PHOTOSYNTHETICA 37 (4): 615-619, 1999

BRIEF COMMUNICATION

Flavonoid responses in wheat grown at elevated CO_2: green *versus* senescent leaves

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Abstract

We compared flavonoids in green, mature, and senescing flag leaves of wheat grown under ambient (AC - 370 μmol mol^{-1}) and elevated (EC - 550 μmol mol^{-1}) concentrations of CO_2 in a FACE (Free Air CO_2 Enrichment) system. The concentrations of flag leaf flavonoids (*e.g.*, isoorientin and tricin) decreased to one third in mature leaves, and the majoritary isoorientin almost disappeared in senescing leaves. Flavonoid concentrations increased in green well-developed flag leaves under EC (46 % isoorientin and 55 % tricin), whereas the differences disappeared in mature and senescing flag leaves. Predictions of changes in litter phenolic concentrations and their effects on decomposition rates under EC based on changes in green leaves need to be revised.

Additional key words: carbon based secondary compounds (CBSC); isoorientin; leaf age; litter; phenolics; tricin; *Triticum aestivum*.

Most source-sink theories predict a larger accumulation of carbon based secondary compounds (CBSC) in plant tissues in response to increasing atmospheric CO_2 concentrations (Bryant *et al.* 1983, Herms and Mattson 1992, Peñuelas and Estiarte

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Received 15 July 1999, *accepted* 30 August 1999.

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Acknowledgments: We acknowledge the technical help and the cooperation of the staff of the U.S. Water Conservation Laboratory, USDA, at Phoenix, Arizona, and the Univ. of Arizona Maricopa Agricultural Center. This research was made possible by grants CICYT CLI97-0334 (Spain) and CIRIT (Catalonia) and financial aid from USDA-ARS, and from the US Dept. of Energy, Atmospheric and Climate Research, Office of Energy Research under Interagency Agreement DE-AIOS-88ER-69014.

Abbreviations: CBSC - carbon based secondary compounds; DM - dry mass; FACE - free air CO_2 enrichment; SDM - structural dry mass; TNS - total nonstructural saccharides.

1998). Although some groups of CBSC such as terpenes do not seem to vary under elevated CO_2, the green leaf concentration of one important group, phenolic compounds, tends to increase in most reported studies (for reviews see Peñuelas *et al.* 1997, Peñuelas and Estiarte 1998). These phenolic compounds may play a crucial role in nutrient cycling, among other reasons, because of their effects on decomposition rates (Palm and Sanchez 1990), because of their negative correlations with net mineralization (Palm and Sanchez 1991), and/or because polyphenols may control N release from litter in dissolved organic forms relative to mineral forms (Northup *et al.* 1995). However, in spite of such a possible important effect on nutrient cycling, available data for phenolics under elevated CO_2 are mostly from green leaves and there is almost no data from leaf litter (Peñuelas and Estiarte 1998). For leaf litter there is much more information on lignin and structural polysaccharide concentrations, with almost all reports showing no significant differences due to elevated CO_2. Although the ratio lignin/nitrogen increases as a consequence of diminished nitrogen concentrations, there are also doubts about whether these changes occur in the field (O'Neill and Norby 1996). Most experiments done in various ecosystems have reported little change in litter chemistry under elevated CO_2 concentrations (Norby and Cotrufo 1998).

In this work we studied specific phenolics such as flavonoids in wheat under elevated CO_2 concentration (EC) supplied by a FACE system and under different water supplies. We aimed to test (*a*) whether there was any difference between green and senescent leaves, (*b*) whether there were phenotypic increases in flavonoid concentrations of green leaves under EC, and in case there were, (*c*) whether these increases remained in senescing leaves and therefore in litter. We also tested the direct and the interactive effects of water availability, as one of the main environmental factors interacting with CO_2.

Spring wheat (*Triticum aestivum* L. cv. Yecora rojo) leaves were collected from a crop grown in an open field at Maricopa, Arizona, USA in a Trix clay loam soil [fine-loamy, mixed (calcareous) hyperthermic Typic Torrifluvent]. Wheat was planted on 15 December, and emerged on 1 January. Four 20-m-diameter plots of the field were subjected to 550 μmol mol^{-1} of CO_2 from a FACE system during 24 h d^{-1} from emergence until harvest. Four plots at ambient concentrations of CO_2 (AC - about 370 μmol mol^{-1} during daytime) served as controls. The plots were split into semicircular subplots, half of which were well-watered using a drip irrigation system by frequent replacement of the water lost by potential evapotranspiration (WET treatment) and half of which received half as much water as the wet plots (DRY treatment). Thus, there were a total of 16 plots (two concentrations of CO_2, two levels of water supply, four replicates). A pre-plant granular fertilization and applications of N with irrigation provided nutrients at ample rates [a total of 27.6 g(N) m^{-2} and 4.4 g(P) m^{-2}]. For more details, see Estiarte *et al.* (1999), Kimball *et al.* (1995), Lewin *et al.* (1994), Peñuelas *et al.* (1996), and Pinter *et al.* (1996). Leaf samples were taken in three dates during the flag leaf cycle (DOY 90, 118, and 140). At each date, 10 flag leaves per treatment were cut at 13:00 h (Mountain Standard Time), pooled, and kept on dry-ice for up to 5 h until transport to the laboratory. Leaves were kept overnight in a cooler and the day after they were freeze-dried, ground through a 0.4 mm mesh in a *Wiley Mill*, and stored in the freezer over a desiccant. Leaf powder (0.25 g) was extracted three times with 30 cm^3 of methanol in a sonic bath for 10 min. Extracts were pooled, and methanol was removed under vacuum. The residue was redissolved in 2 cm^3 of an HPLC-grade methanol solution of 250 mg kg^{-1} quercetin as internal standard, filtered through a 0.22 µm nylon micro-spin filter (*LIDA*, Kenosha, WI, USA), and stored in the freezer at -20 °C. HPLC analyses of flavonoids (Waterman and Mole 1994) were performed following a chromatographic program that combined gradient and isocratic elutions, peak identification was conducted by ^{13}C-NMR and ^{1}H-NMR on isolated compounds, and commercial isoorientin (*Extrasynthese*, Genay, France) was used to obtain standard curves at 350 nm absorbance as reported in Estiarte *et al.* (1999). We expressed flavonoid concentrations on structural dry mass (SDM) basis because the expression of concentration on a dry mass (DM) basis can mask or diminish the changes in the concentration of compounds, specially for EC-grown plants in which there are usually large increases in Total Nonstructural Saccharides (TNS) concentrations (Poorter *et al.* 1997), and specially on organs such as leaves with large daily TNS fluctuations due to accumulation during light period and export at night (*e.g.*, Hendrix and Grange 1991). TNS were analyzed as reported in Estiarte *et al.* (1999). Analysis of variance (ANOVA) was used to examine temporal and treatment (CO_2 and irrigation) effects on flavonoids. ANOVA was performed using *STATVIEW 4.5.* (*Abacus Concepts*, Berkeley, CA, USA).

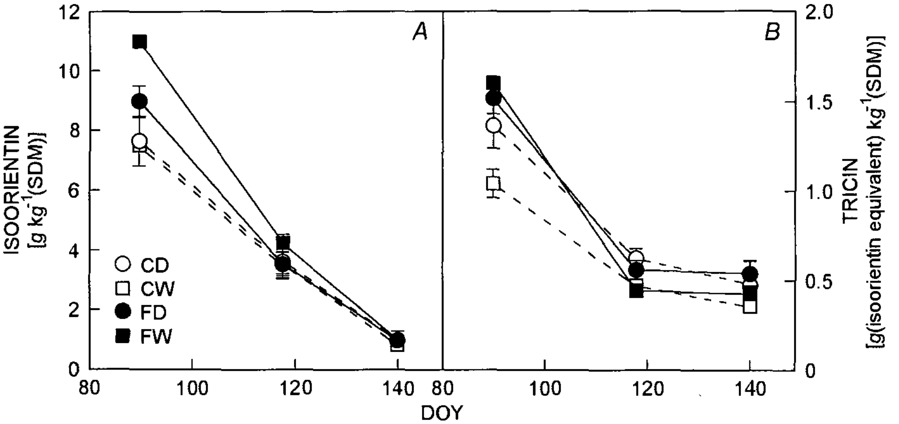

Fig. 1. Variation of the concentrations of the flavonoids isoorientin (*A*) and (*B*) tricin in well developed (DOY 90), senescing (DOY 118), and senesced (DOY 140) wheat flag leaves under different CO_2 and irrigation treatments (C - control, 370 mmol mol^{-1} CO_2, F - FACE 550 mmol mol^{-1} CO_2, W - WET, D - DRY). DOY - day of year. Means±SE are shown ($n = 4$).

The leaf age, expressed as DOY, had significant effects on the phenolic concentrations of all flavonoids ($p<0.001$). They decreased as the leaves senesced (Fig. 1). The concentration of tricin decreased to one third in senescing leaves, that of isoorientin even more. Similar patterns were found for the other flavonoid compounds (results not shown).

Atmospheric CO_2 enrichment had significant effects on these flavonoid compounds in green well-developed flag leaves. The concentrations of isoorientin and tricin (and all the other minor flavonoids) were significantly higher at EC than at AC (p<0.01), by 46 % for isoorientin and by 55 % for tricin in the WET treatment, whereas it was not significant in the DRY treatment. However, all these differences in flavonoid concentrations at EC disappeared in mature (DOY 118) and senescing (DOY 140) leaves (Fig. 1).

The effects of irrigation on flavonoid concentrations of green well-developed flag leaves (at DOY 90) were less significant than those of EC. Moreover, they were different depending on the flavonoid compound. Isoorientin had 27 % higher concentrations in WET treatment than in DRY treatment ($p = 0.01$), but only under EC. The opposite was found for tricin, which had slightly significant ($p = 0.07$) 29 % higher concentrations in DRY than in WET treatment but only under AC. The effects of irrigation on minor peaks were also variable. All these variable effects also disappeared in senescing flag leaves.

The effects of leaf senescence on flavonoid contents have already been reported. Seedling leaves of barley contain high concentrations of flavonoids since emergence until near senescence, when the concentration decreases (Liu *et al.* 1995). Lower flavonoid concentrations in old leaves than in young leaves were also reported in field-grown barley (Blume and McClure 1979) and in *Avena sativa* (Popovici and Weissenböck 1977). Lees *et al.* (1995) found that condensed tannins disappeared from vacuoles in sainfoin senescing leaves and stated that they were catabolized at senescence. Thus, phenolics concentration seems a trait highly dependent on leaf senescence.

The responses of green leaves to EC coincided with the majority of available data on concentrations of phenolic compounds in plant tissues (Peñuelas and Estiarte 1998). The increase in flavonoid concentrations under EC (Fig. 1) was parallel to a decrease in the N concentration and to an increase in the TNS concentration (Estiarte *et al.* 1999), indicating higher availability of carbon to be invested in CBSC, which is also in accordance with source-sink theories for CBSC (Bryant *et al.* 1983, Herms and Mattson 1992, Lambers 1993, Peñuelas and Estiarte 1998). The responses of flavonoids to water treatment and the interactions with EC were very variable depending on the compound and also in agreement with the very variable results reported in the literature (Estiarte and Peñuelas 1999). These differences produced by EC (and also by water treatments) in green leaves disappeared in senescent leaves, in parallel to the large decrease in phenolic concentrations. Thus, if these results are confirmed, predictions of changes in litter phenolic and CBSC concentrations at EC would have to be revised, and decomposition rates would not be as much affected as previously predicted based on green leaf responses. In fact, most experiments carried out in various ecosystems have not found significant changes in decomposition rates at EC (Norby and Cotrufo 1998).

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