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Moving Plant Toxicology from the Greenhouse to the Field: A Method that Incorporates the Positive Attributes of Each

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Single species, greenhouse/laboratory plant toxicity testing has been the norm for many years in the United States under test regulations formulated from the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA 1978), the Toxic Substances Control Act (TSCA 1976) and the Clean Air Act (CAA 1991). Also, internationally similar test protocols exist under the Organization for Economic Co-operation and Development (OECD 1984) guidelines. These test requirements are intended to protect non-target plants from chemicals being introduced into the environment. For many years there have been recommendations for having tests take place under more realistic (field) growing conditions with more representative plant species (Fletcher and Ratch 1991; SAP 2001). Using the current guidelines selected agricultural species (generally 10) are grown in pots in a greenhouse for up to 28 days. The results are hypothetically used to protect all plants and plant communities in the United States and in many cases throughout the world.

Open top chambers (OTC) have been highly successful and have been used throughout the world to study the effects on plants from a variety of air pollutants including ozone, nitrogen oxides, sulfur oxides and acid precipitation. Part of their success can be attributed to their simple design and cost effectiveness. Over the years, modifications have been added for such things as data collection and exposure control (Hogsett et al. 1985). Recently, there has been increased interest in studying complex ecological effects of air pollutants on plant communities and interactions occurring within them between plants and/or other organisms (US EPA 1996) which have not been traditionally studied using OTC because the chambers lack a controlled rooting environment. Considerable research has been conducted using OTC with agricultural species in pots (Heagle, 1989) and to a lesser extent over agricultural fields (Fuhrer, 1994). Effects of ozone on native plants using OTC has been studied to a much lesser degree than agricultural species (Davision and Barnes, 1998) but again with potted plants (Pleijel and Danielsson 1997, Warwick and Taylor, 1995) and rarely with field plots (Barbo et al., 1998). To meet our research objectives of performing ecological studies investigating the effects of pollutants on plant community dynamics, we modified the OTC's to have a homogeneous rooting environment that simulated natural conditions. The objective of this communication is to describe modifications made to OTC's to expand their usefulness from mainly

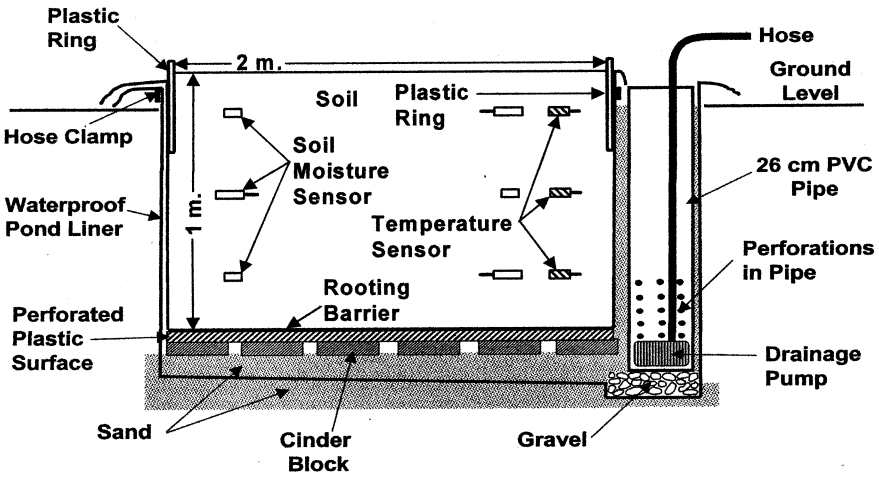

Figure 1. Diagram of modifications to the Open Top Chambers' rooting environment. Probes were located at depths (15, 45, and 75 cm) from the top. Temperature probes were located only on the north side of the chamber while moisture probes were located on both the north and south sides. Each soil moisture probe at a particular depth was turned 90 degrees to the other one at that depth.

physiological studies using individual plants to ecological studies using plant communities while reducing the variability of most field studies and retaining the environmental control found in greenhouse studies.

MATERIALS AND METHODS

This study was conducted at the U.S. Environmental Protection Agency Laboratory using the Field Ecological Research Facility (FERF), in Corvallis, Oregon (Hogsett et al. 1985). Nine open top chambers were modified to grow plant communities (Figure 1). A hole was dug 1.25 m down and 2.25 m across under each OTC. Adjacent to this large hole on the north side, a smaller hole was dug for a drainage pump. Any standing water would drain towards the drainage pump area, the slope of the bottom was adjusted by adding a layer of sand. The hole was lined with an impenetrable water proof fabric (EPDM pond liner, Tetrapond) and a drainage pump (Little Giant, 4500 l/hr) was installed inside a 26 cm dia PVC pipe. The bottom 20 cm of the pipe was drilled with 0.5 cm holes and covered with screen to allow water, but not other materials to drain into the pipe. The base of the pipe was held in place by river gravel (Figure 1). Another thin layer of sand was placed inside the pond liner to level the bottom. Ten cm thick cinder blocks were placed on top of the sand. The blocks were spaced to support reinforced, perforated plastic greenhouse bench tops, (Benchmaster). The hole was then lined with a fabric (Typar Geotextile) that allowed passage of water but not roots. This arrangement allowed for drainage but prevented movement of groundwater into the chambers. The resulting soil area was one meter deep and two meters across. Any open spaces remaining between the

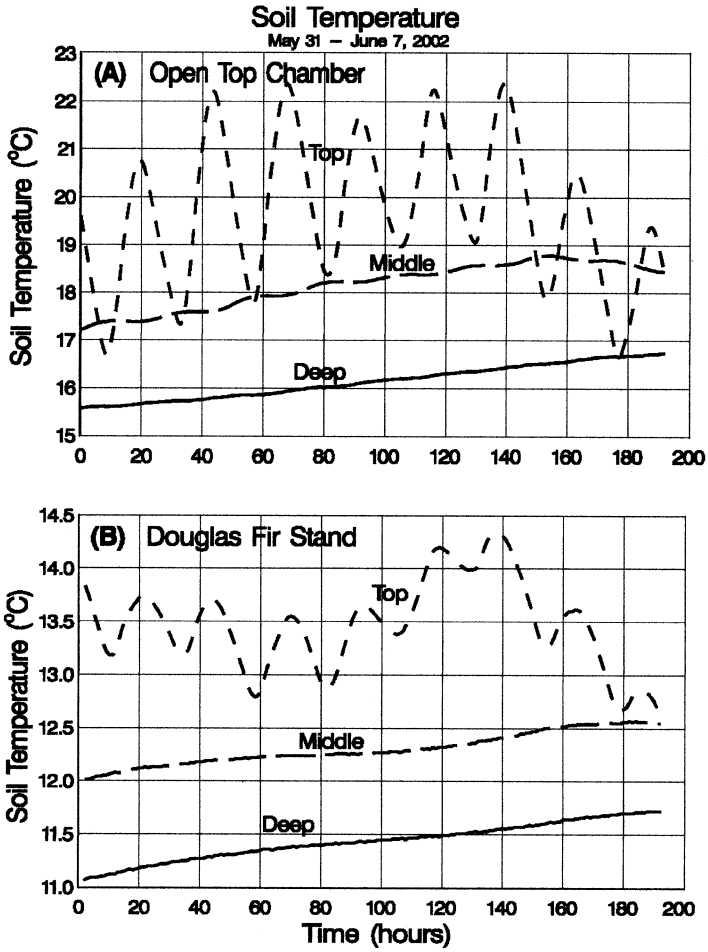

Figure 2A. Soil temperatures from three depths for eight days starting on May 31 and ending on June 7, 2002. Temperature probes were located at Top 15 cm, Middle 45 cm and Deep 75 cm from the soil surface. The OTC were in full sun.

Figure 2B. Soil temperatures under a Douglas-fir stand 50 m away from the OTC at three depths for eight days starting on May 31 and ending on June 7, 2002. Temperature probes were located at Top 10, Middle 70 and Deep 90 cm below the soil surface.

pond liner and the ground (side walls) were back filled with sand. The area inside the fabric was filled with sieved (1 cm) Willamette Valley loam, an agricultural soil typical of the central Willamette Valley of Oregon. As the soil area was being filled, two soil moisture sensors (Campbell Scientific, Inc) and one temperature sensor (Campbell Scientific, Inc) were placed at depths of 15, 45 and 75 cm from

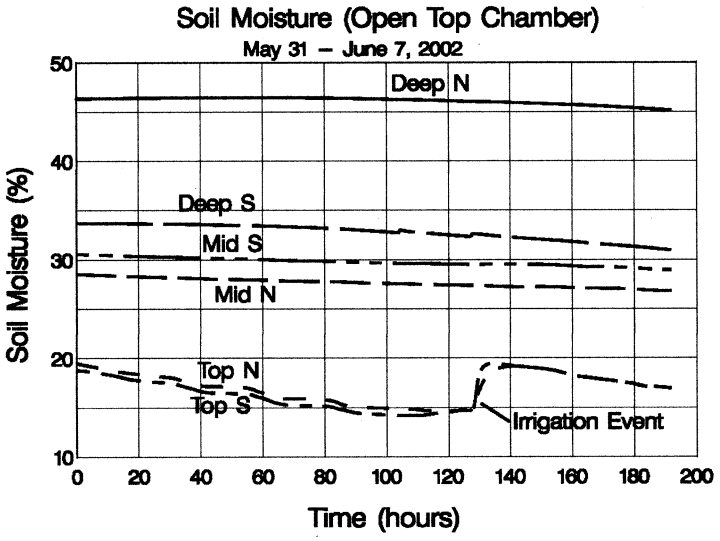

Figure 3. Amount of soil moisture at three depths for eight days starting on May 31 and ending on June 7, 2002. Moisture probes were located at Top 15 cm, Middle 45 cm and Deep 75 cm from the soil surface. N = north side of the chamber, S = south side of the chamber. Probes at similar depths were placed 90 degrees from each other. At approximately 130 hours, an irrigation event occurred when the top soil moisture measured below 15 percent.

the top (Figure 1). The top of the soil column rose 20 cm above the surrounding ground and was supported by a polyethylene plastic ring. The ring was 46 cm wide. More than half the ring was below ground level. The pond liner and the geotextile were held against the plastic ring above the soil by a stainless steal hose clamp (Figure 1).

A 2.5 cm layer of seed bank soil was placed on top of the loam and misted twice a day until germination. The seed bank soil came from a field at the Oregon State University Botany and Plant Pathology Farm, which is disturbed annually by tillage to encourage the growth of a diverse population of herbaceous plants for use in plant taxonomy classes. After the first year, no additional seeds or soil were added.

The soil was surface irrigated when the top soil moisture sensors indicated the top 15 cm of soil had less than 15 percent moisture. Between growing seasons, the subsurface water was recharged to 40 percent soil moisture by pouring measured amounts of water into holes where soil samples had been removed.

The resulting nine replicated plant communities were exposed to three different levels of ozone (0, 90, 120 ppb episodic ozone, three chambers/treatment). Each chamber received the same episodic ozone exposure (Lefohn et al. 1986) every year.

RESULTS AND DISCUSSION

An example of soil temperature recorded from one OTC from the first week of June 2002 is shown in Figure 2A. Diurnal temperature patterns exhibited at the soil surface in the OTC are similar cycles to those in natural environments (Figure 2B; Pearson's correlation coefficient r = .65). The data presented in Figure 2B are from under a stand of Douglas fir trees 50 m from the OTC. Daily temperature changes under the trees were less severe and cooler due to shading versus full sun exposure of the OTC. The damping of the diurnal patterns with soil depth in the OTC also is present in natural plant communities (Figures 2A and 2B).

An example of soil moisture recorded from one OTC from the first week of June 2002 is shown in Figure 3. The subsurface water supply is slowly depleted over the growing season with moisture remaining longest at the greatest depths. Removal of subsurface water across the growing season as shown by the soil moisture profiled from the OTC was similar to the loss of ground water in the natural areas. The upper layer of soil was maintained between 15 and 20 percent soil moisture by adding known amounts of water when the soil moisture was less than 15 percent (Figure 3).

Modified OTC operated without problems through four growing seasons. The pond liner barrier successfully prevented movement of groundwater in the chambers allowing soil moisture control. Soil temperature patterns mimic those found under natural conditions in contrast to soil temperatures in potted plants. The existing soils under the OTC could have been used instead of reconstructing the soil profile. However, the OTC were located on a highly disturbed agricultural field which upon excavation was found to contain the remains of a field drainage system. The modifications we made reduced the amount of soil heterogeneity and its associated variability that often is found in field studies while adding environmental complexity lacking in greenhouse studies. For example, the mean coefficient of variation between replicate treatment chambers for aboveground biomass over four years was 13.8 percent with a range of 2.0 to 28.0 percent and a standard deviation of 7.4. In one of the few studies using OTC and naturally occurring plants (Barbo et al. 1998, Table 3), plant cover measures between replicate treatments had a mean coefficient of variation of 72.2 percent with a range of 11.5 to 232.8 percent and a standard deviation of 53.2.

Laboratory toxicity testing is done in leu of field testing because it is less expensive and easier to perform. However, real exposures are in the field. If laboratory toxicity testing has no relevance to field situations then the results are of questionable value. However, the US EPA Office of Pesticide Programs found that results from wildlife field experiments were so excessively expensive with such highly variable results that they no longer routinely request them. What is needed are a series of test scenarios that bridge between the complexity of field tests and the lack of realism found in laboratory tests. The approach presented here uses the experience gained from years of research performed in open top chambers (OTC) to propose a method

Table 1. Plant species common to all treatments (0, 90, 120 ppb episodic ozone). Means are aboveground biomass at harvest for three years (1999-2001) and include all treatments.

Species	Common Name	Dry Weight (gm/m^2) Mean (sd)
Amaranthus powelli Wats.	Powell's amaranth	28.0 (41.0)
Sonchus spp. L.	Perennial sowthistle	5.0 (4.0)
Capsella bursa-pastoris (L.) Medic.	Shepards purse	9.2 (11.5)
Spergula arvensis L.	Corn spurry	44.1 (50.7)
Vicia tetrasperma (L.) Moench.	Slender vicia	13.2 (12.7)
Erodium cicutarium (L.) L'Her.	Redstem filaree	50.3 (44.1)
Digitaria sanguinalis (L.) Scop.	Large crabgrass	2.5 (2.8)
Eragrostis orcuttiana Vasey	Orcutt's lovegrass	2.0 (3.1)
Panicum capillare L.	Witchgrass	4.0 (5.6)
Calandrina ciliata (R.&P.) DC.	Red maids	121.6 (35.2)
Veronica biloba L.	Bilobed speedwell	13.8 (9.5)
Solanum nigrum L.	Black nightshade	5.2 (4.7)

that takes advantage of some of the experimental control found in greenhouse experiments such as homogeneous soil and environmental conditions along with some of the reality found under field conditions, i.e. high species diversity. For example, during the course of the four year study, 60 different plant species were identified representing 22 different families. However, only twelve species were found in all treatments every year (Table 1). The most dominate species as measured by aboveground biomass were *Amarantu powelli*, *Spergula arvensis*, *Erodium cicutarium* and *Calandrina ciliata* (Table 1). Plant species responded differently to ozone, neighboring plant interactions and environmental conditions. We use these four species as examples of the complexities common in multispecies plant communities found in natural habitats but not demonstrated in single species testing (Table 2). *A. powelli* has a C_4 photosystem which would be expected to do well in the hot, dry environment of the OTC, however, after the first year its presence decreased substantially. It also was more productive in the highest ozone treatment two of the three years measured (Table 2). In contrast, *S. arvensis* increased productivity with each year regardless of treatment but was most productive in the 0 ozone treatments. *E. cicutarium* was most productive the third year and in the 90 ozone treatments. While, *C. Ciliata* was most productive the first year having a variable response to treatments (Table 2). A positive response to exposure was most likely an indirect effect from smaller neighbors (i.e., less competition) and/or more space for colonization. These responses are not likely to be found in simple laboratory tests and would be difficult to attribute causality in natural habitats. Our modifications have expanded the type of experiments we are able to conduct from simple single species tests to complex ecological studies under realistic environmental conditions. We investigated effects of ozone exposure on plant community dynamics and found that results from community level studies are not necessarily predictable from single species tests (Table 2).

Table 2. Aboveground biomass means for dominate species by year and treatment (0, 90, 120 ppb episodic ozone). Means = gm dry weight/m^2

	1990			2000			2001		
Species	0	90	120	0	90	120	0	90	120
Ampo	63.1	81.5	99.4	2.5	2.5	0.6	0.2	0.8	1.2
Apar	4.2	2.2	1.1	76.1	33.2	20.0	148.8	90.1	20.9
Erci	3.7	8.5	5.5	61.8	68.0	23.0	75.4	137.0	68.8
Caci	175.4	144.9	155.7	111.0	111.6	132.0	55.7	103.8	104.0

Traditional plant testing under FIFRA, TSCA or OECD have used potted plants. The limited soil volumes found in pots provides a habitat that has very little resemblance to native soil conditions. The soil temperatures and moisture levels in pots large have daily fluctuations. In contrast, the large block of native soil used in the OTC's are much more stable, more closely simulating natural conditions. Therefore, plants growing in the modified OTC with native soils and normal soil temperature and moisture will have a soil microbiology more closely resembling native populations. These populations are important for the normal functioning of plants. Results from plants grown in the modified OTC would have more credibility as they resemble the natural conditions of the areas trying to be protected. Studies using modified OTC could be used instead of large scale Tier III field studied at times requested prior to pesticide registration under FIFRA.

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