

Fig. 3. Effect of 5 concn of GA₃, IAA and kinetin on the total contents of chlorophyll, chlorophyll a and b, and photosynthesis.

^zExtracted from the leaves of 12-day old seedlings.

^yDetermined with pea epicotyls that emerged and had grown for 72 hr in the dark.

epicotyls emerged in the aerated conditions (Table 1). The increased synthesis of chlorophyll, higher rate of photosynthesis and vigorous growth due to kinetin at 0.1 and 0.01 mg/liter and inhibitory effects exhibited at 10 ppm or higher (Fig. 3) indicate that anabolic

metabolism was enhanced by lower concn and inhibited at higher concn (10).

Literature Cited

1. Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts: polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.* 24:1-15.

Ethephon-Endothall as a Chemical Abscissor of Bean Leaves¹

J. T. Davis, J. P. Sterrett and G. R. Leather²

Department of the Army, Fort Detrick, Frederick, Maryland

Abstract. 7-Oxabicyclo (2.2.1) heptane, 2-3-dicarboxylic acid (endothall) alone was not an effective chemical abscissor of bean leaves (*Phaseolus vulgaris* L. cv. Red Kidney) until a concn of 30 to 40 mg/liter was attained, but in the presence of 800 mg/liter (2-chloroethyl)phosphonic acid (ethephon) abscission began at 5 mg/liter endothall. Low pH (1.5) significantly lowered the break strength of abscission zone explants of leaves sprayed with ethephon and endothall compared to pH 6.0, and endogenous ethylene production from endothall alone was higher at pH 1.5 than pH 6.0.

Strong evidence indicates that ethylene is involved in the abscission

process by accelerating senescence through its action on plant cell walls (2, 7). Successful defoliation of plants by ethephon has been reported by several workers (3); however, large quantities and repeated applications are usually required for satisfactory defoliation (10). Low levels of endothall may cause mild leaf injury and induce endogenous ethylene production (7, 8). This study was designed to determine the ratios of ethephon to endothall (acid) which would defoliate bean and the effect of acidity on this combination.

Seeds sown in 10-cm pots filled with soil were germinated in the greenhouse then transferred into a growth chamber at 24±1°C, 65±10% relative humidity and 1200 ft-c of light intensity (16-hr photoperiod). The primary leaves of each plant were sprayed to runoff with a DeVilbiss atomizer (No. 163) at 352 g/cm² (5 psi) when the 2nd trifoliolate leaves were emerging (15 days). It was determined from preliminary experiments that 1.6% poloxyethylene sorbitan monolaurate (hereafter referred to as Tween 20) and 4% glycerin

2. Barton, L., and McNab, J. 1956. Relation of different gases to the soaking injury of seeds. III. *Contr. Boyce Thompson Inst. Pl. Res.* 18:339-356.
3. Bonner, D. M., A. J. Haagensmit, and F. W. Went. 1939. Leaf growth hormones, I. A bioassay and source for leaf growth factors. *Bot. Gaz.* 101:128-144.
4. Grineva, G. M., and Z. S. Burkina. 1966. The effect of anaerobic conditions on absorption and distribution of heavy water (H₂O¹⁸) in maize and sunflower plants (in Russian). *Fiziol. Rastenii.* 13:612-615.
5. Koski, V. M. 1950. Chlorophyll formation in seedlings of *Zea mays* L. *Arch. Biochem. Biophys.* 29:339-343.
6. Kozinka, V. 1970. Inhibition of water uptake by high concentrations of auxin like substances. *Biol. Plant* 12:180-190.
7. Larson, L. A., and Kyogaba Lwanga. 1969. The effect of seed soaking on seedling growth of *Pisum sativum*. *Can. J. Bot.* 47:707-709.
8. MacKinney, G. 1941. Absorption of light by chlorophyll solutions. *J. Biol. Chem.* 140:315-322.
9. Orphanos, P. I., and W. Heydecker. 1968. On the nature of the soaking injury of *Phaseolus vulgaris* seeds. *J. Exp. Bot.* 19:770-784.
10. Overbeek, J. V. 1966. Plant hormones and regulators. Gibberellins, cytokinins, and auxins may regulate plant growth via nucleic acid and enzyme synthesis. *Science* 152:721-731.
11. Stauffer, J. F. 1964. Applications of the differential respirometer, photosynthesis. p. 81-85. *In* W. W. Umbreit, R. H. Burris, and J. F. Stauffer (4th ed.) *Manometric techniques*. Burgess Publ. Co., Minneapolis, Minn.

enhanced the abscission response; therefore, they were included in each treatment. The study was divided into 2 experiments.

Dose response expt. Due to limited space in the growth chambers the dose response experiment was divided into 4 sub-experiments (A to D) which were conducted over a 4-week period. Several of the treatments were used in all 4 sub-experiments to provide a common basis of comparison. One bean plant with 2 primary leaves was considered a treatment and each treatment was replicated 10 times in a randomized block design within each sub-experiment. Treatments consisted of 0, 800, 2,400, and 4,000 mg/liter ethephon (Amchem 68-240, propylene glycol base) combined with 11 concn of technical endothall (acid) ranging from 0 to 80 mg/liter. The pH of all treatments was adjusted to 1.5 with 0.1 N HCl. Germination of sub-experiment B occurred under cloudy weather conditions which caused slightly taller plants at the time of treatment. This resulted in higher mean defoliation and greater variability among replication; therefore, only sub-experiments A, C, and D were pooled for analysis. Defoliation was determined as the % primary leaves which dropped naturally. Each sub-experiment was terminated after 72 hr since virtually all abscission occurred before this time. Desiccation was determined visually at 24 hr by

¹Received for publication April 10, 1972.

²Horticulturist and Plant Physiologists respectively. Senior author is now located at Cave Spring, Georgia.

The authors gratefully acknowledge the statistical assistance of Dr. Walter D. Foster and Mrs. Marian W. Jones. Thanks is extended to Dr. F. B. Abeles for advice. Appreciation is expressed for the technical endothall acid donated by the Pennwalt Corporation and Ethrel (Amchem 68-240) furnished by Amchem Products, Inc.

estimating % desiccated tissue on both primary leaves compared with leaves of untreated plants. Statistical analysis consisted first of selecting an appropriate dose-response model by which to relate % defoliation (or % desiccation) to level of endothall (9). A modified logistic function fitted by least squares proved satisfactory. The curves for different levels of ethephon were compared statistically.

Figure 1 shows the 3-day defoliation response of bean plants treated with combinations of ethephon and endothall. Endothall alone did not become effective until a concn of 30 to 40 mg/liter was reached; in the presence of 800 mg/liter ethephon, abscission began at a much lower concn of endothall (5 mg/liter). When 2,400 or 4,000 mg/liter ethephon was applied without endothall, some defoliation was noted. However, with the addition of small amounts of endothall, defoliation increased markedly. Mixtures containing 30 mg/liter of endothall and 2,400 or 4,000 mg/liter of ethephon gave 90 to 100% defoliation. The response functions describing defoliation of these 2 higher levels of ethephon could not be distinguished from each other statistically.

The desiccating effect of endothall was enhanced by the addition of ethephon since the curves which represent 800, 2,400 or 4,000 mg/liter ethephon are significantly different from the curve without ethephon (Fig. 2). Desiccation of the leaves treated with endothall alone at 40 mg/liter or less was low (under 10%, Fig. 2); therefore, this level of endothall may be considered a sublethal dose since the leaves were only slightly damaged. This same dose of endothall (40 mg/liter) yielded approx 40% defoliation, in combination with 800 mg/liter ethephon the defoliation was nearly 80%, and with an ethephon concn of 2,400 mg/liter or more the defoliation was over 95% (Fig. 1).

Although not shown in Fig. 1 and 2, leaves treated with zero ethephon and endothall at pH 1.5 were 8% desiccated and did not abscise.

Acidity expt. To determine the effect of acidity (pH 1.0, 1.5, 3.0, and 6.0) on ethephon and endothall, one combination with glycerin and Tween 20 was sprayed on bean plants in a manner similar to the dose response experiment. The pH was adjusted with 0.1 N HCl or 0.1 N KOH. Before adjustment, the pH of the spray solutions containing endothall alone (30 mg/liter) was 4.0 ± 0.1 and ethephon (1,000 mg/liter) was 2.0 ± 0.1 . Break strength of distal explant abscission zones from 10 leaves was measured after 72 hr using the abscissor described by Craker and Abeles (5). Ethylene production was determined for pH 1.5

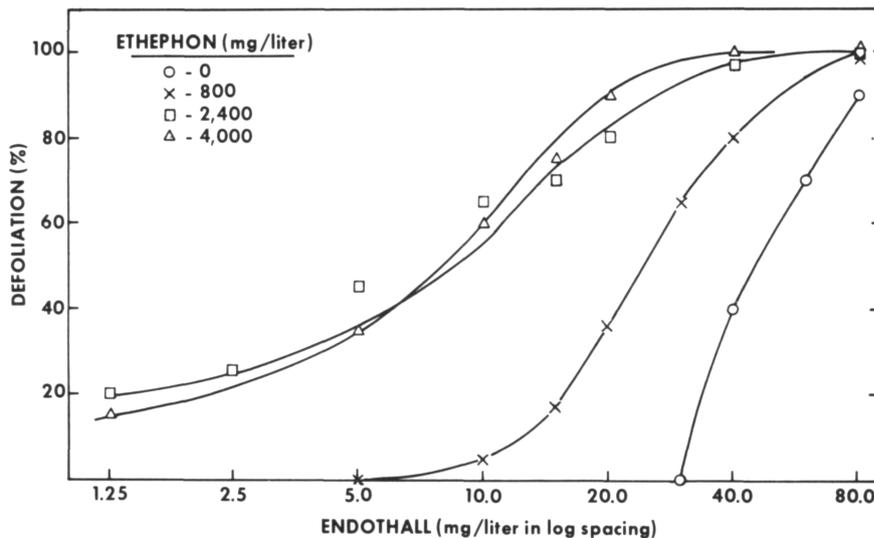


Fig. 1. Defoliation dose-response curves for combinations of endothall and ethephon on bean after 72 hr. All curves are significantly different at the 5% level using the rank sum test except those representing 2,400 and 4,000 mg/liter ethephon.

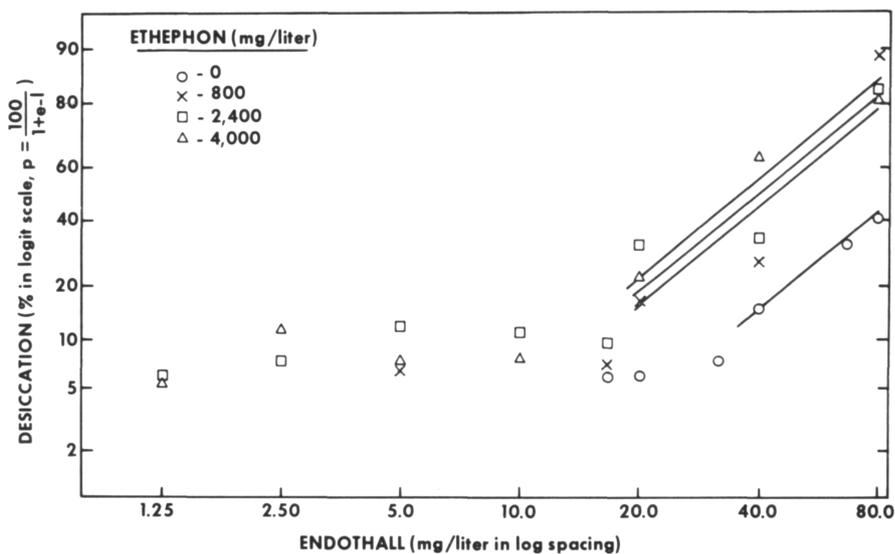


Fig. 2. Desiccation dose-response curves for combinations of endothall and ethephon on bean after 24 hr. The curves were truncated near endothall levels of 20.0 mg/liter because of an apparent residual level of desiccation which was independent of dose. The curves which represent 800, 2,400 or 4,000 mg/liter ethephon are significantly different from the curve without ethephon at the 5% level using the rank sum test but are not different from each other. The logistic model was fitted under the restraint of parallelism for all 4 ethephon levels.

and 6.0 by placing 2 leaf discs (16 mm diam) from a primary leaf selected at random into a gas collection bottle fitted with a vaccine cap. Measurements (nl/liter) at 3 hr were made by sampling 2 ml of the gas phase on a gas chromatograph as described by Abeles and Rubinstein (1). Ethephon degradation at 72 hr was determined for pH 1.5 and pH 6.0 in a similar manner. Ethylene determinations were replicated 3 times. Both experiments were repeated.

Break strength of explant abscission zones was influenced greatly by pH when treated with the combination of ethephon and endothall (Table 1). This effect of acidity on abscission was also observed by Barmore and Biggs (4) who demonstrated that some mineral and

organic acids accelerated abscission on citrus plants. The production of ethylene from the application of endothall alone was significantly higher at pH 1.5 than 6.0; however, when ethephon and endothall were applied in combination, the production of ethylene was not significantly different from ethephon applied alone (Table 1). Apparently, the small amount of ethylene production stimulated by endothall was masked by the large quantity of ethylene evolved from ethephon. The degradation of ethephon was still occurring on leaf discs after 72 hr at both pH 1.5 and 6.0, and the ethylene evolved was higher at pH 6.0 than pH 1.5.

Ethephon not only provided a large source of ethylene gas in these studies

Table 1. Effect of acidity with ethephon and endothall on break strength of explants after 72 hr and ethylene production of bean leaves 3 hr after treatment².

pH	Ethephon (mg/liter)	Endothall (mg/liter)	Break strength ^Y (g)	Ethylene ^Y (nl/liter/3 hr)
Control	Untreated		325c	9a
1.0		30	327c	
1.5		30	328c	165c
3.0		30	315c	
6.0		30	303c	44b
1.0	1,000		324c	
1.5	1,000		327c	1,333d
3.0	1,000		332c	
6.0	1,000		321c	1,298d
1.0	1,000	30	57a	
1.5	1,000	30	135b	1,651d
3.0	1,000	30	161b	
6.0	1,000	30	264c	2,005d

²All treatments included 4% glycerin and 1.6% Tween 20 except control.

^YValues in a vertical column not followed by the same letter are significantly different at the 5% level using Duncan's Multiple Range test for break strength and rank sum test for ethylene production.

but added to the desiccation caused by endothall, possibly, by lowering the pH of the combination which enhanced the abscission process. The damaged cuticle was most likely more permeable which facilitated the entry of both endothall and intact ethephon (6).

Glycerin, which was added to the spray solution for the purpose of reducing droplet evaporation, appeared to enhance wilting of the leaves; however, it did not promote abscission when applied without ethephon and endothall.

The ethephon-endothall combination has many of the characteristics of a rapid acting, nonherbicide chemical abscissor. Sublethal concn caused abscission in 3 days which is extremely fast, and except for some temporary growth suppression, little damage to the plant was observed after leaf drop. To provide minimal ecological disturbance it is desirable that a chemical abscissor be nonherbicide to a broad spectrum of plants. Based on these results, studies are in progress with woody plants to determine nondamaging ratios of

ethephon to endothall as related to species sensitivity and stage of plant development.

Literature Cited

1. Abeles, F. B., and B. Rubinstein. 1964. Regulation of ethylene evolution and leaf abscission by auxin. *Plant Physiol.* 39:963-969.
2. ———, and G. R. Leather. 1971. Abscission: Control of cellulase secretion by ethylene. *Planta* (Berl.) 97:87-91.
3. American Society for Horticultural Science. Ethylene, Fruit Abscission Symposium, *HortScience* 6:353-392.
4. Barmore, C. R., and R. H. Biggs. 1970. Salt and acid effects on citrus leaf abscission. *J. Amer. Soc. Hort. Sci.* 95:211-213.
5. Craker, L. E., and F. B. Abeles. 1969. Quantitative measurement with a recording abscissor. *Plant Physiol.* 44:1139-1143.
6. Foy, C. L. 1964. Foliar penetration. *Agr. and Food Chem.* 12:473-476.
7. Hall, Wayne C. 1952. Evidence on the auxin-ethylene balance hypothesis of foliar abscission. *Bot. Gaz.* 113:310-321.
8. Jackson, J. M. 1952. Physiology of leaf abscission. *Ark. Acad. Sci.* 5:73-76.
9. Kempthorne, O., T. A. Bancroft, J. W. Gowen, and J. L. Lush. 1954. Statistics and mathematics and biology. Iowa State Col. Press, Ames, Iowa. 632 p.
10. Sterrett, J. P., J. A. Baden, III, and J. T. Davis. 1971. Defoliation of oak, maple and other woody plants with 2-chloroethylphosphonic acid and potassium iodide. *Proc. 25th Northeastern Weed Sci. Soc.* p. 376 (Abstr.)

Influence of Brassins on the Growth of Woody Plants¹

J. F. Worley and D. T. Krizek²

U. S. Department of Agriculture, Beltsville, Maryland

Abstract. A single application of brassins isolated from rape (*Brassica napus* L.) pollen and placed on a 1 – 3.5 mm internode of young paper birch (*Betula papyrifera* Marsh.), Siberian elm (*Ulmus pumila* L.), and tea crabapple (*Malus hupehensis* (Pamp.) Rehd.) seedlings greatly accelerated elongation of the treated internodes.

Previous reports on the growth regulating activity of brassins have been limited largely to the bean second internode assay (1, 2, 4, 5). This report describes the response of 3 woody species, paper birch, Siberian elm, and tea crabapple to a single application of

brassins to the internodes of the young seedlings.

Seed were germinated in a sterile peat-vermiculite mixture or in sterilized composted soil in the greenhouse. Long days (15 hr) were provided by use of natural daylight supplemented with light from 1500 ma cool white fluorescent lamps. When the seedlings had produced their first true leaves they were transplanted to 7.5 cm pots and subjected to the 15 hr days with min night temp of 18°C. One to 4 weeks later, depending on the species, the most uniform seedlings were selected and comparable sets were treated either with brassins in a fractionated lanolin carrier or with the carrier alone (3). The procedures for obtaining purified extracts of brassins from rape pollen have been described previously (4). A mixture of 50 µg of brassins in 250 µg of fractionated lanolin was applied

quantitatively and unilaterally to the shortest internode of the plant that was at least 1 mm long (2nd internode for elm and 2nd, 3rd or 4th for birch and crabapple) as described for the bean second internode bioassay (3). The preparation was spread evenly along the internode with the aid of a microscope. Length of the internode (from stipule to stipule) was recorded with an ocular micrometer. The treated internode was kept in the path of the microscope light until the carrier had melted and penetrated between the epidermal hairs thereby making contact with the stem. Measurements of internode length and overall height were taken initially and 4-7 days after treatment. The experiments were repeated at least 3 times for each species.

Brassin treatment accelerated the growth of all 3 woody species tested (Fig. 1A,B,C). Increase in the overall height of the plants was due mostly to elongation of the treated internode but the internode above was stimulated in some cases. The internode below the treated internode showed little or no increase in elongation over the control plants, suggesting that the influence of brassins on differentiated tissue and/or transport of brassins in these woody plants was limited. In all 3 species,

¹Received for publication August 4, 1972.

²We wish to thank Dr. Richard H. Zimmerman for supplying the crabapple seed, Dr. Frank S. Santamour, Jr., for the elm seed and Mr. Thomas S. Pinney, Jr., for the birch seed.