Up-regulating Plant Defenses in *Populus* Increases their Phytoremediation of Carbon Tetrachloride

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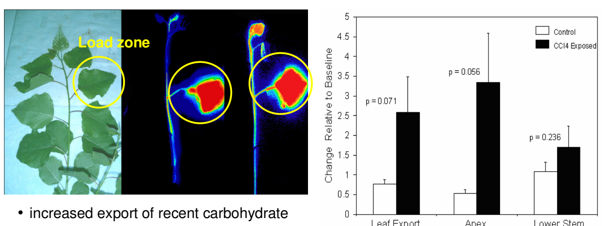
Introduction

Chlorocarbon compounds such as carbon tetrachloride (CCl_4) present a health risk as a potential carcinogen and a pollutant capable of depleting the ozone layer. Although the use of poplar trees to remove chlorocarbon contaminants from groundwater is cost-effective, we still face the challenge of minimizing foliar emissions. *Populus* species metabolize CCl_4 to 1,1,1-trichloroacetic acid (TCA), a non-volatile and less toxic substance, but little is known of what resources the plant uses to perform this process.

Objectives

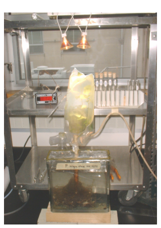
We hypothesized that plant metabolism of CCl_4 involves recently fixed carbon in leaf chloroplast. If true, it should be possible to enhance metabolism through chemical treatment. Recent work by BNL scientists in collaboration with Tufts University biologists has demonstrated that up-regulation of plant defenses through exogenous treatment with jasmonate, a defense hormone, causes significant changes in leaf biochemistry increasing sugar substrate pool size at the expense of starch. Knowing this, we further hypothesized that treatment with jasmonate will increase plant metabolism of CCl_4 reducing leaf emission.

1. Effect of CCl_4 on Recent Carbon Partitioning using $^{11}CO_2$



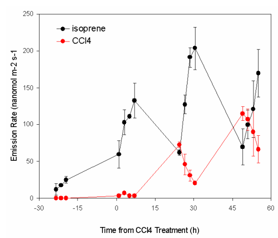
- increased export of recent carbohydrate to younger leaves seen 12 hr after CCl_4 exposure.

2. Time Profile of Leaf Emissions after CCl_4 Exposure

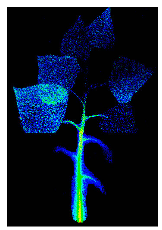


Plant volatile emissions

- immediate increase in isoprene emissions (stress response?).
- CCl_4 emissions begin 24 hr after exposure.
- leaves receive a signal of stress from roots long before the CCl_4 itself arrives.
- CCl_4 and isoprene emissions exhibit opposing diurnal patterns.

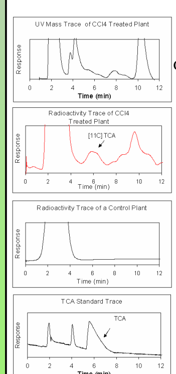


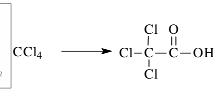
3. *In Planta* Transport of CCl_4



- radiographic image of poplar sapling 1 hr after $^{11}CCl_4$ was supplied to the cut stem.
- contaminant transport is rapid *via* the transpiration stream.

4. Biochemical Transformation





(TCA)

- metabolite analysis of leaf tissue via HPLC showed [^{11}C]TCA after $^{11}CO_2$ administration.
- evidence for recent carbon's role in plant metabolism of CCl_4.
- TCA is non-volatile and less toxic than CCl_4.

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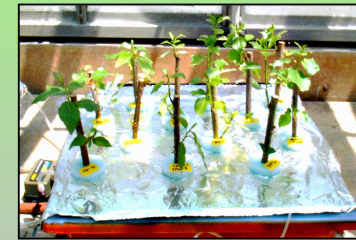
General Approach



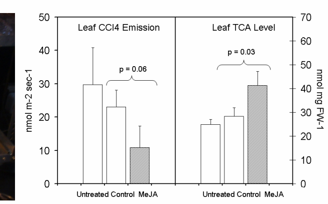
- **Plant growth —** Hardwood cuttings (OP637) were grown hydroponically using Hoagland nutrient solution. Age-matched plants at 4 weeks were used in all studies and maintained using halogen lamps (500 µmol m^{-2} s^{-1}).
- **CCl_4 Exposure** – Excess CCl_4 was introduced into the hydroponics solution providing a saturation concentration of 520 ppm.
- **Radiotracer Administration** – Carbon-11 ($t_{1/2}$ 20.4 m) as $^{11}CO_2$ was administered to a select intact source leaf of individual plants using a lighted leaf chamber. $^{11}CCl_4$ was administered to cut stems.
- **Metabolite Analysis** – 30 min after tracer administration, the exposed leaf was excised at the petiole, extracted in 80% aq. ethanol at 80°C and analyzed by radio-HPLC (C18 column; 250 x 4.6mm using a mobile phase of 50mM monoammonium phosphate--pH adjusted to 3.3 with phosphoric acid).
- **Imaging** – Tracer was administered to leaf or stem, and after 1 hr phosphor plate imaging (Fuji BAS model 2500) was used to capture 2D radiographs of the radioactivity distribution.
- **Leaf Emissions** – Plant volatile emissions were captured on 2 hr intervals using Tenex cartridges attached to the air outflow (150 mL min^{-1}) of plastic bags affixed around the upper stem. Contents were analyzed by gas chromatography.

Summary

1. Plants respond quickly to CCl_4 exposure by partitioning their recent carbon to younger leaves (Box 1).
2. Plant metabolism of CCl_4 involves recently fixed carbon (Box 4). The extent of metabolism appears dependent on recent carbon pool size (Box 2).
3. Plants also respond to CCl_4 with a rapid elevation of leaf isoprene emission (Box 2). This may be a stress response linked to plant re-allocation of starch/sugar pools in leaf tissue. Isoprene synthesis makes use of sugar intermediates.
4. Transport of CCl_4 via the transpiration stream is rapid (Box 3), yet leaf emissions show a clear lag in CCl_4 emission relative to isoprene emission (Box 2) and recent carbon partitioning (Box 1). We suspect root uptake of the contaminant is a factor.
5. Plant tolerance to CCl_4 stress can be enhanced through treatment with jasmonate, a plant hormone linked with the plant defense train, and recently shown to increase leaf chloroplastic sugar intermediates. Treatment enhances plant metabolism of CCl_4 to TCA thereby decreasing leaf emissions of the contaminant (Box 5).

5. Building Plant Tolerance to CCl_4 Stress

CCl_4 + Jasmonate (*not wilting*) **CCl_4 only** (*wilting*)



- treatment reduces leaf CCl_4 emissions.
- increases leaf metabolism of CCl_4 to TCA.