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Up-regulating Plant Defenses in *Populus* Increases Phytoremediation Capacity for Carbon Tetrachloride.

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ABSTRACT: Phytoremediation is a technology that exploits plants to clean up polluted soil and water, with a potential for enormous benefit to mankind. It is cost-effective and certainly more aesthetically pleasing than aggressive land reforming approaches. Such technology is proving successful, but could benefit from a stronger research base. For example, our landmark results suggest that remediation by poplar could be made more efficient by exploiting the plant's own natural defense mechanisms. This raises the prospect of bio-engineering plants to enhance defense mechanisms and improve their remediation of ground-water contaminants including organic compounds and heavy metals. Our experimental approach to research plant function is also unusual, relying on non-invasive nuclear imaging of carbon-11, a short-lived positron emitting radioisotope that allows us to trace the fate of newly acquired carbon within intact plants and perform repeat tests on the same plant over time. Such information is not available by using carbon-14 or carbon-13.

INTRODUCTION

There is a growing concern over continued elevated levels of halocarbon contaminants in groundwater which present human health risks as potential carcinogens. Carbon tetrachloride (CCl_4), one of several compounds in this class of contaminants, has been targeted for environmental remediation. Unlike many organic compounds, halocarbons can persist in the environment for decades (Wackett *et al*., 1989). Use of poplar trees to clean up such groundwater contaminants has proven to be a cost-effective (Schnoor *et al*., 1995; Kassel *et al*., 2002; Nyer and Gatliff, 1996; Newman *et al*., 1998), but we still face the challenge of minimizing foliar emissions of volatile chlorocarbon compounds as they can become atmospheric pollutants capable of depleting the ozone layer (Van der Lelie *et al*., 2002; Schwitzguebel *et al*., 2002; Ma and Burken, 2003). However it turns out that chlorocarbons can be metabolized, and the plants need not act only as agents to transfer chlorocarbons from soil to atmosphere. Our research is focused on elucidating the mechanistic pathways through which plants naturally metabolize halocarbon compounds, and on improving these processes. One strategy we have been exploring is to heighten a plant's natural defense system using treatments of specific compounds known to be biosynthesized as part of the plant's defense processes. We recently showed that treatment of poplar saplings using jasmonic acid, a potent plant hormone involved in responses to wounding and other environmental cues, will result in a significant rise in sugar intermediates at the expense of starch biosynthesis with

consequential increases in both leaf export of mobile sugar and emission of isoprene (Ferrieri *et al*., 2005; Babst *et al*., 2005). This suggests that jasmonate may serve as a cue for redirection of recently fixed carbon.

We hypothesize that plant utilization of carbon newly acquired through leaf photosynthesis will have major effects on plant metabolism of CCl_4, thereby altering foliar emissions. Through labeling of intact leaves of poplar clones (OP637) with the radioactive isotope carbon-11 ($t_{½}$ 20.4 m) using ^{11}C-labeled carbon dioxide gas, we have been able to test this hypothesis owing to the extremely short half-life of the tracer which reflects only those processes involving newly acquired carbon. Further, the short half-life of the tracer affords a unique opportunity to retest same plants over time and treatment.

MATERIALS AND METHODS

Plant growth **—** Hardwood cuttings from poplar clone (OP637) were greenhouse grown in hydroponics using Hoagland nutrient solution. Age matched plants at 4 weeks were used in all studies and maintained using metal halide lamps (1000 µmol m^{-2} s^{-1}).

CCl_4 Exposure – Excess CCl_4 was introduced into the hydroponics solution providing a saturation concentration of 520 ppm.

Leaf Emission – Air flowing at 150 mL m^{-1} passed through a plastic containment bag tied around the entire foliage of the plant. Volatile emissions were captured on molecular sieve cartridges (Tenex GR carbon-based molecular sieve) placed on the outflow. Samples were collected on 2 h intervals during the lighted period.

Gas Chromatography Analysis of Volatile Emissions – The glass cartridges were designed to insert directly into the heated injector of a Hewlett Packard 5280A gas chromatograph equipped with a flame ionization detector. Volatile components were thermally desorbed from the cartridge, and individual components isolated using a 8 ft x 1/8 in o.d. stainless steel column packed with Porapak T (Analabs). Data for both isoprene emission and CCl_4 emission was obtained from a single analysis.

Gas Chromatography Analysis of Leaf TCA Levels **–** Frozen leaf tissue was ground up, extracted into 80% aqueous methanol at 80°C, and samples analyzed by capillary gas chromatography using a 25 m x 0.32 mm o.d. Carbowax 20M column coupled with a flame ionization detector.

Radiotracer Administration – Carbon-11 ($t_{½}$ 20.4 m) was produced on a JSW 41 in. cyclotron using the $^{14}N(p,\alpha)^{11}C$ nuclear transformation. The isotope was extracted from the target gas as $^{11}CO_2$ was administered directly to an intact source leaf using a lighted leaf chamber (Ferrieri *et al*., 2005).

Positron Autoradiogaphy – In some experiments, 90 min after $^{11}CO_2$ was administered to a leaf the plant was transferred to a phosphor plate for contact-exposure, and the image recorded (Fuji BAS model 2500) to show radioactivity distribution throughout the plant due to [^{11}C]carbohydrate transport. Fuji Image Gauge software was used to quantify radioactivity partitioning between leaf load zone, apex, and lower stem.

Metabolite Analysis – In other plants, 30 minutes after tracer administration, the exposed leaf was surgically excised at the petiole and extracted using methods described above. Contents were analyzed by radio-HPLC on a C18 column (250 x 4.6mm) using a mobile phase of 50mM monoammonium phosphate (adjusted to a pH of 3.3 with phosphoric acid) at 1.2 mL m^{-1} flow rate. A UV absorption detector was used for mass measurements at 254 nm, and a gamma sensitive radiation detector was used on the outflow of the UV detector.

RESULTS AND DISCUSSION

Using whole-plant radiographic imaging (see Figures 1 & 2), we have shown that there is an increase in leaf export of [^{11}C]carbohydrate from mature leaves to the apex of the plant within 24 hrs exposure to 520 ppm of CCl_4 in the root solution, relative to control plants, suggesting that rapid changes occur in foliar metabolism and whole-plant partitioning of newly acquired carbon upon exposure to the contaminant.

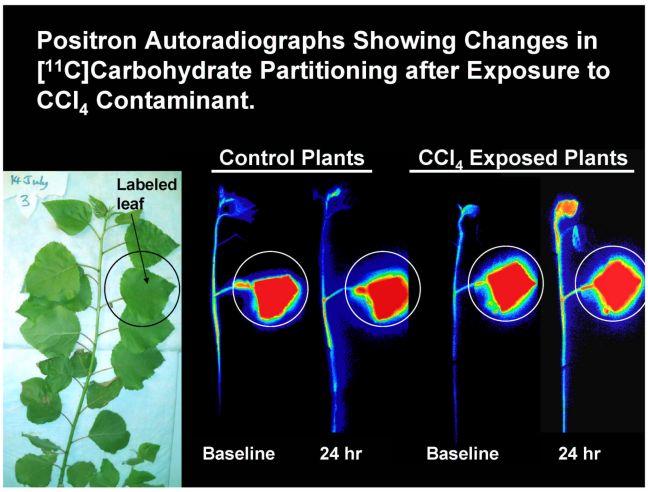

FIGURE 1. Photograph on left shows a poplar clone (OP637) set on the imaging bed. Radiographic images, on the right, show extent of [^{11}C]partitioning of a control plant acquired at baseline (time zero) and 24 hours later, and of the CCl_4 exposed plant shown in the photograph over the same time period. Separate doses of $^{11}CO_2$ were administered to the selected leaf encircled.

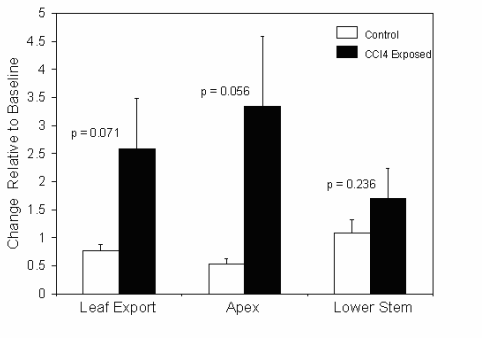

FIGURE 2. Partitioning (shown means ± SE from n=3 replicates) of [^{11}C]carbohydrate from poplar clones (OP637).

The metabolic profile of foliar tissue revealed that carbon-11 was incorporated into trichloroacetic acid (TCA), a known metabolite of CCl_4 (L. Newman, *pers. comm.*) that is not volatile, nor carcinogenic.

Further, we observed that there is diurnal variation in leaf emissions. Foliar emissions of CCl_4 were noted to be highest in the morning hours when the carbohydrate pool size was low, but decreased as the day progressed and the pool size increased (Figure 3). As expected, isoprene emission also increased during the course of the day as leaf carbon pools increased (Funk *et al*., 2003).

These observations support a direct association between the plant's ability to metabolize CCl_4 and its newly acquired carbon stores, and suggest that it should be possible to amplify contaminant metabolism, thereby lowering foliar emissions, by altering the way plant's utilize these stores.

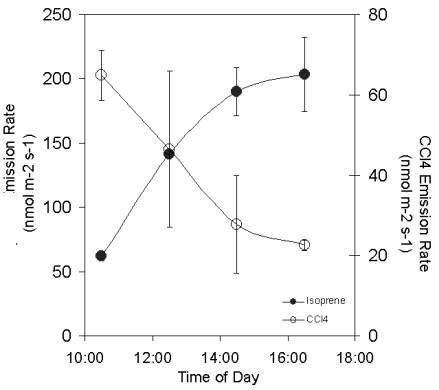

FIGURE 3. Leaf emissions of isoprene and CCl_4 on Day 3 of chronic CCl_4 exposure. Data presented as a function of the time of day (shown means ± SE for n=3 replicates).

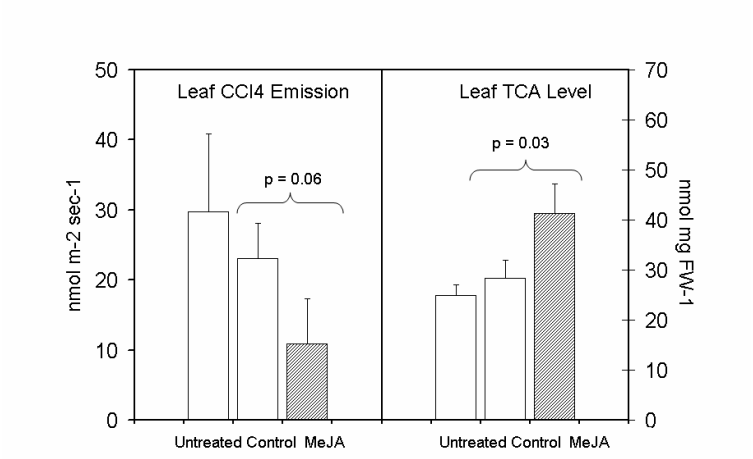

FIGURE 4. Left-side panel shows average leaf CCl_4 emission rates for untreated, control treated and 1 mM MeJA treated plants exposed to 520 ppm CCl_4 for 24 h. Right-side panel shows average leaf TCA levels for untreated, control treated and 1 mM MeJA treated plants at the same point in time.

We recently showed that exogenous treatment with jasmonate, a phytohormone implicated in signal transduction within the plant's defense train, increases sugar intermediates in leaves at the expense of starch storage (Ferrieri *et al*., 2005; Babst *et al*., 2005). Indeed, when we treated the entire foliage of intact poplar plants with a 1 mM solution of methyl jasmonate (MeJA), foliar CCl_4 emissions were reduced 2-fold relative to controls while TCA levels were increased 1.7-fold relative to controls (Figure 4).

CONCLUSIONS

We have demonstrated that plant metabolism of CCl_4 by poplar clones (OP637) involves recently fixed carbon. Additionally, the size of the leaf carbon pool – and particularly the size of the mobile sugar pool or its intermediates – affected CCl_4 metabolism. Knowing this, we tested whether foliar treatment with jasmonate, a plant hormone linked with the plant defense train, and recently shown to increase leaf chloroplast sugar intermediates, will enhance plant metabolism of CCl_4 thereby decreasing leaf emissions, and did indeed see this result in preliminary studies. Future research will explore the long-term effects on phytoremediation capacity with persistent up-regulation of a plant's defenses through continuous jasmonate treatment.

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