Appraisal of Ethylene Production as a Test for Defoliants

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Evaluations were made of ethylene production and defoliation responses of 9-day-old red kidney beans from foliar-spray applications of 94 test chemicals made singly or in combination with ethephon. Test chemicals, including defoliants, herbicides, and growth retardants, were found to exhibit three types of ethylene production as related to untreated controls: increased production or ethylene positive, decreased or ethylene negative, and negligible effect or ethylene neutral. All chemicals causing defoliation when applied singly were ethylene positive, but varied defoliation responses were obtained when chemicals were applied in combination with ethephon. Chemical groups such as endothalls, ureas, and phosphorus compounds were mostly ethylene positive; other groups including triazines and thiophenols caused reduced ethylene production. It was concluded that ethylene production is a valid criterion for defoliants.

Research on ethylene as summarized by Abeles (1973) has shown that defoliation of plants is associated with internal production of ethylene. Applications of herbicides and defoliants have been found to enhance ethylene production, as illustrated by endothall (Abeles, 1967), picloram (Baur and Morgan, 1969), and 2,4-D (Morgan and Hall, 1962; Holm and Abeles, 1969). Ethephon [(2-chloroethyl)phosphonic acid], when applied to plant tissue, degrades at cytoplasmic pH to release ethylene and subsequently stimulates leaf abscission (de Wilde, 1971). Combinations of known defoliants such as endothall and ethephon act synergistically to enhance abscission (Davis et al., 1972).

The relatively rapid evolution of ethylene in plant tissue (within 24–48 hr) following applications of herbicides and defoliants formed the basis for the present survey of chemicals to test the hypothesis of a direct relationship between the capacity of a chemical to cause defoliation and its enhancement of endogenous ethylene production. Included in the schematic approach were evaluations of plant response from applications of the test compound in combination with ethephon. The array of 94 chemicals in the test included known defoliants, herbicides, growth retardants, and other chemicals previously shown to exhibit defoliant activity.

PROCEDURE

Bean (*Phaseolus vulgaris* L. cv. Red Kidney) plants were grown in 10-cm pots containing a sterile soil mix (2:2:1 sand–loam–peat on a volume basis) under greenhouse conditions, supplemented with fluorescent and incandescent light with a photoperiod of 16 hr. On the ninth day, plants were thinned to two plants per pot and three pots were used for each treatment.

Test chemicals were applied in water or acetone solutions at 100 ppm with 1% Tween 20. Applications were made with a DeVilbiss atomizer. Primary leaves were sprayed to the point of runoff; a spray volume of 7 ml totally covered the 12 primary leaves in each treatment. Each experiment included the following treatments as controls and standards: (a) untreated control; (b) control sprayed with solvent and 1% Tween 20; (c) endothall at 100 ppm in water and 1% Tween 20; (d) ethephon at 1800 ppm in water and 1% Tween 20; (e) endothall at 40 ppm and ethephon at 800 ppm in water and 1% Tween 20. Candidate compounds were tested at 100 ppm applied singly and in combination with 1800 ppm of ethephon. The standard treatments (c, d, and e, above) yielded an average defoliation response of 60, 5, and 90%, respectively. These standards were included to account for week-to-week variations in the test plant responses.

Following treatment, six disk samples (17 mm diameter) were removed from the primary leaves with a no. 10 cork borer, placed two each on moist filter paper in three 45-ml gas collection vials, sealed with rubber vaccine caps, and incubated for 24 hr in a growth chamber at 25 °C and constant fluorescent and incandescent light (7535 lx). After incubation, 2-ml gas samples were obtained from each vial with a hypodermic syringe and ethylene determinations made with a gas chromatograph having an alumina column and flame ionization detector. Treatments sprayed with ethephon were not sampled.

After removal of the leaf-disk samples, plants were placed in the greenhouse under natural illumination and the percent defoliation of primary leaves recorded at 3 and 7 days for all treatments.

Criteria Used in Data Analysis. Data on ethylene production from test compounds were transformed to a ratio of parts per million ethylene production from the test compound to parts per million ethylene production from untreated controls in order to standardize the results from the several series of test applications. Three classes of ethylene production responses were distinguished: ethylene positive, ratio greater than 2.0; ethylene neutral, ratio of 1.0 to 2.0; ethylene negative, ratio of less than 1.0.

Defoliation ratings at 7 days based on the percentage of leaves defoliated were used to distinguish three classes of defoliation response: class I, defoliated when applied singly and in combination with ethephon; class II, defoliated when applied in combination with ethephon but inactive when applied singly; class III, inactive as defoliants when applied either singly or in combination with ethephon.

Test Materials. The following groups of chemicals were included in the test array of 94 compounds evaluated by this procedure: endothalls, 51 (esters and salts, 16; N-substituted amidic acids, 21; amides, 7; miscellaneous, 7); triazines, 12; growth retardants, 8; substituted ureas, 6; thiophenols, 3; organometallic compounds, 3; phosphorus compounds, 3; miscellaneous, 8.

RESULTS AND DISCUSSION

The general relationships between ethylene production ratios and the defoliation response classes of the entire group of 94 compounds are shown in Table I. The tabular data represent the numbers of chemicals in the designated combinations of ethylene production ratios and defoliation classes.

A discussion of the results obtained from the individual classes of compounds follows.

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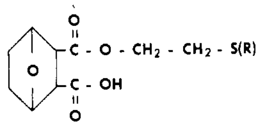
Table I. Correlation of Ethylene Production Ratio and Defoliation Classes of the Test Array of 94 Chemicals

Ethylene production ratio	Defoliant class			Total
	I	II	III	
Positive	24	19	14	57
Neutral		6	15	21
Negative		13	3	16
Total	24	38	32	94

Endothall Derivatives. Derivatives of endothall (7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid) comprised the largest and most active group of defoliants of the compounds tested in this program. Four subgroups of endothall compounds were distinguished: (1) esters and salts; (2) N-substituted amidic acids; (3) amides; and (4) miscellaneous.

(1) *Esters and Salts.* Of 16 compounds in this subgroup, 7 were class I defoliants with the following chemical structure:



Radical R attached to this structure ranged from a single ethyl group to long-chain hydrocarbons. The greatest defoliation activity in this subgroup was noted for the mono(2-ethylsulfinylethyl) and mono(2-isopropylthioethyl) esters. Without exception all thio or mercapto compounds in the endothall group were found to be class I defoliants.

Maximum values for ethylene production in this subgroup included a ratio of 19.3 for the mono(2-ethylsulfinylethyl) ester and 18.0 for the mono(2-ethylthioethyl) ester. Both of these compounds were class I defoliants. High ethylene production also occurred from applications of 1:2 salt of the dibasic acid (endothall) with phenylhydrazine, a class II defoliant in the disubstituted salt category.

Class III defoliants included three ethylene-positive endothall compounds (dimethyl ester, 1:1 salt with phenylhydrazine, and 5-sulfo-, trisodium salt) and two ethylene-neutral compounds (monohydrazide and monomethyl ester).

(2) *N-Substituted Amidic Acids.* The N-substituted amidic acids comprised 21 compounds, 12 of which were class I defoliants. Three chemicals, mainly ethylene neutral, were inactive as defoliants.

Of the 12 class I defoliants, 8 were equivalent in degree of defoliation to the endothall acid standard when applied singly (75% or more defoliation). All 12 of the class I defoliants were classified in the ethylene-positive group.

Maximum ethylene production noted for the 3-isopropylaminocarbonyl moiety was correlated with complete defoliation when the compound was applied singly or with ethephon.

Examination of the chemical structures of the six class II defoliants shows the group to have unbranched side chains, the moiety varying in complexity from methyl to chlorophenyl.

(3) *Amides.* Amides comprised 7 of the total of 52 endothall compounds tested and were relatively ineffective as defoliants. Two compounds, 2-(2-chloroethylsulfonyl)hexahydro-4,7-epoxy-1*H*-isoindole-1,3(2*H*)-dione and octahydro-*N*,*N*-dimethyl-1,3-dioxo-4,7-epoxy-1*H*-isoindole-2-sulfonamide, gave class I defoliation; the remaining 5 amides were class III defoliants, none of which contained S. It is suggested that the N–S linkage in the above two compounds is unstable and may be associated with the defoliation activity of these compounds. The latter compound also showed maximum ethylene production associated with complete defoliation.

(4) *Miscellaneous Endothall Compounds.* Five of the seven chemicals in this group were inactive as defoliants. Trimethylsulfonium, 1:1 salt with endothall, had high ethylene production associated with its rating as a class I defoliant. *N*-(3-Carboxyl-7-oxabicyclo[2.2.1]hept-2-ylcarbonylalanine gave class II defoliation associated with an ethylene neutral rating. The inactivity in defoliation of the remaining chemicals in this group appears to be associated with the stability of paired side-chain groups.

Triazines. Ten commercially formulated triazines and two additional compounds available as technical grade materials all proved to be class II defoliants. Minimal defoliation values were obtained with trietazine (2-chloro-4-(diethylamino)-6-(ethylamino)-*s*-triazine), terbuthylazine (2-*tert*-butylamino)-4-chloro-6-(ethylamino)-*s*-triazine), and 2-*sec*-butylamino-4-ethylamino-6-methoxy-*s*-triazine.

This group of chemicals is of interest in that 10 of the 12 chemicals were ethylene negative or ethylene neutral. A major exception in ethylene production ratios was noted for ipazine (2-chloro-4-(diethylamino)-6-(isopropylamino)-*s*-triazine) with a value of 13.9. In general, as the ethylene production decreased, the defoliation response increased when the chemical was applied with ethephon.

No apparent relation was noted between either the ethylene production ratio or the defoliation ratings when applied with ethephon and the type of triazine based on substituents of chlorine, methoxy, or methyl and mercapto or thiomethyl.

Growth Retardants. A group of eight growth retardants and related plant growth-regulator chemicals included seven compounds which were ethylene positive or ethylene neutral but none of the compounds qualified as class I defoliants. *N*,*N*-Dimethyl-3-nitro-2-fluorenamine, a chemical related to chlorflurenol, was the only growth retardant in the ethylene-negative group.

Relatively high ethylene production occurred from applications of TIBA (2,3,5-triiodobenzoic acid), a class II defoliant, and ethyl hydrogen-1-propylphosphonate, a class III defoliant. Three other retardants, ancymidol (*a*-cyclopropyl-*a*-(4-methoxyphenyl)-5-pyrimidinemethanol), Alar (succinic acid, mono(2,2-dimethylhydrazide)), and DPX 1840 (3,3a-dihydro-2-(*p*-methoxyphenyl)-8*H*-pyrazolo[5,1-*a*]isoindol-8-one), showed no defoliant activity when applied with ethephon.

Substituted Ureas. Of six substituted ureas evaluated, including commercial formulations of monuron, diuron, and fenuron, four were class II defoliants of which three were ethylene positive. 1-(*p*-Chlorophenyl)-3-(2-propynyl)urea had the greatest ethylene production ratio of the group but showed no defoliant activity. Other tests (Frank, 1973) indicate this chemical to be an active defoliant on Black Valentine bean when applied at 0.1 lb/acre. 4-Chlorophenylcarbamic acid, 2-propynyl ester, showed no defoliant activity in the current test or in earlier primary screening tests.

Thiophenols. Three thiophenols evaluated showed ethylene-negative ratios. Interest in this chemical group was stimulated when early tests of 4,5-dibromo-2-thiophenecarboxylic acid applied at 0.1 lb/acre with ethephon resulted in complete defoliation in contrast to the lack of defoliant activity when applied singly. Additional dose–response data obtained on this compound showed that maximum defoliation occurred within 3 days when it was applied at a rate of 400 ppm in combination with 1800 ppm of ethephon. This finding indicates that the selected standard of 100 ppm may not give sufficient discrimination in defoliation response when the compound is applied with and without ethephon.

Organometallic Compounds. Three compounds in this category were evaluated. The triethyl derivative of *a*,*a*-

dimethyl benzyl hydroperoxide qualified as a class I defoliant with moderate evolution of ethylene. The other two compounds, containing tin and lead, caused no defoliation when applied singly or with ethephon.

Phosphorus Compounds. Three chemicals containing phosphorus were found to be class II defoliants. Dibutyl 1-(dibutoxyphosphinyl) butyl phosphate and dibutyl 1-(dibutoxyphosphinyl)propyl phosphate were ethylene positive; the remaining compound, di-*N*-butyl-(2,4-dichlorobenzyl)phosphonate, was ethylene negative.

Miscellaneous. Eight miscellaneous chemicals included a single class I defoliant, allylpropylarsinic acid. Two other chemicals, the 2,2-diphenyl-2-(ethylamino)ethyl ester of pentanoic acid and 1,6-diisothiocyanatohexane, were class II defoliants and ethylene negative. The remaining five chemicals gave negative defoliation ratings when applied singly or with ethephon.

GENERAL DISCUSSION

At the onset of this research, an initial hypothesis was proposed that a direct relationship existed between ethylene production and the degree of defoliation caused by foliar applications of a given chemical (Webb and Leather, 1973). Initial tests emphasized the derivatives of endothall, a defoliant known to stimulate ethylene production when applied foliarly.

Esters, salts, and N-substituted amidic acid derivatives of endothall showed a high correlation between the amount of ethylene produced and the degree of defoliation when applied to bean plants. Thus, of the 37 compounds in these two groups, 19 were class I defoliants with ethylene-positive ratios. This correlation of ethylene positive and defoliation rating is not without exception as 10 of the 51 endothall derivatives were ethylene neutral and 11 of 18 chemicals, respectively, were class II and class III defoliants.

The group of triazine compounds studied was found to cause the opposite effect with a reduction in the amount of ethylene produced as compared with the controls. Although none of the triazines caused defoliation when applied singly, all caused leaf abscission when applied in mixture with ethephon. Thiophenols were similar to triazines in having reduced ethylene production or an ethylene-negative ratio.

The study thus confirms the reports summarized by Abeles (1973) that chemicals have varied effects on ethylene production. This range of variation is summarized in Table II in which the ethylene-production ratios are separated into eight groups instead of three as in Table I. The first two groups comprise the ethylene-negative category with a ratio of less than 1.0, falling mostly into class II defoliants. Ethylene-neutral chemicals with a ratio of 1.0 to 2.0 belong mainly in class III. Ethylene-positive chemicals with ratios of 2.0 to 8.0 times that of the control occur in all three defoliation classes. Chemicals with ethylene-production ratios exceeding 8.0 included principally those causing defoliation when applied singly. Further analysis of the data in Table II indicates that the initial hypothesis was valid in that 42% of the ethylene-positive compounds were class I defoliants. An additional 33% were class II defoliants; thus a modified test regime utilizing ethylene production as the criterion for a defoliant is justified.

Table II. Matrix Table of Test Compounds Classified as to Ethylene Production Ratios and Defoliation Classes

Ethylene production ratio	Defoliation class			
	I	II	III	Total
0–0.5		3	2	5
0.5–1.0		10	1	11
1.0–2.0		6	15	21
2.0–4.0	5	13	10	28
4.0–8.0	12	3	4	19
8.0–12.0	4	1		5
12.0–16.0	1	2		3
16.0–20.0	2			2
Totals	24	38	32	94

Supplementary Material Available. A listing of chemical names and experimental data on endothall derivatives and other chemicals will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Business Office, Books and Journals Division, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for $4.50 for photocopy or $2.50 for microfiche, referring to code number JAFC-75-1113.

LITERATURE CITED

Abeles, F. B., *Physiol. Plant.* **20**, 442 (1967).

Abeles, F. B., "Ethylene in Plant Biology", Academic Press, New York, N.Y., 1973, pp 98–100.

Baur, J. R., Morgan, P. W., *Plant Physiol.* **44**, 831 (1969).

Davis, J. T., Sterrett, J. P., Leather, G. R., *HortScience* **7**(5), 478 (1972).

de Wilde, R. C., *HortScience* **6**(4), 364 (1971).

Frank, J. R., Primary Screening Data, Vegetation Control Division, Ft. Detrick, 1973.

Holm, R. E., Abeles, F. B., *Planta* **78**(3), 293 (1969).

Morgan, P. W., Hall, W. C., *Physiol. Plant.* **15**, 420 (1962).

Webb, D. T., Leather, G. R., *Proc. Northeast. Weed Sci. Soc.* **27**, 142 (1973).

Received for review February 19, 1975. Accepted July 18, 1975.