or atmospheric treatment. Flavonoid compounds are labeled as: Q = quercetin, K = kaempferol, G = genistein, and D = daidzein. Numbers indicate a separate glycoside.

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Introduction

Atmospheric carbon dioxide levels have risen since the start of the industrial revolution, from 280 ml l^{-1} to 370 ml l^{-1} today. This rise is expected to double within the next 100 years. Japanese beetles (*Popillia japonica* Newman) fed soybean foliage grown under elevated CO_2 experienced increased longevity. Secondary chemicals that increase in other plant species grown under elevated CO_2 include flavonoids, compounds with known antioxidant properties. Dietary flavonols, for example, enhance insect longevity (Wang et al., 2003). Isoflavonoids, however, may function as defenses against herbivore attack and other stresses (Liu et al., 1992). Thus, increased beetle longevity could be due to increases in antioxidant flavonols, and/or to decreases in isoflavonoids. We measured the levels of individual flavonoids in undamaged and beetle-damaged soybean foliage grown under elevated levels of CO_2, and elevated levels of O_3, another atmospheric pollutant of concern to soybean growers.

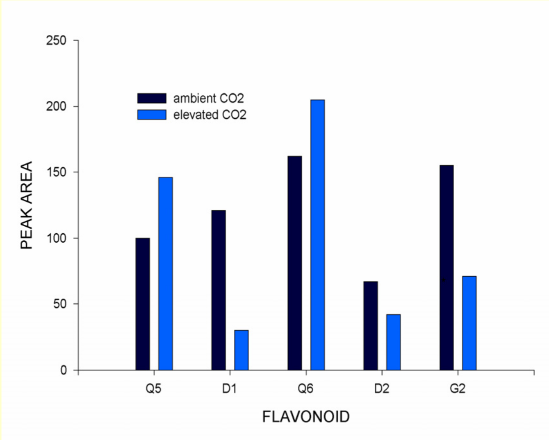


Figure 3 – Flavonoid profiles for significantly different plants grown under ambient and elevated levels of CO_2, regardless of date or damage. Compound labeling is the same as in figure 2.

Methods

Research was conducted at the SoyFACE (soybean free air gas concentration enrichment) facility in Savoy, Illinois (Figure 1a). Flavonoids were measured in beetle-damaged and undamaged foliage at multiple time points for all treatments. Soybean plants (Pioneer 93B15) in the field were covered with mesh cages (Figure 1b) that either excluded all herbivores or were stocked with 60 Japanese beetles (*Popillia japonica* Newman). Leaf disks measuring one cm diameter were collected from the field on 6/30/05 and 7/19/05. Six disks from each treatment were placed in a 2-ml centrifuge tube and homogenized by adding a six mm glass bead and shaking in a bead beater for 15 seconds. Methanol (500 ul) was added to each sample which was then shaken again for another 15 seconds to mix. Extraction continued undisturbed for one hour at room temperature. Samples were then sonicated for one minute and centrifuged for two minutes. Flavonoid compounds in the supernatant were separated in a reverse-phase HPLC (712 Wisp, Waters Corporation, USA) with a 250 x 4.6 mm ID 5 um Capcell Pak C18 column (AG120, Shiseido Fine Chemicals, Japan). Flavonoids were identified by comparing UV spectra with those of pure compounds. Peak areas were compared by repeated measures two-way ANOVA, with damage and date repeated and CO_2 and O_3 as fixed effects.

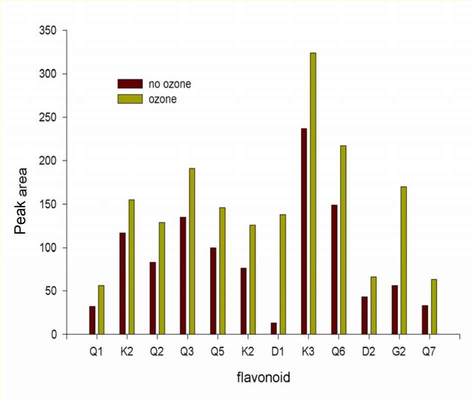
Results

Four classes of flavonoid compounds were identified--the isoflavones genistein and daidzein and the flavonols quercetin and kaempferol. The aglycones as well as several ester conjugates were found in all samples. A significant main effect of beetle damage was found for two quercetin compounds, a genistein glycoside, and a daidzein glycoside (figure 2). Two quercetin glycosides significantly increased in foliage grown under elevated CO_2 conditions, while two daidzein and a genistein compound significantly decreased (figure 3). Almost all compounds significantly increased in foliage grown under elevated O_3 conditions (figure 4).

Discussion

Atmospheric and herbivore treatments led to variations in soybean foliage flavonoid profiles. The large increases seen in most flavonoids measured under the elevated O_3 treatment were expected as O_3 is toxic to plants and damage may be ameliorated by flavonoids. Increases in flavonoids after beetle damage also can be expected as herbivory is another plant stress. The lack of substantial variation under the elevated CO_2 treatment is also not surprising as elevated CO_2 has been shown to increase plant productivity and photosynthesis (Hamilton et al., 2005). Quercetin, a known antioxidant compound, is one of the few flavonoids showing an induced response to elevated CO_2. Increased Japanese beetle longevity on plants grown under elevated CO_2 may be due to elevated levels of the flavonols, such as quercetin.

Figure 4 - Flavonoid profiles for significantly different plants grown under ambient and elevated levels of O_3, regardless of date or damage. Compound labeling is the same as in figure 2.



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