An Explanation for the Synergistic Interaction of Endothall and Ethephon on Foliar Abscission

J. P. Sterrett, G. R. Leather and W. E. Tozer

Vegetation Control Division, Fort Detrick, Frederick, Maryland

Abstract. To explain the synergistic interaction on abscission between 7-oxabicyclo(2,2,1)heptane-2,3-dicarboxylic acid (endothall) and (2-chloroethyl)phosphonic acid (ethephon), absorption and translocation of foliarily-applied 14C-ethephon alone or together with endothall were determined with (Phaseolus vulgaris L. cv. Red Kidney) plants using liquid scintillation, autoradiography, and gas chromatography. More 14C-ethephon remained in the treated leaf when applied in combination with endothall than when applied alone. These results suggest that the synergistic interaction between endothall and ethylene can be attributed to transport inhibition thereby increasing the availability of ethylene for the abscission process.

Combinations of endothall and ethylene synergistically accelerated foliar abscission of bean and several woody species (5, 12). Endothall applied up to 40 µg/ml or ethylene applied as high as 2,400 µg/ml induced little or no abscission of bean primary leaves but combined applications resulted in 100 percent abscission in 72 hr (5). Endothall is not phloem-mobile and reduces baxipetal transport of labeled assimilates and several growth regulators (7). Ethephon, which generates ethylene at cytoplasmic pH (9, 13), is transported in the phloem in a source-to-sink manner (14). To explain the synergistic interaction on abscission of endothall and ethylene, absorption, transport, and ethylene generation of foliar-applied 14C-ethephon alone were compared with foliar applications of 14C-ethephon combined with endothall.

Materials and Methods

Propagation. ‘Red Kidney’ bean seeds sown in 10-cm pots filled with 2:1:1 loam:sand:peat moss (v/v/v) were germinated in the greenhouse, then transferred to a growth chamber at 23 ± 2°C, 68 ± 10% relative humidity, and 12,000 lux light intensity with a 16-hr photoperiod. Illumination was supplied by a mixture of fluorescent and incandescent lights. The 10-day-old plants were transferred to a hood in the radiological laboratory 1 day before treatment. The hood was illuminated by fluorescent lights at 3,200 lux and temperature was maintained at ca 25°C. Plants were arranged in randomized blocks.

Absorption. Technical grade endothall acid and 14C-ethephon (specific activity 4.1 mc/mmol) were applied to the primary leaves with 1.0% Tween 20 and water. Fifty-µl quantities of the following chemicals and concentrations were spread uniformly over an area 3-cm in diameter around the same secondary vein of both primary leaves: 14C-ethephon (120 µg), and 14C-ethephon (120 µg) plus endothall (2 µg). Unlabeled ethephon4 was added to 14C-ethephon to increase the concentration. Each treatment contained 0.11 mc 14C-ethephon.

After 5, 30, and 54 hr the treated leaves were excised between the blade and distal abscission zone, washed with 20 ml of 0.5% Tween 20 (pH 4.0) for 1 min and dried at 60°C for 20 hr. Dried samples were ground and sonified at 7 amps for 1 min in 10 ml of distilled water. Both the Tween 20 wash and water extracts of the leaves were quantitated by liquid scintillation. Count rates were corrected for quenching by the channels ratio method and the resultant disintegrations per min (dpm) were converted to µg of ethylene per wash and per leaf. The mean fresh wt and standard deviation of the leaves were 2.4 ± 0.4 g. Each treatment was replicated 6 times and means were compared by the Duncan multiple range test following analyses of variance.

Translocation. Treatments were similar to the absorption experiment except only 1 time period (30 hr) was used and 1 primary leaf per plant was treated. Treated leaves were excised between the leaf blade and distal abscission zone and washed with 20 ml of 0.5% Tween 20 (pH 4.0) for 1 min. Roots were separated from the shoots and washed in water to remove soil. The 3 plant parts were then oven-dried at 60°C for 20 hr, and ground and sonified at 7 amps for 1 min in 0.5% Tween 20 (pH 4.0). Aliquots of the extracts were quantitated by liquid scintillation and the remainder concentrated under vacuum for thin-layer chromatography in the degradation study. Count ratios were corrected for quenching by the channels ratio method, and the resultant dpm’s were converted to µg of ethylene per treated leaf, shoot, and root. The fresh wt means and standard deviations of plant parts were: treated leaf 2.0 ± 0.3 g, shoot 5.4 ± 0.4 g, and roots 2.8 ± 0.4 g. Shoot and root values were combined for statistical analyses. Treatments were replicated 4 times and analyzed with the rank-sum test.

To confirm the results of this experiment, 5 replications of plants treated in a similar manner were freeze-dried under vacuum for 14 days. Shoots and roots of 3 replicates were ground and sonified at 7 amps for 1 min and quantitated by liquid scintillation. The remaining plants were prepared and autoradiographed in duplicate according to techniques described by Crafts and Yamaguchi (4).

Metabolism. Concentrated extracts (100 µl) of the treated leaf, shoot and root from the translocation experiment were streaked on thin-layer chromatographic (TLC) Analtech plates coated with Avicel, 250 µ in thickness, in duplicate and developed for 10-cm in benzene-glacial acetic acid-water (8:3:5, v/v/v, lower layer). Reference extracts were prepared by fortification of control plant extracts with labeled 14C-ethephon and streaked adjacent to unknowns on all plates. One set of chromatograms was autoradiographed for 4 weeks. The absorbent from the other set of developed chromatographic plates was removed in ten 1-cm increments and radioassayed using liquid scintillation.

Ethylene evolution. Ethylene was diluted 1:100 in potassium phosphate buffer, pH 6.5 (0.1 M), the pH of leaf
extracts. A 5-ml aliquot of this solution was pipetted into gas collection bottles. The final amount of ethephon was 84 μMoles per samples.

The bottles remained vented in a constant temperature chamber during the experimental period. At each sampling period the bottles were stoppered with vaccine caps, and after 1 min, 1-ml aliquots of the gas phase were obtained with a syringe and injected into a gas chromatograph having an alumina columns and a flame ionization detector. All samples were compared to a 1 μl/liter standard, and the rates were calculated in μl/liter per hr. The volume of solution in each bottle was maintained at a level of 5 ml using phosphate buffer.

**Results and Discussion**

Significantly more 14C-ethephon was recovered in the extracts of treated primary leaves at all time periods when applied in combination with endothall than when applied alone (Table 1). Six-fold more 14C-ethephon was detected in the shoots and roots at 30 hr when applied separately than in combination (Table 2). Autoradiographs confirmed these results in that 14C-activity was minimally visible in the shoots and roots after 28 days exposure to X-ray film from the combined treatments of 14C-ethephon and endothall (Fig. 1). Conversely, radioactivity was obvious in the shoot and roots when treated with 14C-ethephon alone. The effect of the combination on translocation was also evident in the quantity of 14C activity found in the wash; i.e., less 14C-ethephon was removed from the surface of leaves treated with 14C-ethephon alone than when combined with endothall (Table 1). No attempt was made to account for all of the 14C-ethephon applied since that was not the objective of the study. No doubt, much of the unaccounted-for 14C-activity was released as 14C-ethylene.

No metabolites of 14C-ethephon were evident from the liquid scintillation assay (Table 3) or the autoradiographs. These results do not show conclusively that no metabolites are produced by the bean plants, since the spots were not sharply defined, but they do indicate that a high percentage of the 14C activity detected in the bean plant was intact ethephon. Several other workers who radioassayed 14C-ethephon with thin-layer chromatography from grape, walnut, apple, and cherry extracts were unable to detect metabolites (6, 8, 14), but metabolites were found in peach and rubber trees (1, 3). The condensed shoot extracts from the 14C-ethephon and endothall treatment contained too little radioactivity for accurate detection from 1-cm increments of the chromatogram.

### Table 1. Percent 14C-ethephon recovered for 3 time periods after treatment of bean primary leaves. Treatments consisted of 120 μg of 14C-ethephon applied alone or in combination with 2 μg of endothall in 50 μl of water and 1.0% Tween 20.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% 14C-ethephon recovered/leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wash</td>
</tr>
<tr>
<td>14C-ethephon</td>
<td>38 b^2</td>
</tr>
<tr>
<td>14C-ethephon + endothall</td>
<td>48 a</td>
</tr>
<tr>
<td>14C-ethephon</td>
<td>31 c</td>
</tr>
<tr>
<td>14C-ethephon + endothall</td>
<td>53 a</td>
</tr>
<tr>
<td>14C-ethephon</td>
<td>32 c</td>
</tr>
<tr>
<td>14C-ethephon + endothall</td>
<td>51 a</td>
</tr>
</tbody>
</table>

^2 Mean separation, within columns, by Duncan's multiple range test at the 5% level.

### Table 2. Percent 14C-ethephon recovered 30 hours after treatment of bean primary leaf. Treatments consisted of 120 μg of 14C-ethephon applied alone or in combination with 2 μg of endothall in 50 μl of water and 1.0% Tween 20.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% 14C-ethephon recovered^2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treated leaf</td>
</tr>
<tr>
<td>14C-ethephon</td>
<td>19.0</td>
</tr>
<tr>
<td>14C-ethephon + endothall</td>
<td>26.8</td>
</tr>
</tbody>
</table>

^2 Treatments within plant parts are significantly different at the 1.4% level using the rank-sum test.

^3 Shoot does not include untreated primary leaf.
adsorbent; therefore, thin-layer chromatography was not attempted.

The rate of ethylene evolution from ethephon is shown in Fig. 2. Beginning at the moment active acidity was adjusted to pH 6.5, the ethylene evolved at a rate of 649 µl/liter per hr for each sample containing 84 µMoles, reached a peak of 950 µl/liter per hr after 1 day, then rapidly declined over a 6-day period. Minute quantities of ethylene were still detectable after 20 days. Endothall, when added to the solution, did not affect the rate of ethylene evolution when measured over a 4-day period.

These results provide evidence that the synergistic interaction between endothall and ethephon may be related to a reduction in ethephon transport. Since endothall did not affect the rate of ethylene evolution from ethephon in gas collection bottles in this study or in leaf tissue (5), it seems reasonable to assume that ethylene was evolved from the endothall/ethephon combination after absorption by the leaf. Because greater concentrations of ethephon were trapped in the leaf, a high concentration of ethylene gas should be evolved and available during the time period required for abscission to occur in bean (72 hr). In an unpublished experiment5, ethylene gas, injected into a sealed chamber containing bean plants, enhanced foliar abscission after treatment with endothall.

Consideration was given to the thesis that synergism was due to increased foliar absorption. It was thought that endothall/ethephon caused more leaf tissue damage than ethephon thereby facilitating entry (5). And although there was less radioactivity in both leaf wash and extract from 14C-ethephon applied alone, this thesis was discounted, because 14C-ethephon alone was absorbed and transported more readily than when combined with endothall. The ethephon concentration within the leaf when combined with endothall was higher due to lack of transport than in the ethephon only treatment. Loss of more 14C-ethylene directly from the leaf surface when 14C-ethephon was applied alone versus the combination was ruled out since endothall did not alter the rate of ethylene evolution from ethephon in gas collection bottles or in the study with bean leaf tissue (5).

No doubt, reduced ethephon transport is not the entire explanation for synergism. Auxin synthesis may have been reduced as a result of direct tissue damage, and transport of endogenous auxin could have been affected since translocation of 2,4-D and assimilates is known to be reduced in the presence of endothall (7). This auxin effect would also tend to help tip the auxin-ethylene balance toward ethylene and eventual abscission. It has been proposed that when auxin levels decline and ethylene levels increase, a point is reached where ethylene affects abscission directly (2, 10, 11).

### Literature Cited