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Received for review December 8, 1964. Accepted May 17, 1965. Paper of Journal Series, New Jersey Agricultural Experiment Station.

TERMINAL GROWTH CONTROL

Apical Dominance in Bean Plants Controlled with Phthalamic Acids

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Nine new phthalamic acids were found to be active growth regulators when applied to stems of young bean plants, six approximating the activity of the known regulator *N*-(1-naphthyl)phthalamic acid when applied at the rate of 125 μg. per plant. These compounds inhibited terminal growth and accelerated growth of lateral buds. Applied to the stem, *N*-(3-nitro-1-naphthyl)phthalamic and *N*-(5-acenaphthenyl)phthalamic acids caused an abscission zone to develop in the second internode and abscission of the terminal bud. A fraction of 1 μg. of the nitro-substituted acid induced abscission when applied to that area of stem capable of developing an abscission zone. Although very effective when applied to stems or roots, this acid did not affect terminal growth when applied to leaves. *N*-(5-Acenaphthenyl)phthalamic acid increased growth of lateral buds and decreased the amount of fruit that developed. Some of the phthalamates were apparently inactivated rapidly by the plant.

Some growth-regulating compounds induce abscission when used to thin flowers and fruits and to defoliate plants. Others retard abscission of some kinds of fruits and leaves when used to prevent preharvest drop and to improve the appearance of decorative plants (*1*, *4*). There is need, however, to control other kinds of abscission. For example, control of the abscission that results in seed shatter would reduce losses in such crops as carrots and lettuce. A means of defoliating tomato and grape would facilitate development of machines to harvest these crops.

In an earlier study of abscission, 30 compounds, some with widely different structures, retarded terminal growth and induced formation of an abscission zone in the stem near the terminal bud when applied to stems of young bean plants. Abscission of the terminal buds usually followed, thus inducing growth of lateral buds (*2*).

The purpose here is to describe the effects of some phthalamic compounds on apical dominance and terminal bud abscission. The practical significance of phthalamic compounds when used to induce the responses described is not implied, however, since little is known regarding their toxicity.

Experimental

Sixty-four phthalamic acids were selected to determine their effects on apical dominance. Each compound was mixed separately with melted lanolin and the mixture cooled. Equimolar concentrations were not used in most cases, since differences in weight were relatively slight and marked plant responses were involved. However, in comparing the activity of *N*-(3-nitro-1-naphthyl)-phthalamic acid, the most effective compound in terms of abscission with

the known regulator *N*-(1-naphthyl)-phthalamic acid, equimolar concentrations of these substances were employed. It was necessary to use the sodium salts of both acids, since *N*-(3-nitro-1-naphthyl)phthalamic acid is relatively insoluble in water. The required weight of the salt was dissolved in a volume of distilled water which could be mixed uniformly with a measured amount of lanolin to form a smooth paste. Different dosage levels were obtained by varying the amount of lanolin in the mixtures. Thirteen milligrams of the mixture were applied to the test plant with a microinjector (*3*).

Bean plants (Pinto variety) used in most of these experiments had developed primary leaves 3 to 4 cm. wide and trifoliate leaves that were still folded in the terminal buds. In some experiments, plants in four different stages of development were used, those emerging from the soil representing the earliest stage and others with the first trifoliate leaf unfolded from the terminal bud representing the latest stage.

A portion of the lanolin paste about one half the size of a wheat seed and containing approximately 125 μg. of the chemical was placed around the stem of the test plant as a band a few millimeters wide and midway between the first and second nodes. In experiments involving plants at different stages of development, 65 μg. of the chemical were applied in lanolin paste to each plant as a band a few millimeters wide around the hypocotyl 1 cm. below the cotyledons. Very small amounts of the test chemical were applied unilaterally in lanolin to partially elongated second internodes to determine sensitivity based on terminal bud abscission (*5*).

In other experiments, primary leaves of the test plants were treated by spreading evenly approximately 125 μg. of the chemical in the lanolin paste over 1 sq. cm. of the upper surface near the petiole attachment.

In studying the effect of root application, the sodium salt of *N*-(3-nitro-1-naphthyl)phthalamate was dissolved in the required amount of water and then applied so as to wet thoroughly composted soil in which the plants were growing.

Results

Of the new compounds tested, nine were active. These compounds induced to varying degrees the following responses when applied to stems: (1) growth suppression of the trifoliate leaf in the terminal bud and epinasty of the primary leaves; (2) formation of an abscission zone (Figure 1) and abscission of the terminal bud with accelerated lateral bud growth due to loss of apical dominance; (3) sometimes death of the terminal bud (without abscission) and suppression of lateral bud growth when relatively large amounts of the compound were used.

The active phthalamates were compared on the basis of their effect in retarding terminal growth, a response usually associated with growth of lateral buds, and their effectiveness in inducing terminal bud abscission.

The six most active phthalamates retarded terminal growth of young bean plants to a marked degree when applied at the rate of 125 μg. per plant. The magnitude of the response induced by these substances at this dosage approximated that induced by the regulator *N*-(1-naphthyl)phthalamic acid which was used for comparison (Table I).

In addition to suppressing terminal bud growth when the compound was applied to first internodes, *N*-(3-nitro-1-naphthyl)phthalamic acid and *N*-(5-acenaphthenyl)phthalamic acid caused an abscission zone to develop in the second internodes resulting in abscission of terminal buds. The nitro-substituted compound was the more effective.

Effectiveness of the phthalamates in inducing abscission did not parallel their effectiveness in inhibiting terminal bud growth. For example, sodium *N*-(1-naphthyl)tetrachlorophthalamate very effectively inhibited terminal bud growth when compared with sodium *N*-(3-nitro-1-naphthyl)phthalamate at various dosage levels (Table II), but the nitro-substituted phthalamate was the more effective in causing abscission of terminal buds.

The effectiveness of *N*-(3-nitro-1-naphthyl)phthalamic acid in inducing terminal bud abscission was greatly increased by applying the compound directly to the second internode where the abscission zone developed rather than to the first internode some distance from the responsive area. Applied in this way, for example, 0.03 μg. of the regulator induced terminal bud abscission in each of six replications and 0.015 μg. caused abscission in all but one of six replications.

Loss of apical dominance and accelerated growth of lateral buds was demonstrated by applying *N*-(5-acenaphthenyl)-phthalamic acid to stems of test plants. The above-ground parts of plants treated with this chemical (exclusive of fruits) weighed approximately 60% more within 1 month than did comparable untreated ones. This difference was due mainly to accelerated lateral bud growth at the expense of fruit development. An even greater increase in vegetative growth (108%) resulted when treatment of the stem was repeated at the end of 4 days following the initial treatment. A single delayed application was, however, ineffective (Table III).

Response to phthalamates applied to leaves differed greatly from responses to stem applications. For example, when *N*-(3-nitro-1-naphthyl)phthalamic acid was applied to the primary leaves of the test plant, there was no apparent response

Table I. Regulating Activity of Phthalamic Compounds

Compared on basis of retarding effect on growth of terminal buds when applied to first internodes

Compound	Relative Degree of Inhibition[a]
N-(1-Naphthyl)phthalamic acid[b]	+++
N-(2-Nitro-1-naphthyl)phthalamic acid	+++
N-(3-Nitro-1-naphthyl)phthalamic acid[c]	+++
Sodium-*N*-(3-nitro-1-naphthyl)-phthalamate	+++
N-(4-Nitro-1-naphthyl)phthalamic acid	+++
N-(4-Isoquinolyl)phthalamic acid	+++
N-(5-Acenaphthenyl)phthalamic acid[c]	+++
N-(4-Nitro-2-naphthyl)phthalamic acid	++
N-(7-Nitro-1-naphthyl)phthalamic acid	++
N-(8-Nitro-2-naphthyl)phthalamic acid	+

[a] + Slight; ++ moderate; +++ marked.
[b] Included for comparison.
[c] Induced abscission of terminal buds.

Table II. Regulating Activity of Sodium *N*-(3-Nitro-1-naphthyl)-phthalamate and Sodium *N*-(1-Naphthyl)phthalamate on Basis of Terminal Bud Inhibition

Dosage Level, μg.	Magnitude of Response: Sodium N-(3-nitro-1-naphthyl)-phthalamate	Magnitude of Response: Sodium N-(1-naphthyl)-phthalamate
65	Moderate	Marked
32.5	Slight	Marked
16.3	Slight	Marked
8.2	None	Marked

Table III. Effect of *N*-(5-Acenaphthenyl)phthalamic Acid on Vegetative Growth and Pod Development

Average per plant (10 replications) 1 month after application of chemical

Treatment Date	No. of Axillary Buds[a]: Cotyledonary	No. of Axillary Buds[a]: Primary	Weight of Above-Ground Vegetable Growth, G.	Weight of Pods, G.
None	0	1.9	10.9	17.1
March 18	0	2.6	17.5	8.6
March 18 and 22	1.4	2.8	22.7	7.4
March 22	1.8	2.0	10.7	18.0

[a] Developing as branches.

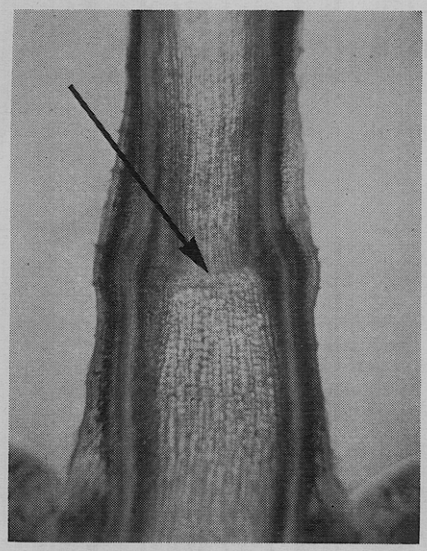

Figure 1. Effect of sodium 3-nitro-1-naphthyl carbamate

1.4 μg. applied to first internode of young bean plant. Abscission zone (arrow) formed in second internode below terminal bud during following week and internode eventually separated at abscission zone

in the terminal bud and no abscission occurred. Applied in this way, 125 μg. of the compound induced moderate epinasty within 24 hours and had no other apparent effect. This indicated that the compound apparently was not translocated from the leaves downward into the stem in an active form. On the other hand, when an equal amount of the chemical was applied to the stem, an epinastic response resulted indicating that the regulator was absorbed and translocated upward into the primary leaves.

The epinastic response in primary leaves was temporary, regardless of whether the compound was applied to the leaves or stems, and the persistence of the response varied depending on the chemical used. For example, primary leaves of the test plants developed epinasty within 24 hours after *N*-(3-nitro-1-naphthyl)phthalamic acid was applied to the stems. This response persisted for 3 or 4 days. In contrast, *N*-(1-naphthyl)phthalamic acid induced similar epinasty which persisted for approximately 21 days (Table IV).

Terminal growth was inhibited and growth of lateral buds was accelerated when sodium *N*-(3-nitro-1-naphthyl)-phthalamate was applied to roots. For example, terminal growth was inhibited 19, 38, and 41%, respectively, when 1, 1.5, and 3 mg. of the compound were used. Growth of lateral branches was 10, 8.5, and 7.5 times that of the untreated plants when the same amounts of the compound were used.

The degree to which bean plants responded to sodium 3-nitro-1-naphthyl-phthalamate and sodium *N*-(1-naphthyl)-tetrachlorophthalamate depended upon the stage of development of the plant at the time the chemical was applied. For example, response to both salts was very pronounced when 125 μg. of either were applied as the plants emerged from the soil and the resulting epinasty was persistent. The magnitude of the response decreased when plants at later stages of development were treated and the epinastic response was temporary.

Consider briefly the molecular structure of the naphthylphthalamic compounds: substitution of a nitro group at different positions on the naphthalene ring demonstrated that the 2, 3, and 4 positions resulted in compounds that were most active. Substitution of nitrogen in place of the nitro group in the 3 position of the naphthalene ring also resulted in an active compound—*N*-(4-isoquinolyl)phthalamic acid—under the conditions used. The activity of the nitro-substituted phthalamic acids was reduced when the phthalamic side chain was shifted from the 1 to the 2 position of the naphthalene ring [*N*-(3-nitro-2-naphthyl)phthalamic acid and 4-nitro-2-naphthylphthalamic acid]. 3-Nitro-1-naphthaleneacetic and 3-nitro-1-naphthoxyacetic acids were inactive.

Table IV. Inactivation of *N*-(3-Nitro-1-naphthyl)phthalamic Acid and *N*-(1-Naphthyl)phthalamic Acid

Based on persistence of epinasty in primary leaves after treatment of stems

Compound	Dosage Level, μg.	Days Following Application of Chemical[a] 1	2	3	4	5	6	7	21
N-(3-Nitro-1-naphthyl)-phthalamic acid	130	+++	+++	++	+	0	0	0	0
	65	+++	+++	++	0	0	0	0	0
	32.5	++	+++	+	0	0	0	0	0
N-(1-Naphthyl)phthalamic acid	130	+++	+++	+++	+++	+++	+++	++	+
	65	+++	+++	+++	++	++	++	+	0
	32.5	+++	+++	++	+	+	+	0	0
No treatment	0	0	0	0	0	0	0	0	0

[a] 0 None; + slight; ++ moderate; +++ marked.

Table V. Phthalamic Acids and Related Compounds Inactive When Applied to Stems of Bean Plants

3-Acetoxy-2-naphthoic acid
5,6-Benzsulfonfluorescein
5,6-Benzsulfonphthalein
1-(5′,8′-Dichloro-2′-naphthylazo)-5,8-dichloro-2-naphthol
1-(5′,8′-Dichloro-2′-naphthylazo)-2-hydroxy-3-naphthoic acid
1-(5′,8′-Dichloro-2′-naphthyl)-3,3-dimethylurea
2′,4′-Dichlorophenyl 3-hydroxy-2-naphthoate
6,7-Dinitro-1,4-naphthoquinone-1-oxime
Ethyl 8-nitro-2-naphthyl carbamate
3,4,5,6,7,8-Hexachloro-2-naphthoxyacetic acid
3-Hydroxy-2-naphthanilide
3-Hydroxy-2-naphthoic acid
3-Hydroxy-2-naphthohydrazide
3-Methoxy-1-naphthaleneacetic acid
3-Methoxy-2-naphthoic acid
Methyl 3-acetamido-1-naphthaleneacetate
Methyl 5,8-dichloro-2-naphthoxyacetate
Methyl 1-(5′,8′-dichloro-2′-naphthylazo)-hydroxy-3-naphthoate
Methyl 3-hydroxy-2-naphthoate
Methyl 3-methoxy-2-naphthoate
Methyl 3-nitro-1-naphthaleneacetate
Methyl 3-nitro-1-naphthoxyacetate
Methyl 3-nitro-2-naphthoxyacetate
N-(5,8-Dichloro-2-naphthyl)phthalamic acid
N-(3,4,5,6,7,8-Hexachloro-2-naphthyl)-phthalimide
N-(3-Nitro-1-naphthyl) amide-acids from pyromellitic dianhydride
N-(3-Nitro-1-naphthyl) amide-acids from trimellitic anhydride
N-(3-Nitro-1-naphthyl) benzamide
N-(8-Nitro-2-naphthyl) glycine hydrochloride
N-(6-Nitro-2-naphthyl) maleamic acid
1,2-Naphthoquinone-2-oxime
1,2-Naphthoquinone-2-oxime dimethylcarbamate
1,2-Naphthoquinone-2-oxime tosylate
N-(2-Naphthyl)phthalamic acid
N-(1-Naphthyl)tetrahydrophthalamic acid
3-Nitro-1-naphthaleneacetic acid
N-(3-Nitro-1-naphthyl)maleamic acid
3-Nitro-1-naphthoxyacetic acid
3-Nitro-1-naphthyl nitromethane
N-(1-Nitro-2-naphthyl)phthalamic acid
N-(3-Nitro-2-naphthyl)phthalamic acid
N-(5-Nitro-1-naphthyl)phthalamic acid
N-(5-Nitro-2-naphthyl)phthalamic acid
N-(6-Nitro-1-naphthyl)phthalamic acid
N-(6-Nitro-2-naphthyl)phthalamic acid
N-(7-Nitro-2-naphthyl)phthalamic acid
N-(8-Nitro-1-naphthyl)phthalamic acid
N-(3-Nitro-1-naphthyl) phthalimide
N-(3-Nitro-1-naphthyl)tetrahydrophthalamic acid
N-(3-Nitro-1-phenyl)phthalamic acid
N-(3-Nitro-5,6,7,8-tetrahydro-1-naphthyl)phthalamic acid
1-Phenylazo-3-hydroxy-2-naphthanilide
N-(3-Pyridyl)phthalamic acid
Sodium *N*-(1-naphthyl)tetrachlorophthalamate
2,4,6,7-Tetranitro-1-naphthol

Other inactive phthalamic acids and related compounds are listed in Table V.

Summary

Bud growth was selectively controlled with *N*-(3-nitro-1-naphthyl)phthalamic acid and *N*-(5-acenaphthenyl)phthalamic acid. Applied to the stems, these compounds were apparently absorbed and

translocated into the terminal buds and subtending internodes in amounts that inhibited terminal bud growth and induced internodal abscission. During the development of these responses, the lateral buds apparently did not absorb sufficient *N*-(3-nitro-1-naphthyl)phthalamic and *N*-(5-acenaphthenyl)phthalamic acids at the dosage levels used to inhibit their subsequent growth. It is probable that any residual amounts of these compounds became inactive during this period as far as growth inhibition was concerned, since there was no evidence of inhibitory or formative effects on axillary buds that developed subsequently.

Acknowledgment

The authors appreciate the cooperation of the Fundamental Research Co., Berkeley, Calif., which supplied the compounds.

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Received for review October 12, 1964. Accepted January 29, 1965.

DEFOLIANT RESIDUES

Determination of Cyanamide Residues on Ginned Cottonseed

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A simple and sensitive colorimetric method for the determination of cyanamide residues on the surface of cottonseed has been developed. Residues are extracted from the seed with water and the extracts treated with activated charcoal to remove interfering substances. A red complex is formed by addition of sodium pentacyanoammine ferroate and measured at 530 mμ. Recoveries have averaged approximately 85% over the range from 0.03 to 0.20 p.p.m. The method is capable of detecting net cyanamide residues of 0.03 p.p.m. on cottonseed.

Calcium cyanamide and, more recently, hydrogen cyanamide have been used for the defoliation of cotton plants to facilitate harvesting of the bolls. Work with C^{14}-labeled cyanamide by Miller and Hall (*3*) has shown that under the conditions of their experiments cyanamide was rapidly metabolized by cotton plants and that the parent compound was undetectable in the plants as little as 8 hours after topical application to the leaves. Consequently, no cyanamide was found in the seeds of these plants. Therefore, the work reported here was directed toward the determination of surface residues which might result from late application or from accidental contamination during harvest.

Early attempts to apply the colorimetric procedure of Buyske and Downing (*2*) directly to aqueous extracts of cottonseed were unsuccessful because of the presence of sample extractives which caused high control values for untreated samples.

Attempts to remove the interfering extractives by partition against various organic solvents, ranging in polarity from 1-butanol to chloroform, were unsuccessful. Of a number of adsorbents evaluated for column cleanup of the extracts, only alumina and acid-washed alumina removed appreciable quantities of the interfering extractives. However, flow rates through these columns were very slow unless they were operated under pressure, in which case turbid effluents were obtained.

Treatment of the effluents with activated charcoal followed by filtration through a bed of diatomaceous earth proved satisfactory for the elimination of the turbidity. Further work with these adsorbents showed that the charcoal–diatomaceous earth treatment by itself produced solutions which were satisfactory for use with the colorimetric procedure.

The chromogenic reaction proved to be extremely sensitive to light in the presence of cottonseed extractives remaining after the charcoal treatment, and it was necessary to carry out the reaction in the dark in order to minimize color loss. Although the time for maximum color development in the absence of cottonseed extractives was about 40 minutes, in the presence of such materials the color tended to fade rapidly and the optimal color-development period was about 10 minutes. Under these conditions the color responses of known quantities of cyanamide added to processed control extracts just prior to the color development step were approximately 92% of those obtained for the standard curve prepared as described below. The standard curve obeyed Beer's law from 4 to 40 μg. of cyanamide per 21 ml. with a slope of 16.0 (absorbance *vs.* milligrams of cyanamide).

Method

Reagents. Water, extraction, pH 5. Adjust CO_2-free distilled water to pH 5 with 0.5*N* hydrochloric acid.

Decolorizing charcoal, Darco S-51, Atlas Powder Co.

Diatomaceous earth, Hyflo Super-Cel, Johns-Manville.

Buffer solution, pH 10.4. Mix 3 parts of 0.1*N* sodium carbonate solution with 1 part of 0.1*N* sodium bicarbonate solution.

Cyanamide Standard Solution. Dilute 60% hydrogen cyanamide solution (American Cyanamid Co., P. O. Box 400, Princeton, N. J.) with distilled CO_2-free water to contain 4 μg. of cyanamide per ml. Adjust to pH 5 with 0.5*N* hydrochloric acid. Store in a refrigerator; prepare fresh solution every 2 days.

Color Reagent. Prepare a 4% solution of trisodium pentacyanoammine ferroate, $Na_3[Fe(CN)_5NH_3]$ (K and K Laboratories, Inc., Jamaica 33, N. Y.), in distilled water. Filter the solution through a folded Whatman No. 12 filter