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Protocol for Measuring the Relative Toxicity of Substances on Plant Foliage

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Increasing numbers of synthetic chemicals and naturally occurring substances are being utilized in many newly developed processes and in so doing become part of our environment. To find out how these compounds may be affecting vegetation, a standard test was needed for comparative evaluation of these materials so that government control officials could comply with the recently enacted "Toxic Substances Control Act."

Plants normally produce low levels of ethylene but in response to a variety of biotic and abiotic stresses, they produce greatly elevated amounts of ethylene designated as stress ethylene (ABELES 1973). Stress ethylene is produced by injured, but metabolically active plant tissue.

TINGEY et al. (1976) found that when various plant species were exposed to ozone, ethylene production increased proportionally to the ozone concentration and that this response can be modeled using $\mathrm{Log_e}$ [ethylene concentration] = $\mathrm{Log_e}$ A + B [concentration of toxicant]. In this equation A is the ethylene produced by untreated plants and B the slope parameter expressing the increase in ethylene production in relation to the concentration of the toxicant.

Because increased ethylene production is also induced by a wide array of other stresses (TINGEY 1980) this response was selected to quantitate the toxicity of substances applied to foliage of cultured test plants in aqueous solution. Using the above mentioned stoichiometry, the slope parameter serves as a measure of the relative toxicity of substances. Presented here below is a protocol describing a procedure designed to test the effects of a variety of toxic substances on standard test plants.

MATERIALS AND METHODS

1. Equipment Required

1.1 Plant growth chamber which will provide light intensity of 350 μ Einsteins/m^{-2}/sec^{-1}; temperature controls, 20-35 $\pm$ 2°C; relative humidity controls, 50-90 $\pm$5% and automatic off-on light controls. Cool white fluorescent lights must be supplemented with incandescent bulbs to provide the longer wavelengths.

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1.2 Analytical balance sensitive to 0.1 mg.

1.3 Sprayer--Pendulum sprayer (DAY et al. 1963) or other equipment capable of delivering dosages of solutions reproducible to $\pm 2\%$

1.4 Dark incubation chamber with temperature control $25 \pm 2°C$.

1.5 Gas chromatograph with flame ionization detector and column capable of separating and measuring 0-5000 parts per billion (ppb) ethylene with precision of ± 2 ppb plus strip chart recorder and ancillary tank gases.

1.6 Computer with program or a calculator for determination of slope parameter, intercept and correlation coefficient.

2. Test Plants

Bean, Phaseolus vulgaris (pink kidney) and/or cucumber Cucumis sativus L. (Pickling SMR-58)

2.1 Culture

Test plants are propagated in 310-mL styrofoam cups with drainage holes. Seeds are planted 2 cm deep in moist Jiffy Mix (Jiffy Products of America, Chicago, IL). The cups are placed in trays filled with tap water inside a growth chamber. No top watering is done until the seeds are completely germinated. Plants are then watered with one-half strength N.C. State Univ. phytotron nutrient solution (DOWNS & BONAMINIO 1976). Single plants are grown in each pot. Light intensity is 322 μEinsteins/m^2/sec with a 12 h photoperiod. Day and night temperature and relative humidities are maintained for beans at 27°C, 21°C and 65%, respectively, and for cucumbers 30°C, 26°C and 80%. Temperatures should not vary more than $\pm 2°C$ and humidity $\pm 5\%$. Pink kidney beans will have fully expanded primary leaves in 9-10 days and be ready for foliar spraying. Cucumbers will require 13-14 days.

2.2 Spray application

2.3.1 Compound preparation. Water soluble compounds are weighed on an analytical balance, dissolved in distilled water and a nonionic wetting agent such as X-77 (Colloidal Products Corp., Sausalito, CA) is added to give concentration of 0.6 mL/L. Oil soluble compounds are dissolved in the smallest volume possible of acetone and/or olive oil, a wetting agent added and dispersed with agitation in water.

2.3.2 Determination of dosage range

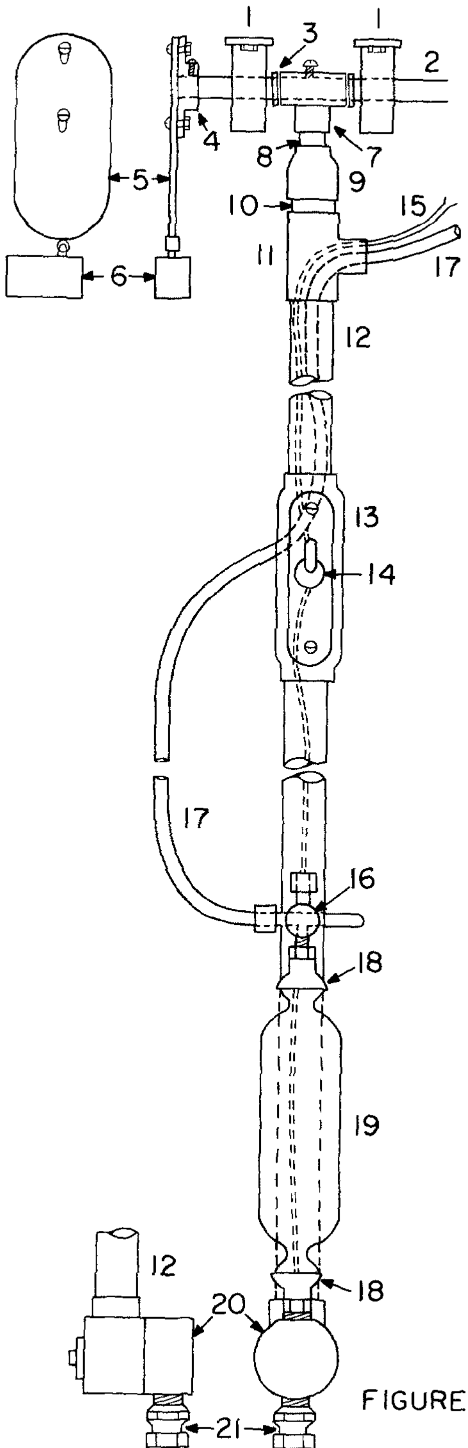
Because the Log_e of the ethylene response to the toxicant is somewhat sigmoid the initial objective is to find those concentrations that embrace the linear portion of this response through one or more range finding tests. If the concentration or dosage of the substance entering the environment is known as with herbicides a range finding test is carried out using, for example, 1 X; 1/2 X; 14 X; and 1/8 X this level where X is the recommended field dose. Substances of unknown toxicity may require initial testing over a much wider range. Six to eight plants are used per concentration. Once this range is established a definitive test is carried out using 7 equally spaced concentrations with 4 to 5 plants per concentration.

2.3.3 Spraying procedure. To assure reproducibility and ease of operation a pendulum sprayer (DAY et al. 1963) or other comparable equipment is modified for this purpose. It consists of a miniature compressed air sprayer mounted on a 1.7 m pendulum equipped to automatically spray a plant placed beneath the center of its arc of swing. Figure 1.

Character of the deposit and deposition rate are determined by: pressure at the nozzle tip, nozzle type and size, height above the leaf surface and the speed with which the nozzle passes over the plant. The objective is a generous wetting of the leaf surface without runoff through manipulation of these variables. The following conditions give reproducible results. The pendulum is released 60 cm from the center of its arc of swing, measured at the nozzle tip. Pressure at the nozzle is 30 psi. Nozzle height above the leaf surface is 30 cm. The nozzle is a Tee-Jet (Spraying Systems Co., Wheaton, IL) flat spray tip No. 4001. The delivery rate is determined by spraying preweighed paper towels or filter paper and weighing afterwards. One spray application consists of two passes of the pendulum. See Table 1 for reproducibility of dosage

Table 1. Reproducibility of Sprayer Delivery

Date	8/22/79	1/24/79	11/29/78	12/27/78
Nozzle #	4001	4001	6501	6501
Distance from Sprayer Tip (cm)	30	30	45	45
Delivery (mg/cm^2)	2.06	2.63	1.18	1.77
	2.07	2.67	1.26	1.77
	2.14	2.70	1.31	1.79
	2.09	2.66	1.26	1.78
		2.79	1.31	1.76
			1.28	1.77
			1.31	1.79
			1.22	1.68
			1.33	1.75
			1.31	1.77
Mean	2.09	2.69	1.28	1.76
Std. Dev.	0.04	0.06	0.05	0.03



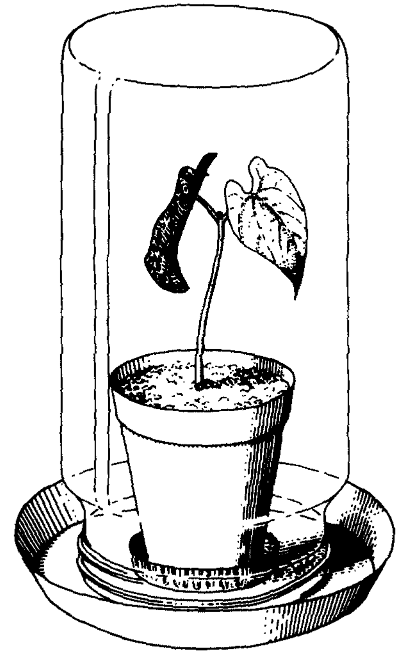


FIGURE 2. SIDE VIEW OF GLASS ENCAPSULATION SYSTEM.

FIGURE 1. PENDULUM SPRAYER WITH INTERVAL CONTROL.

2.3.4 Regimen of spray application and ethylene determination

Plants are taken from the growth chamber after 2 h of light. They are sprayed and placed back in the growth chamber to dry for 0.5 h. To trap the ethylene produced the dry plants are encapsulated as follows: a small aluminum weighing dish is placed in the bottom of a 6 in plant saucer (Super Saucer, Childs and Associates, Mill Valley, CA). The pot with the treated plant is placed in the dish and covered with an inverted 2-qt wide-mouth Masin jar. The saucer is filled with water to seal the opening (Figure 2). Ethylene is accumulated during 24 h in a dark chamber at 24°C. After this period samples for ethylene analysis are taken from the jar with a gas tight syringe equipped with a bent 6-in. hypodermic needle inserted beneath the jar. The values are recorded as ppb. Standard ethylene gas samples of 1000 ppb are run before and after each series of determinations. Typical results for treatment of beans with endothal are shown in Figure 3.

PARTS DESCRIPTION FOR FIGURE 1

1. Pillow blocks with 0.5 in. bearing.
2. 0.5 in. shaft.
3. Teflon washers
4. Flange with set screw (Lab frame foot).
5. Aluminum cam. 2 x 4.5 in.: attached to flance to activate microswitch (6). Cam is vertically adjustable through slotted holes.
6. Microswitch.
7. 0.25 in. galvanized TEE bored out to accommodate 0.5: shaft. A hole is drilled and tapped for a setscrew to fix TEE to the shaft.
8. 0.25 in. nipple.
9. Galvanized reducer 0.5: x 0.25:.
10. 0.5 in. galvanized pipe.
13. Conduit fitting 0.5 in.(type C).
14. Main switch (toggle type).
15. Electrical wiring for the microswitch, solenoid (20), main switch circuit.
16. 3-way valve. One position allows the reservoir to be filled with the spray formulation and the other the air to pressurize the system.
17. 0.25 in. polyethylene tubing for compressed air supply.
18. Stainless steel ball joints (18 mm) modified to fit the valve and solenoid.
19. Glass reservoir; 40 mm diameter, 220 mm long (ball joints included).
    Reservoir is normally exposed to 30 lb pressure.
    Ball joint size is 18 mm and the clamps (not in sketch) are the screw lock type.
20. Solenoid valve with stainless steel valve body.
    110-115 volt; 100-psi; 0.125 in. orifice; 10 watt; valve no V52 DA 2100 Code no VC7.
    Skinner Electric Valve Div. New Britain, Conn. USA

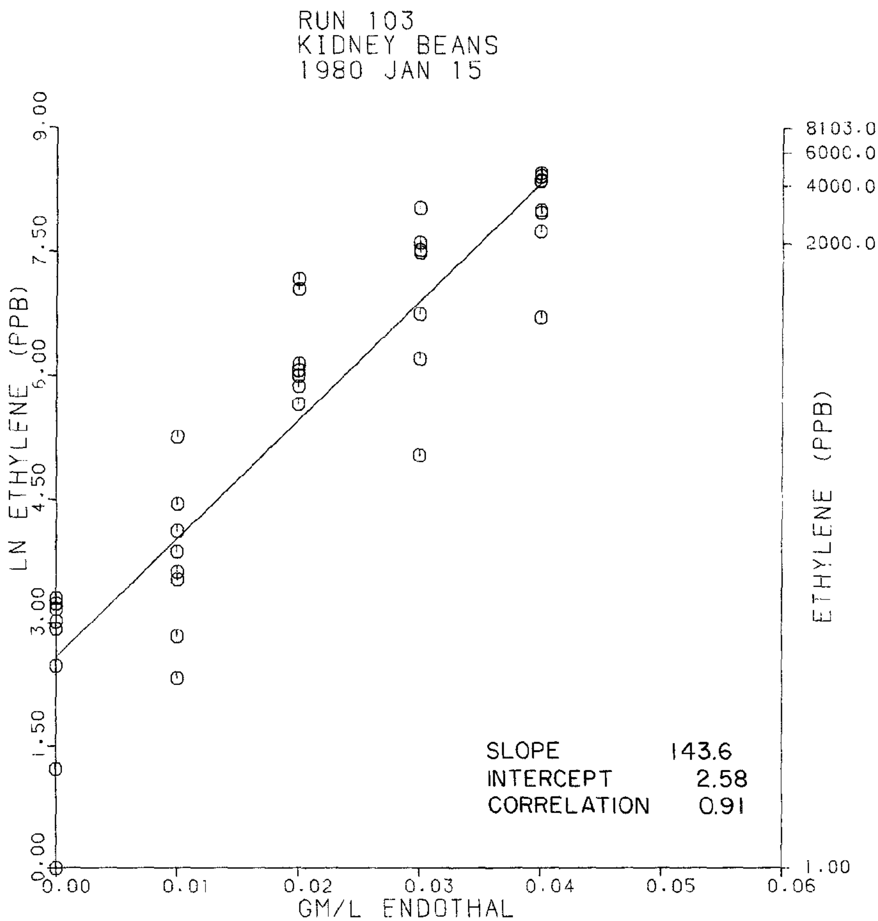


FIGURE 3. ETHYLENE EVOLUTION FROM BEANS TREATED WITH ENDOTHAL

21. L/4 TT Tee-jet stainless steel spray nozzle assembly with interchangeable tip. The tip used in this application is Tee-Jet flat spray tip no 4001.

2.3.5 Data processing

Analysis of the data consists of determining the slope of the linear regression of the Log_e ethylene on the toxicant concentration and the correlation coefficient. This procedure is commonly carried out with the help of a computer or calculator.

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REFERENCES

ABELES, F. B.: Ethylene in Plant Biology, Academic Press, New York, (1973).

DAY, B. E., L. S. JORDAN and R. T. HENDRIXSON: "A Pendulum Sprayer for Pot Cultures," Weeds 11, 174 (1963).

DOWNS, R. J. and D. P. BONAMINIO: Phytotron Procedural Manual for Controlled Environment Research at Southeastern Plant Environment Laboratories, North Carolina Ag. Exp. Station Bull. #244 1-37 (1976).

TINGEY, D. T., C. STANDLEY and R. W. FIELD: Atmos. Environ., 10, 969 (1976).

TINGEY, D. T.: Stress Ethylene Production--A Measure of Plant Response to Stress, Hortsci., In Press (1980).