Constructing Specialized Plant Growth Chambers for Gas-exchange Research: Considerations and Concerns

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Specialized chambers for whole-plant gas exchange have been constructed by individual researchers to provide capabilities that are otherwise difficult to achieve in typical commercial chambers. For example, many investigators have built controlled-environment chambers to measure gas exchange of whole-plant canopies in completely isolated environments to determine how CO, assimilation is affected by hydrocarbons such as ethylene, or to determine the effects of toxic chemical-plant interactions (Akers et al., 1985; Campbell, 1979; Dutton et al., 1988; Knight et al., 1988; McFarlane and Pfleeger, 1987). Materials used in the construction of such chambers include plastics (especially acrylic, polyvinyl chloride, and polyethylene), metals, rubber, and sealants. As self-made chambers have been used over the past few years, many component materials have been identified as interfering with normal plant growth and developmental processes. Major features limiting investigator-constructed systems include construction materials that release harmful substances and materials that alter the gaseous composition of the atmosphere within the chamber.

Materials that release harmful substances

Some chamber materials contain or release harmful components such as heavy metals, plasticizers, hydrocarbons, Hg, xylene from paints, and epoxy resins, any of which may contaminate the atmosphere or rhizosphere within a plant growth environment. Typically, gas-assimilation chambers or cuvettes contain plastic components to impart flexibility to the system as well as to avoid toxic metal ions from metal fittings and metal containers. Teflon or glass fittings are preferred over metal in applications where the fitting would come in contact with nutrient solution. When nutrient solution is repeatedly circulated over metals, such as Cu, galvanized Zn, Al, or brass, micronutrient toxicities may occur (Winsor, 1980). For instance, Al injury in plants often is attended by reduced Ca uptake and symptoms resembling Ca deficiency (Foy et al., 1973). Nickel contained in the sheathing materials of electric immersion heaters was found to be toxic to tomato plants (Graves and Adatia, 1979). Additionally, Cu toxicity problems occurring in growth chambers have been traced to Cu components of humidifiers (Cary A. Mitchell, Purdue Univ., personal communication). Moreover, certain types of foam strips used for supporting plants contain Cd, Pb, Cu, Zn, and Ba and may be harmful to plant development (Graves, 1983).

The second most qualitatively important, and often the most abundant, constituent of plastics belongs to a group of compounds referred to as plasticizers (Mathur, 1974). This group includes fatty acid and vegetable oil derivatives that are subject to degradation by microorganisms, and the less-available esters of phthalic, maleic, and phosphoric acids. External plasticizers are added to convert the glass-like solid polymer linkages to an elastomer, in which state the plastic can be molded. These plasticizers are only loosely linked to the polymers and, therefore, are slowly vaporized with time as volatile out-gassing components.

Often, flexible plastic items [polyvinyl chloride (PVC), polyethylene, hosepipe] contain the plasticizer di-butyl phthalate (DBP). When plastic containing DBP has been enclosed in chambers or greenhouses, DBP vapors have reached levels that are toxic to plants [British Agr. and Hort. Plastics Assn. (BAHPA), 1979; Hannay, 1980; Hardwick et al., 1984; Lokke and Bro-Rasmussen, 1981; Shea et al., 1982]. Symptoms of DBP toxicity include general chlorosis, cotyledons and expanded leaves that first turn grayish-green and then become necrotic and bleached, as well as severe growth restriction (Hardwick et al., 1984). One study reported that plastic screening used to reduce photosynthetic photon flux (PPF) in a growth chamber caused marginal chlorosis on the first true leaves of mung bean plants (Tibbitts et al., 1977). The problem was observed only when a bonded screen was used, and a diethylephthalate was identified only in the bonded material. Although most research has dealt with the effects of DBP in the vapor phase, some evidence indicates that DBP also may cause phytotoxicity in the aqueous

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phase. For example, Ries and Houtz (1983) proposed that waterborne DBP may have secondary effects on plant development, such as by interfering with the action of exogenous triacontanol.

Other investigations have linked damage to chamber-contained plants with certain plastics, but toxic principles were not identified (BAHPA, 1979; Austin et al., 1969). Certain kinds of thermocouple wires, low-grade polyethylene, and clear PVC also give off DBP vapor. Rigid PVC; acrylonitrite butadiene styrene; teflon tubing and fittings; ultra-high molecular weight, high-density, linear polyethylene; polyethylene fittings; and stainless steel do not give off DBP and are considered acceptable as chamber components.

Some chamber components give off hydrocarbons, such as ethylene and ethane. Even a very low concentration of ethylene (55 nl·liter^{-1}) was found to drastically reduce fresh and dry weight of growth chamber-grown lettuce *(Lactuca sativa* L.) under various temperature and PPF regimes (Mortensen, 1989). Thus, variation in ethylene concentration during CO_2-exchange studies may lead to erroneous conclusions, especially in chambers with very low atmospheric turnover rates.

O-ring materials, including natural rubber, neoprene, and viton, were found to release hydrocarbons on the order of 4 to 5 nl·h^{-1} (Bassi and Spencer, 1979) when exposed to a balanced spectrum of radiation. Teflon O-rings were recommended as an excellent construction material, with silicone rubber seals as a second material of choice since neither product released appreciable amounts of hydrocarbons. Conventionally used sealants such as lanolin, Apiezon grease, and mixtures of paraffin (petroleum jelly, Terostat, and vacuum grease) produced substantial amounts of hydrocarbons (0.20 to 30.90 nl·h^{-1}). Silicone sealant, which does not release acetic acid upon curing, is suitable for use as a sealant.

Another significant source of hydrocarbons is the use of brush motors within chambers to drive fans or pumps. (Brushless motors are better, but give off significant amounts of heat.) Both the hydrocarbon and excess heat problems have been bypassed in some systems by positioning the fan and motor shaft inside the chamber, while the motor itself is located outside the chamber (Bassi and Spencer, 1979; Knight et al., 1988).

Physiologically active levels of the hydrocarbons ethylene and ethane (>0.12 µg·liter^{-1}) also were produced from polyethylene, polypropylene, clear PVC, and nylon sheeting (Scott and Wills, 1972). Furthermore, many air and CO_2 cylinders contain hydrocarbon contaminants. Contaminated air can be passed through a column of potassium permanganate-coated aluminum pellets (Air Repair Products, Stafford, Texas) to remove unsaturated hydrocarbon impurities, but this material must be constantly monitored and replaced as it takes up moisture and impurities (Morison and Gifford, 1984); also, it does not remove saturated hydrocarbons.

Accounts of the toxic effects of Hg vapor on plants were first made a century ago (cited by Waldron and Terry, 1975). The primary source of Hg contamination is broken glass thermometers, and these should be avoided whenever possible. Other sources of Hg contamination include broken fluorescent lamps, switches, thermostats, water baths, and some cleaning agents (Tibbitts et al., 1977). Mercury contamination is very difficult to detect and remove once a chamber has been contaminated.

Paints release potentially phytotoxic materials upon drying. The solvent xylene has been identified as particularly harmful to various plant species (Tibbitts et al., 1977). The most acute problems have been found with rust-resistant paints applied to radiators and pipes. In addition, epoxy resins used to seal nutrient containers should be avoided, or they must be completely cured before hydroponic systems are operated (BAHPA, 1979). Some researchers have reported toxic effects from epoxy resins for up to 4 months after application. To prevent contamination of chamber atmosphere from an outside source, the air supply should be from a source that is isolated from potential polluters (e.g., furnaces, chemistry laboratories, compressor rooms).

Polyurethane foam plugs used to collar plants in solution culture have been identified as sources of contamination (Wheeler et al., 1985). The plugs may contain residual tertiary amines or isocyanate compounds trapped in the air cells. The injury appears as a brownish discoloration on the stem tissue of plants (young plants are particularly susceptible) within 30 h. Toxicity varies among batches of plugs and decreases as the plugs age. Rinsing plugs with 95% ethanol or acetone was effective in eliminating visible toxic effects. Recently, variability in sensitivity of plant species to foam plugs was discovered; some species required that plugs receive several rinses with 95% ethanol plus heating at 100C for 1 h to remove toxicity (Wagoner, 1989). No toxicity symptoms were reported after using reticulated ether-based foam plugs (Advanced Packaging Co., Baltimore). Another alternative to conventional open-cell foam plugs is a closed-cell plug cut from insulation tubing (e.g., Ethafoam Sealabacka; Seward Sales, Indianapolis). This material tubing has been used with several plant species (lettuce, tomato, rice), which have shown no toxicity symptoms (unpublished data).

Materials that alter gaseous or water vapor concentration

Some materials commonly used in equipment for measuring CO_2 exchange either adsorb or are permeable to CO_2 and/or water, and thus may lead to large experimental errors. Carbon dioxide is soluble in water and its solubility increases with decreasing temperature (Forsythe, 1969). Consequently, materials that adsorb water also may adsorb CO_2, thereby causing humidity-dependent fluxes of water and CO_2 even in empty chambers.

Most rubber materials are unacceptable for use in gas-assimilation chambers due to problems with both high water adsorption and gas permeability (Bloom et al., 1980; Dixon and Grace, 1982). It should be noted, however, that some rubber materials are better than others. For example, O-rings made of silicone rubber are much more suitable for use than nitrile rubber products (Dixon and Grace, 1982). Viton would be even more desirable than silicone rubber since the latter is too compressible as an O-ring material and creates variation in sealing pressure.

PVC (Tygon) tubing is commonly used in CO_2 and water-exchange systems, although it adsorbs significant amounts of water and, consequently, CO_2 (Bloom et al., 1980). Water adsorption is decreased in black Tygon tubing and black Norprene tubing by as much as 75% (John Towne, Cole-Parmer, personal communication), but a serious drawback of all flexible plastic tubing is the use of plasticizers to increase flexibility. Clear Tygon tubing contains even more plasticizers than black tubing to increase transparency. With enhanced water adsorption and increased organic plasticizer content, microbial growth also is encouraged within flexible plastic tubing. Teflon or nylon tubing contains fewer plasticizers and adsorbs less water and CO_2 than Tygon tubing; thus, teflon or nylon is preferable for use in CO_2-exchange studies (Millar, 1971).

The vast majority of cuvettes and larger, whole-plant chambers are constructed from acrylic plastic (Plexiglas, Lucite, and Perspex). Acrylic plastic often is the material of choice because it uniformly transmits 92% of incident radiation in the photosynthetically active range (400-700 nm). Some acrylics also allow transmittance of a portion of ultraviolet-A and ultraviolet-B [Plexiglas G, >320 nm; Acrylite OP-1 (Cyro Industries), >250 nm]; polycarbonate (Lexan) and other plastics do not (Acrylite GP: Light Transmittance and Reflectance, Cyro Industries technical bulletin, 1988). Unfortunately, water adsorption is high and the thermal conductivity is low for acrylic (Bloom et al., 1980). For example, acrylic adsorbs twice the weight of water as polycarbonate and is 10% less thermally efficient. Problems of low thermal conductivity for acrylic generally can be overcome by placing a heat exchanger directly in the plant chamber, although Al exchangers also can adsorb large amounts of water. Bloom et al. (1980) postulated errors exceeding 50% in estimating transpiration of plants under specific conditions in acrylic chambers.

The amount of error in estimating transpiration or gas exchange, however, depends on the size of the chamber (Dan Morgan, LI-COR, Lincoln, Neb., personal communication); the larger the chamber (less surface : volume ratio), the smaller the error due to water adsorption. LI-COR has found 10% to 25% error in determining water vapor pressure in small leaf cuvettes (LI-COR, LI-6000-12 chamber) with a surface area : volume ratio of 0.083. According to LI-COR scientists, the error for whole-plant growth chambers (e.g., Minitron II chambers, Purdue Univ.) with a ratio of 0.0098 should decrease substantially, and for Minitron III chambers at the Univ. of Illinois (ratio = 0.0065) the error would be even less (Knight et al., 1988). While it is imperative that small leaf cuvettes be coated with a film of teflon to prevent water adsorption by the chamber, LI-COR claims that large gas-assimilation chambers would not require teflon coating. To my knowledge, no information has been published on the effect of teflon coating on water or gas exchange in large chambers. Thus, it may be advisable to coat the inside walls of all acrylic and polycarbonate chambers with teflon to be safe.

In summary, gas-exchange/chamber systems should provide the researcher with the ability to examine net CO_2 exchange by whole plants without significant interference from component materials. Strict avoidance of potentially harmful materials depends on the type of chamber and its use. The more closed a chamber is, the more critical it becomes to eliminate potentially toxic components from the system. Likewise, a researcher studying the effects of contaminants such as ozone or ethylene on plant growth should make every effort to remove any confounding sources of contamination from the interior chamber environment.

Literature Cited

Akers, C.P., SW. Akers, and C.A. Mitchell. 1985. The Minitron system for growth of small plants under controlled environment conditions. J. Amer. Soc. Hort. Sci. 110:353-357.

Austin. R.B.. R.C. Hardwick. and M.S.M. Maclean. 1969. Controlled environment equipment and cultural methods: Phytotoxic constituents in plastic. Rpt. Natl. Veg. Res. Sta. 1968. p. 56-57.

Bassi, P.K. and M.S. Spencer. 1979. A cuvette design for measurement of ethylene production and carbon dioxide exchange by intact shoots under controlled environment conditions. Plant Physiol. 64:488-490.

Bloom. A.J.. H.A. Mooney. O. Björkman. and J. Berry. 1980. Materials and methods for carbon dioxide and water exchange analysis. Plant, Cell & Environ. 3:371-376.

British Agricultural and Horticultural Plastics Association. 1979. Plastics for hydroponic systems. Non-phytotoxic equipment. British Agr. Hort. Plastics Assn. Code of Practice 002.

Campbell, D.E. 1979. A simple, reliable, and expandable monitor and control for gas-exchange analysis. Analytical Methods 105:287-290.

Dixon, M. and J. Grace. 1982. Water uptake by some chamber materials. Plant, Cell & Environ. 5:323-327.

Dutton, R.G., J. Jiao, M.J. Tsujita, and B. Grodzinski. 1988. Whole plant CO_2 exchange measurements for nondestructive estimation of growth. Plant Physiol. 86:355-358.

Forsythe, W.E. (ed.). 1969. Smithsonian physical tables. 9th ed. Smithsonian Institution Press, Washington, D.C.

Foy, C.D., G.C. Gerloff, and W.H. Gabelman. 1973. Differential effects of aluminum on the vegetative growth of tomato cultivars in acid soil and nutrient solution. J. Amer. Sot. Hort. Sci. 98:427-432.

Graves, C.J. 1983. The nutrient film technique. Hort. Rev. 5:1-44.

Graves, C.J. and M.H. Adatia. 1979. Nickel toxicity in nutrient film culture. Annu. Rpt. Glasshouse Crops Res. Inst. p. 103-104.

Hannay, J. 1980. Fumes from flexible PVC damage young plants. Grower 21(August):28-29.

Hardwick, R.C., R.A. Cole, and T.P. Fyfield. 1984. Injury to and death of cabbage *(Brassica oleracea)* seedlings caused by vapors of di butyl phthalate emitted from certain plastics. Ann. Applied Biol. 105:97-105.

Knight, S.L., C.P. Akers, SW. Akers, and C.A. Mitchell. 1988. Minitron II system for precise control of the plant growth environment. Photosynthetica 22:90-98.

Lokke, H. and F. Bro-rasmussen. 1981. Studies of mobility of di-iso-butyl phthalate (DIBP), di-n-butyl phthlate (DBP), and di-(2-ethylhexyl) phthalate (DEHP) by plant foliage treatment in a closed terrestrial simulation chamber. Chemosphere 10:1223-1235.

Mathur, S.P. 1974. Review and analyses: Phthalate esters in the environment: Pollutants or natural products? J. Environ. Quality 16:361-371.

McFarlane, J.C. and T. Pfleeger. 1987. Plant exposure chambers for study of toxic chemical-plant interactions. J. Environ. Quality 16:361-371.

Millar, B.D. 1971. Improved thermocouple psychrometer for the measurement of plant and soil water potential. I. Thermocouple psychrometry and improved instrumentation design. J. Expt. Bot. 22:875-890.

Morison, J.I.L. and R.M. Gifford. 1984. Ethylene contamination of CO_2 cylinders. Effects on plant growth in CO_2 enrichment studies. Plant Physiol. 75:275-277.

Mortensen, L.M. 1989. Effect of ethylene on growth of greenhouse lettuce at different light and temperature levels. Scientia Hort. 39:97-103.

Ries, S. and R. Houtz. 1983. Triacontanol as a plant growth regulator. HortScience 18:654-662.

Scott, K.J. and R.B.H. Wills. 1972. Ethylene produced by plastics in sunlight. HortScience 7:177.

Shea, P.J., J.B. Weber, and M.R. Overcash. 1982. Uptake and phytotoxicity of di-n-butyl phthalate in corn *(Zea mays)*. Bul. Environ. Contamination & Toxicity 29:153-158.

Tibbitts, T.W., J.C. McFarlane, D.T. Krizek, W.L. Berry, P.A. Hammer, R.H. Hodgson, and R.W. Langhans. 1977. Contaminants in plant growth chambers. HortScience 12:310-311.

Wagoner, R. 1989. Chapter 3 detoxification of polyurethane foam used as plant supports in an aeroponics system and evaluation of substitute foam materials. PhD Diss., Univ. of Illinois, Urbana.

Waldron, L.J. and N. Terry. 1975. Effect of mercury vapor on sugar beets. J. Environ. Quality 4:58-60.

Wheeler, R.M., S.H. Scwartzkopf, T.W. Tibbitts, and R.W. Langhans. 1985. Elimination of toxicity from polyurethane foam plugs used for plant culture. HortScience 20:448-449.

Winson, G.W. 1980. Progress in nutrient film culture. Progress in Agr. 23:7-9.1.