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EFFECT OF OZONE ON PHLOEM TRANSPORT IN COTTON

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Abstract

Pima cotton was grown in field exposure chambers exposed to a range of ozone concentrations. The ratio of sucrose to raffinose series sugars was examined in source and sink tissues and in aphid honeydew as a surrogate for phloem sap. The profile of sugars did not change substantially in source leaves. The ratio of sucrose to raffinose series sugars (raffinose plus stachyose plus verbascose) declined significantly in fine root tissue with increasing ozone. The composition of honeydew also declined, though not as dramatically as fine root tissue. These results are not conclusive but are consistent with the hypothesis that ozone alters the apoplastic loading of sucrose into the phloem more than it alters the symplastic loading of raffinose series sugars.

Abbreviations

12 hm, 12 hour daylight (08:00 – 20:00) mean ozone concentration; CF, Charcoal filtered air; g_s, stomatal conductance; gfwt, grams fresh weight; HO3, high O_3 concentration; LO3, low O_3 concentration; LAR, leaf area ratio (total leaf area/total plant dry weight); LSD, Fisher's Protected Least Significant Difference; LWR, leaf weight ratio (total leaf dry weight/total shoot dry weight); MO3, medium O_3 concentration; OTC, open-top chamber; SLW, specific leaf weight (leaf area/total leaf dry weight).

Introduction

Tropospheric ozone (O_3) is a regional problem, and poses the greatest threat to vegetation of any air pollutant (Krupa *et al.*, 2001; Krupa and Kickert, 1989; Krupa and Manning, 1988). Damage to crops in the United States has been estimated at several billion dollars per year (Heck et al., 1983; Adams et al., 1988). O_3 reduces yields of adapted upland *(Gossypium hirsutum* L.) cotton cultivars by about 20% in the San Joaquin Valley (SJV) of California (Grantz & McCool 1993; Olszyk *et al.* 1993; Oshima *et al.* 1979; Temple *et al.* 1988). Pima (G. *barbadense* L.) cotton cultivars such as S-6 are impacted even more (Grantz & McCool 1993; Olszyk *et al.* 1993). More recent selections from the SJV are probably less sensitive, though data are lacking. Much of the cotton acreage in the SJV is subject to increasing concentrations of O_3.

Ozone affects cotton through direct oxidant damage to physiological processes and ultimately to yield (Grantz, 2003). Direct effects of ozone on photosynthesis (Lehnherr et al., 1987; Pell et al., 1992) do not appear to explain the effects of O_3 on whole plant productivity (e.g. Meyer et al., 1997; Reich and Amundson, 1985; Krupa and Manning, 1988; Rennenberg et al., 1996; Grantz and Yang, 1996; Grantz and Farrar, 1999; McLaughlin and McConathy, 1983) Inhibition of carbohydrate export from source leaves may reduce photosynthesis indirectly while directly impacting carbon allocation and growth. For example, O_3 reduced assimilation in wheat (Meyer et al., 1997), but this was attributed to feedback inhibition of photosynthetic enzymes caused by sucrose accumulation. Simulation studies (Grantz et al., 1999) suggested that inhibited root development (Grantz and Yang, 1996) caused by O_3 may mediate some observed O_3 impacts on shoot function. It is not known if carbohydrate translocation may be a primary target of O_3 inhibition.

Cotton is typical of many plants that translocate mostly sucrose in the phloem, although some raffinose series sugars are present. . It is thought that transport of raffinose series sugars differs from that of sucrose in that phloem loading is symplastic, not involving an apoplastic, transmembrane, step (Bachmann et al., 1994; Kandler, 1967; Dey, 1985; Turgeon and Gowan, 1992). The symplastic mechanism (eg. Madore and Webb, 1981; Turgeon and Gowan, 1992) could afford some protection against oxidant damage. Sucrose uptake was inhibited by the sulfhydryl reagent, PCMBS (p-chloromercuribenzene-sulfonic acid; Madore, 1990). A preliminary comparison of bean (*Phaseolus vulgaris*), a sucrose transporter, with basil (*Ocimum basilicum* L.), a stachyose transporter, demonstrated a lower sensitivity of phloem exudation to O_3 and to PCMBS in basil than in bean (M. Madore; personal communication).

In previous studies under different exposure conditions (Grantz and Yang, unpublished) we found that O_3 inhibited the magnitude of phloem loading in Pima cotton (Grantz and Farrar, 1999) and restricted allocation of newly fixed C to developing roots and fruits (Grantz and Yang, 1996). In previous studies we also found that the ratio of sucrose to raffinose series sugars was reduced dramatically in sink root tissue but unchanged by O3 in source leaf tissue. Here we have used aphid honey dew

as a minimally disruptive probe to examine O_3 impacts on the sugars in phloem sap in Pima cotton grown in field exposure chambers. These are compared with sugar profiles in source and sink tissue obtained under the same exposure conditions.

Materials and Methods

Plant Material

Cotton (cv. Pima S6) was grown from seed in 9-l tapered plastic pots (Treepot; Hummert International, Earth City, MO), filled with sintered clay (6-40 mesh; Quicksorb, A & M Products, Taft, CA).

Pots were randomly assigned to outdoor chambers for exposure to one of three concentrations of ozone. Pots were automatically irrigated to run-through up to three times a day as required by the weather. A complete fertilizer (Miracle Gro; Scotts Miracle-Gro Products Inc., Port Washington, NY) was injected into the irrigation water at 1.3 g l^{-1} weekly.

Ozone Exposure

Experiments were conducted in Open Top Chambers (OTCs; 3.1 m diameter x 2.4 m height; Heagle et al., 1973) at the University of California, Kearney Agricultural Center, Parlier, CA (103 msl, 36.598 N 119.503 W). Ozone was generated by corona discharge (Model G22; Pacific Ozone Technology, Brentwood, CA) from oxygen (Model AS-12; AirSep Corporation, Buffalo, NY). The daily timecourse of O_3 concentration was regulated in a single OTC using a dedicated O_3 monitor (Model 49C, Thermo Environmental Instruments, Franklin, MA). This monitor was interfaced to a computer for feedback control, as described previously (Grantz et al., 2003). The low O_3 (LO3) regime was charcoal filtered air (CF) and was nominally O_3-free (actual 12 hour mean, hm = 15.9 ppb). The medium O_3 (MO3) regime approximated the diurnal profile and maximal concentration observed on exceptionally polluted days at this location (actual 12 hm = 80.6 ppb). The high O_3 (HO3) regime was approximately 1.9-fold greater than the MO3 at each time point (actual 12 hm = 153.6 ppb).

Tissue

All measurements were performed on youngest fully expanded leaves or on fine roots. Roots were sampled after the sintered clay potting medium was removed by agitation in cold water. Tissue was quick-frozen in liquid N_2 and stored at -80°C until lyophilized. Lyophilized tissue was diced finely with a razor blade, weighed and transferred to a 1.7 ml screw cap plastic tube to which 1.0 ml water was added, the tube shaken, then placed in a Branson 85 W sonicator filled with ice water for 15 min (Murray, 1998). The extraction was repeated twice using 1.0 and 0.5ml of water respectively.

Phloem Sap

A colony of cotton aphid (*Aphis gossypii*), was established from individuals obtained from the USDA/ARS aphid colony in Parlier, CA. The aphids were reared on young cotton plants in a greenhouse. Aphids were transferred to the field OTCs in small custom designed "clip cages". These were 3 cm long made from 2.5 cm diameter plastic tubing. Air holes were cut in the sidewall and covered with fine mesh. One end of the tube was closed with a spring loaded clear plastic cover attached to the tube with a large hair clip. The other end of the container was the honeydew collection surface, a piece of aluminum foil formed tightly over the end of the tubing. This was easily removed and replaced during sample collection.

Approximately 10 aphids were transferred to each clip cage using a camel hair brush. Four clip cages were placed in each OTC, attached to the youngest fully expanded leaves of different plants. Containers were supported with a wire frame to maintain orientation with the aluminum foil at the bottom for efficient honeydew collection. Aphids were allowed to adapt to the experimental plants and to purge for 24 hours at which time the aluminum foil was discarded. A new piece of aluminum foil was attached and honeydew collected for 3 days. Containers were washed and dried prior to installation on a plant.

The aluminum foil with deposited honeydew was immediately dried (40 C, 24 hours), then stored in individual Petri dishes sealed with Parafilm, until extraction.

Honeydew was eluted with water, freeze dried and brought to known volume. Carbohydrate analysis was performed by HPAEC-PAD (High pH Anion Exchange Chromatography with Pulsed Amperometric Detection) on a Dionex Bio-LC, with a Dionex CarboPac PA 1 column. The eluent was 150mM NaOH, isocratic for 5 minutes, followed by a linear sodium acetate gradient from 0 to 500mM in 150mM NaOH, for 35 minutes . Retention times are expressed in minutes and detector response in µCoulombs (see Murray et al., 1997, 1999 in these Beltwide Proceedings, for further details).

Chromatographic analysis of honeydew and tissue extracts was performed with Dionex PeakNet software, transferred to an Excel spreadsheet. For known sugars, for which authentic standards were available, standard curves based on peak areas were used. Peak areas or sugar amounts were analyzed for O_3 effects over all samples using a 1-way ANOVA (PROC GLM; SAS Institute, 1990).

Results and Discussion

Growth

Growth of cotton plants was negatively impacted by exposure to ozone. Plants were significantly shorter (Grantz and Shrestha, this volume) and produced significantly fewer leaves and less biomass with increasing ozone.

Sugar Composition

Source. Source leaf tissue of Pima S-6 grown in field exposure chambers exhibited a ratio of sucrose to raffinose series sugars (sum of raffinose, stachyose and verbascose) slightly in excess of 3:1 in LO3 (Figure 1B) This declined in a dose dependent fashion, but only slightly with increasing ozone exposure.

Sink. The content of sucrose in fine root sink tissue declined substantially from LO3 to MO3 and HO3 (Fig. 1A, squares). This ratio declined from about 10:1 to about 6:1, with no further change as ozone increased from MO3 to HO3.

These data, consistent with our previous results (Grantz and Yang, unpublished) demonstrate that sugar profiles in sink and source tissues are uncoupled. The sugar profiles observed in sink tissues represent whole tissue extractions, and may reflect either the composition of the phloem sap feeding the sink tissue, or a consequence of metabolism during transport or in the sink. It was important to examine the composition of the phloem sap, which links the two tissues.

Phloem Sap. Cotton is generally considered to translocate primarily sucrose in the phloem sap. However, it is clear from our data and that of others (Henneberry et al., 2000) that this is an oversimplification. Both sucrose and raffinose series sugars (raffinose, stachyose, verbascose, ajugose and members of greater degree of polymerization (including putative DP-7 and DP-8) are present in the honeydew (Figure 2). DP-7 is the peak appearing just prior to DP-8 (Fig. 2). The chromatograms clearly resolve a large number of known and unknown sugars. Some, particularly the larger species, may be artefacts of honeydew collection and not representative of phloem sap. However, sugar composition of the sap (analyzed as honeydew) responded substantially to ozone exposure (Fig. 1A, circles).

The relative abundance of sucrose declined with increasing O_3 concentration, while the relative content of raffinose and stachyose, as well as DP-7, increased. In this pair of contrasting samples (Figure 1) the relative abundance of sucrose declined only modestly as the O_3 concentration increased. Over all samples (n = 104) the relative abundance of sucrose declined by approximately 18% while that of the known raffinose series sugars increased by 17%.

This resulted in a substantial decline in the average ratio of the identified sugars, sucrose:raffinose series (Figure 2). These data agree well with the sink tissue constituents obtained previously with the same cultivar (above) and not with profiles obtained from source leaf tissue.

The tentative conclusion from these data, and a basis for more detailed experiments, is that the sugar profiles of the sink tissues reflects that of the phloem sap that is unloaded in the sink, rather than that of the source from which the phloem is loaded. The relative reduction in sucrose and increase in raffinose series sugars could implicate the apoplastic step of sucrose loading as a more sensitive site of O_3 attack than the symplastic phloem loading processes of raffinose series sugars.

The data are preliminary in the sense that aphid honeydew is modified relative to its source, the actual phloem sap. Further experiments are planned with cotton and other species to characterize the extent of the modification.

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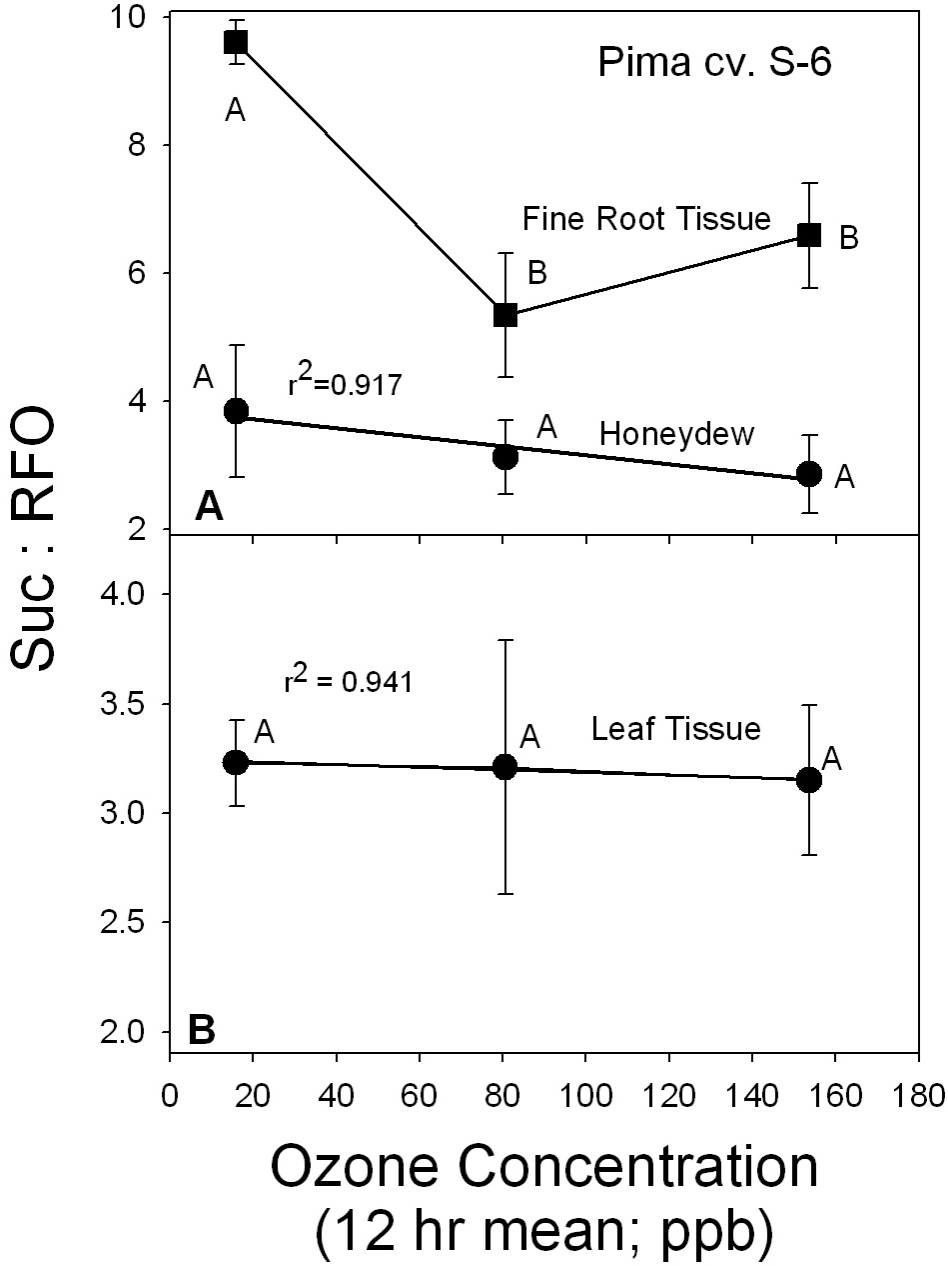

Figure 1. Effect of increasing O_3 exposure on the ratio of sucrose to the principal known raffinose series sugars (RFO; Sum of Raffinose, Stachyose and Verbascose) in phloem sap sampled as aphid honeydew from Pima S-6 plants.

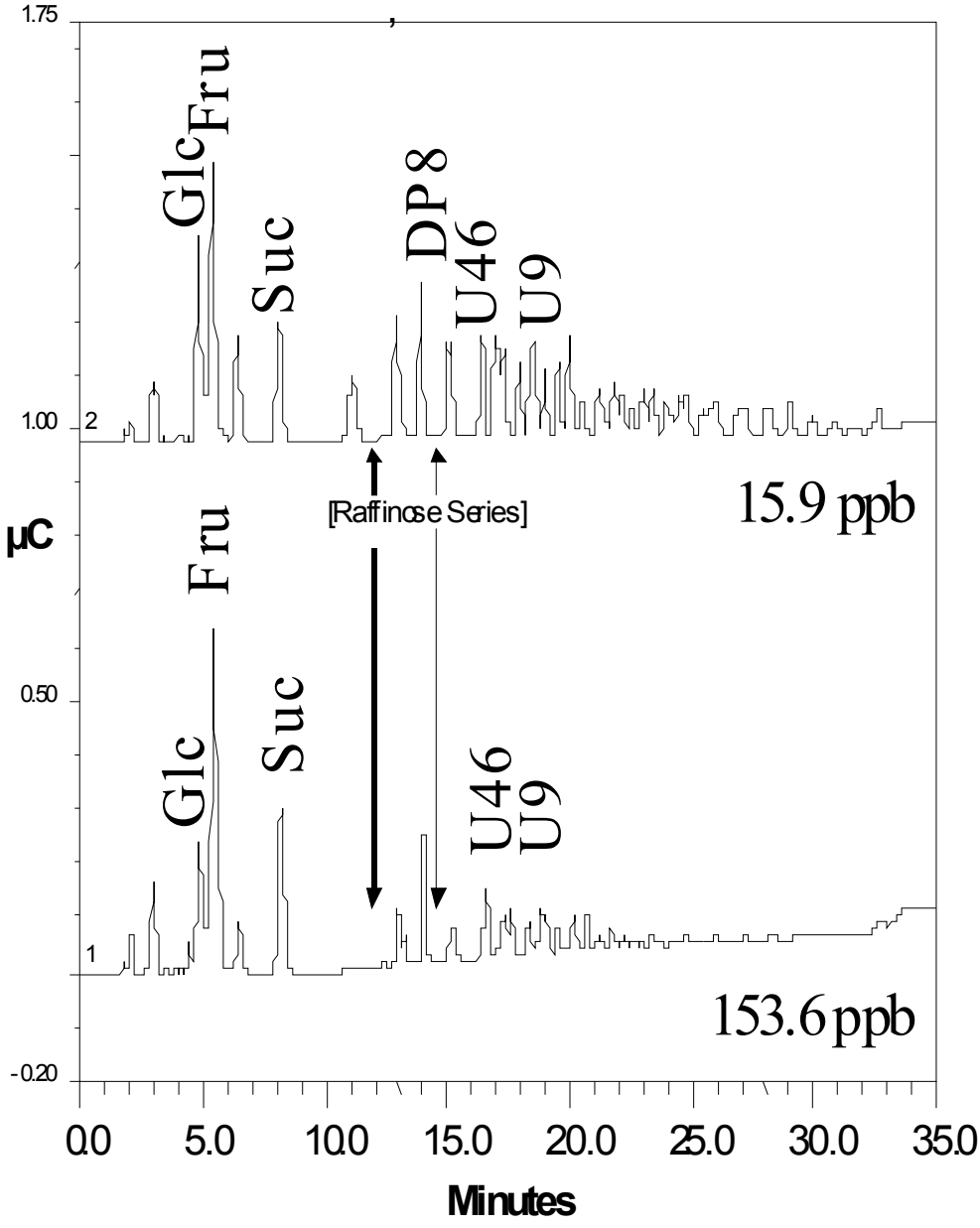

Figure 2. Representative chromatograms resolving a large number of known and unknown sugar constituents in phloem sap sampled as aphid honeydew from Pima S-6 plants exposed to low (top) and high (bottom) concentrations of O_3.