Irregular variations between the *normal* and *iso* forms of the three higher acids are probably due to the fact that both boiling points and solubility in water vary irregularly between the isomers. Mixtures of a higher and a lower acid are detected by the fact that the rate of distillation drops off in the last fractions since the higher acid is practically removed in the first three fractions. Mixtures of formic, acetic and valeric acids, however, can simulate acetic acid as shown by the Viburnum bark distillations given in Tables II and III. Reduction tests indicating formic acid are readily obtained in the Viburnum distillates as well as the odor of the higher acids. The largest amount of acid present seems to be acetic acid.

The *Viburnum prunifolium* barks given in Table II and Table III were representative samples of large commercial lots obtained from four principal dealers, and were authenticated by well-known pharmacognosists.

### CONCLUSION

The results given would seem to indicate the following conclusions:

1. Root bark of *Viburnum prunifolium* contains up to some 20% more total volatile acid following hydrolysis than does stem bark.

2. The proportion and kinds of acid present in the mixture are practically the same in the case of root as compared to stem bark. The greater amount present in the former is due to more acid of all kinds rather than more of the higher acids such as valeric. Variation in the distillation rates of root bark differ as widely between individual samples as they do between root and stem bark.

3. One sample of rossed stem bark had more total acidity than did any sample of root bark. A greater proportion of the lower acids seem to be present in this sample, however, than in the unrossed samples or in the root bark, and such treatment may not be wholly desirable.

4. If the amounts and proportions of the mixture of volatile acid present are any measure of activity, there does not seem to be sufficient difference between stem and root bark of *Viburnum prunifolium* to justify that only the root bark be official.

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# The Growth Effects of Thiamin Chloride, Ascorbic Acid and Phytohormones on Belladonna and Ricinus*

*By Louis C. Zopf*†

Data of considerable value are rapidly accumulating from currently widespread experimentation as to the effects of phytohormones; vitamins and related organic stimulants upon seed germination and general growth of plants (1, 5, 7, 8, 13, 15, 17, 18, 20). Most such experiments have dealt with the effects of a single stimulant upon crop plants. Relatively little information is as yet available as to effects of varying combinations of two or more stimulants employed simultaneously or successively. Neither have medicinal plants been employed to any significant extent in such studies.

Most experiments performed by other investigators on the effects of growth stimulants have merely employed seedlings or very young plants. Very few data are available as to the effects of phytohormones and vitamins upon plants permitted to attain maturity.

An experiment was consequently devised to determine the specific effects of certain hormone and vitamin-like stimulants, singly and in combination upon the entire growth cycle of the official species *Atropa Belladonna* and *Ricinus communis*.

### EXPERIMENTAL

#### MATERIALS AND METHODS

The experimental procedure involved (1) the use of vitamin and hormone-like stimulants applied to seeds in various concentrations in a medium of powdered talc (U. S. P.) of 200 mesh in fineness, and (2) the application of aqueous solutions of the stimulants to growing plants in pure quartz sand cultures.

*1. Seed Treatment.*—Crystalline indole acetic acid and *a*-naphthalene acetic acid were weighed out and added to proper amounts of talc and then made into a uniform mixture by a three-hour treatment in a ball mill. Vitamins $B_1$ and C were

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prepared as aqueous solutions and measured amounts thereof were added to proper quantity of talc, which was air-dried and then transferred to the ball mill. Vitamin and hormone powders were stored in airtight dark glass screw cap jars. Vitamins were used in concentrations of 10 p. p. m. A similar procedure was followed in preparation of the light-sensitizing eosin dye, "Photosensin," which was used in concentrations of 40 p. p. m.

Lots of thirty seeds each of Belladonna and Ricinus were moistened with a wet cloth, and rolled in the various powder mixtures until coated with a uniform light coating of powder as shown in Table I.

Table I.—Dusting Powders for Seed Treatment for Various Lots

*A.* 1/1000 of *a*-naphthalene acetic acid.
*B.* 1/1000 of *a*-naphthalene acetic acid plus 10 p. p. m. of crystalline vitamin $B_1$.
*C.* 1/1000 *a*-naphthalene acetic acid plus 10 p. p. m. of crystalline vitamin C.
*D.* 1/500 of indole acetic acid (heteroauxin).
*E.* 1/500 of heteroauxin plus 10 p. p. m. crystalline vitamin $B_1$.
*F.* 1/500 of heteroauxin plus 10 p. p. m. crystalline vitamin C.
*G.* 1/1000 *a*-naphthalene acetic acid plus 10 p. p. m. vitamin $B_1$ plus 10 p. p. m. vitamin C.
*H.* 1/500 of heteroauxin plus 10 p. p. m. vitamin $B_1$ plus 10 p. p. m. vitamin C.
*I.* 1/20,000 of photosensin.
*J.* 1/20,000 of photosensitizer plus 10 p. p. m. vitamin $B_1$, plus 10 p. p. m. vitamin C plus 1/1000 *a*-naphthalene acetic acid.
*K.* Untreated controls.

All seeds were planted in separate wooden sand germinating trays to avoid mingling of chemicals. The Belladonna seeds were planted approximately twice as deep as the thickness of the seed. Castor beans were covered with three-fourths inch of sand. Sand used was sterilized, acid and distilled water washed until free of phosphates. All groups received nutrient and water by daily watering with Knop's (modified) solution (Table II).

Table II.—Composition of Knop's (Modified) Solution

| | |
|---|---|
| $KNO_3$ | 2.0 Gm. |
| $KH_2PO_4$ | 2.0 Gm. |
| $MgSO_4$ | 2.0 Gm. |
| $Ca(NO_3)_2$ | 8.0 Gm. |
| $H_2O$ distilled | 14000.0 cc. |

Seeds were planted April 22nd and kept under ordinary greenhouse conditions. Seedlings were observed every twenty-four hours. Castor beans, because of their rapid rate of germination and growth, were studied for twenty days after the initial sprout was visible. Since the Belladonna seeds were slow in germination and growth, they were observed for twenty-six consecutive days.

Percentage germination (Table III) and rate of germination are shown in (Figs. 1, 2, 3).

Fig. 1.—*Ricinus communis* Seedlings 20 Days Old (Left) Untreated Control. Slight Inhibitory Effect of 1/500 Indole Acetic Acid Evident in (Center) Indole Acetic Acid Control. Value of Supplementary Vitamin Feeding Exhibited in Indole Acetic Acid 1/500 Plus Vitamin $B_1$ and Vitamin C 10 p. p. m. (Right).

Fig. 2.—*Ricinus communis* Seedlings 20 Days Old, a Comparative Study of Indole Acetic Acid Control 1/500 (Left). Untreated Control (Center). *a*-Naphthalene Acetic Acid 1/1000 (Right).

Fig. 3.—*Ricinus communis* Seedlings 20 Days Old. *a*-Naphthalene Acetic Acid 1/1000 Control (Left). *a*-Naphthalene Acetic Acid 1/1000 Plus Vitamin $B_1$ 10 p. p. m. (Center). Improved Growth Effect Upon Addition of the Two Vitamins is Evident (Right).

Table III.—Percentage Germination of *Atropa Belladonna* and *Ricinus Communis* Seeds Receiving Various Dust Treatments

| | *Atropa Belladonna* | *Ricinus Communis* |
|---|---|---|
| Control | 56.0 | 73.0 |
| *a*-Naphthalene acetic acid | 66.0 | 76.0 |
| Indole acetic acid | 43.0 | 86.0 |
| *a*-Naphthalene acetic acid plus thiamin chloride and ascorbic acid | 13.0 | 93.0 |
| Indole acetic acid plus thiamin chloride and ascorbic acid | 13.0 | 100.0 |
| *a*-Naphthalene acetic acid plus thiamin chloride | 50.0 | 93.0 |
| Indole acetic acid plus thiamin chloride | 56.0 | 93.0 |
| Indole acetic acid plus ascorbic acid | 40.0 | 90.0 |
| *a*-Naphthalene acetic acid plus ascorbic acid | 53.0 | 93.0 |
| *a*-Naphthalene acetic acid plus thiamin chloride plus ascorbic acid plus photosensitizer | 46.0 | 83.0 |
| Photosensitizer | 50.0 | 90.0 |

*2. Growth Study.*—For this study Belladonna and Castor Bean seeds were germinated in separate trays containing a 50/50 sandy loam soil. Transplants were made to white sand contained in two-gallon glazed earthenware pots, equipped with side or bottom drain plugs. The initial set of Castor Bean seedlings were planted two to the pot, and an initial set of Belladonna seedlings were planted five to each jar. This group will hereinafter be referred to as Group I.

A second lot (Group II) of both seeds was germinated under the same conditions as Group I. Transplanting was made in the same manner as above with the exception of the Belladonna seedlings which this time were planted two plants to the pot. Group I consisted of two pots of each species to each nutrient series. Group II contained eight pots of each series.

Water and nutrient solutions were applied directly to the sand. The control and basic nutrient solution was Knop's solution (Table II). Thiamin chloride and ascorbic acid, 10 p. p. m. of each, were added to the control solution in the vitamin series *a*-Naphthalene acetic acid when supplied was added as a separate aqueous solution. The initial solution of the acid was made in 10 cc. of alcohol. Nutrient formulas received by the various series are listed in Table IV.

Table IV. Nutrient Formulas[a]

*A.* Knop's plus *a*-naphthalene acetic acid.
*B.* Knop's plus *a*-naphthalene acetic acid plus 10 p. p. m. each of vitamins $B_1$ and C.
*C.* Knop's plus *a*-naphthalene acetic acid and 10 p. p. m. each of vitamins $B_1$ and C and 1/25,000 of Photosensin.
*D.* Knop's plus 10 p. p. m. each of vitamins $B_1$ and C.
*E.* Control, Knop's solution only.

[a] NOTE: The vitamin $B_1$ (Betabion), vitamin C (Cebione) and *a*-naphthalene acetic acid were supplied through the courtesy of Merck & Co., Inc. Indole acetic acid was the product of the Eastman Kodak Co. All chemicals used as nutrients were of C.P. or analytical quality. Distilled water was used exclusively. The photosensitizer was a product of the Photosensin Co.

In addition to the Knop's and other nutrients supplied, each pot received 500 cc. of a solution of minor elements (Table V).

Table V.—Solution of Minor Elements

| | |
|---|---|
| *Solution A* | |
| $FeSO_4$ | 0.8 Gm. |
| $H_2O$ | 500 cc. |
| *Solution B* | |
| $H_3BO_3$ | 0.8 Gm. |
| $MnSO_4$ | 0.8 Gm. |
| $ZnSO_4$ | 0.8 Gm. |
| $CuSO_4$ (Crystals) | 0.4 Gm. |
| $H_2O$ *q. s.* | 500 cc. |

Used 5 cc. of Solution *A* and 10 cc. Solution *B* to each 19 liters of distilled water.

Addition of photosensitizer was made as separate distilled water solutions.

The non-vitamin series of plants all received regular additions of Knop's solution. Equal amounts of Knop's plus the vitamins were administered to the vitamin series. The nutritional dosage was graduated with the age and requirements of the plants. The average weekly amount for a maturing plant was 500 cc. The solution supplying the minor elements was added twice to each pot in amounts of 250 cc. In order to correct and prevent the appearance of chlorosis, each jar including the Castor Bean series received 150 cc. each of 0.1 per cent ammonium sulfate and 10 p. p. m. of iron and ammonium citrate.

In all series receiving *a*-naphthalene acetic acid, the total amount of acid added was 0.0125 Gm. per pot, administered in two doses. The first dose was 0.0025 Gm., and this being without noticeable effect, the next and last dose was increased to 0.01 Gm. per pot.

Immediately following flowering of the Belladonnas the leaves were picked both from the controls and the control vitamin series. They were dried at room temperature for forty-eight hours when they were transferred to an oven and dried at 38° C. Root examination of both Belladonna and Castor Beans were made at this point by flushing sand from the pot.

## RESULTS

### 1. GERMINATION RESPONSES

*A. Germination Responses of Ricinus Communis.*—Response of *Atropa Belladonna* and *Ricinus communis* seeds treated with the various dusting powders was extremely varied (Table III).

A definite increase in percentage germination of *Ricinus communis* was evident in all seeds receiving the mixture of hormone and vitamin. Hormone treatment alone gave an increase in both *a*-naphthalene acetic acid and indole acetic acid controls over

the untreated controls. A small decrease in *rate* of germination of the Castor Beans was noted upon the addition of vitamins to the *a*-naphthalene acetic acid dust treatment. Percentage germination of the *a*-naphthalene acetic acid plus vitamin series was well above that of the controls.

The only seeds showing 100 per cent germination were those receiving the combined treatment of indole acetic acid, thiamin chloride and ascorbic acid In addition this group exhibited the greatest stem elongation and foliage yield of all groups (Fig. 1). Stem length increases of approximately $^1/_8$ to $^1/_4$ inch over that of the untreated controls are recorded by the indole acetic acid control and the *a*-naphthalene acetic acid control (Fig. 2). In the case of each hormone combined with both vitamins an improved growth response is noted over that of the corresponding hormone control (Figs. 1, 3).

The average growth of *Ricinus communis* seed treated with *a*-naphthalene acetic acid plus thiamin chloride and *a*-naphthalene acetic acid combined with thiamin chloride and ascorbic acid exceeds that of the *a*-naphthalene acetic acid controls by approximately $^3/_4$ and 1 inch, respectively (Fig. 2). Root responses also proved sufficiently definite and varied to justify examination. Results are given synoptically.

*B. Root Responses of Germinated Seedlings of Ricinus Communis.*—(1) Controls: A definite massed root system with many thick primary roots. Many secondary roots of a coarse hunger type uniformly scattered along the primary roots.

(2) Indole acetic acid: The roots of the series receiving indole acetic acid showed a much finer general root system than either the controls or the *a*-naphthalene acetic acid series. A definite branching occurred primarily at the root tips.

(3) Indole acetic acid plus thiamin chloride: In the case of indole acetic acid plus thiamin chloride the roots showed a vertical stratification due possibly to the vitamin effect.

(4) Indole acetic acid plus ascorbic acid: In indole acetic acid plus vitamin C there was a general massing of primary roots in the hypocotyl region. These primary roots were more slender, less stubby than in the indole acetic acid plus thiamin chloride series, with the whole system showing a horizontal stratification.

(5) Indole acetic acid plus thiamin chloride and ascorbic acid: This series previously mentioned as 100 per cent in germination and excelling in growth, presented in general a smaller root system, in comparison to either of the other series. The primary roots were finer and, as was the case in the other indole acetic acid groups, no tertiary roots were evident.

(6) Photosensitizer: Relatively few stubby primary roots and little secondary branching appeared. The roots were massed in the hypocotylary region.

(7) *a*-Naphthalene acetic acid plus vitamin $B_1$, plus vitamin C and photosensitizer: A slight thickening and elongation of roots were noticeable in comparison to that of the corresponding indole acetic acid series.

(8) *a*-Naphthalene acetic acid: The roots of the series receiving *a*-naphthalene acetic acid in their treatment present a shorter, less numerous but thicker primary and secondary root system.

(9) *a*-Naphthalene acetic acid plus vitamin $B_1$: Massing of the roots at the base of the hypocotyl is evident, but not so marked as in the case of the *a*-naphthalene acetic acid plus thiamin chloride and ascorbic acid. A linear stratification of roots is evident.

(10) *a*-Naphthalene acetic acid plus vitamin C: The possible difference in macroscopical examination of these roots is the evidence of the branching along the tap root.

*C. Germination Responses of Belladonna.*—With the exception of the *a*-naphthalene acetic acid controls, all dust-treated Belladonna seeds gave a lower percentage germination than the untreated controls. The Belladonna seeds treated with indole acetic acid plus vitamin $B_1$ equaled the untreated controls and surpassed the indole acetic acid controls in percentage germination (Table III).

All dust-treated Belladonna seeds receiving the combination of a hormone and one or both of the vitamins, with the exception of indole acetic acid plus thiamin chloride, show a noticeable retardation in the rate of germination. The controls were among the first to germinate and led in the rate of germination throughout the experiment.

Top growth was so slow and irregular in the treated plants that it is impossible to record the comparative response with any degree of accuracy.

Presented in the order of their best (general) top growth they are as follows: control (untreated), photosensitizer, indole acetic acid plus thiamin chloride, indole acetic acid plus ascorbic acid. The groups presenting the poorest top growth are *a*-naphthalene acetic acid and indole acetic acid plus *a*-naphthalene acetic acid.

*D. Root Responses of Germinated Seedlings of Atropa Belladonna.*—Extreme irregularities in growth and rate of germination among the various series of Belladonna also made it difficult to study the root system of plants of comparable ages.

In general the root responses of Belladonna could be said to be comparable to that of Castor Bean. All seeds receiving indole acetic acid in their powdered form had a rather extensive but thin secondary root system. The seedlings produced from seeds treated with *a*-naphthalene acetic acid had much shorter and thicker roots. The control (untreated) had slightly less roots than the indole acetic acid series and appeared less fibrous. The roots of the photosensitizer treated seedlings were short but rather numerous. This effect was not noticeable, however, in the group receiving the combined treatment of *a*-naphthalene acetic acid plus vitamin $B_1$, plus vitamin C and photosensitizer.

2. GROWTH RESPONSES

For uniformity, the plants receiving only Knop's solution will be referred to as the control series. The other series will be designated by abbreviations as follows:

Knop's-*BC*—Knop's plus 10 p. p. m. each of thiamin chloride and ascorbic acid.

Knop's-*BC-P*—Knop's plus 10 p. p. m. each of thiamin chloride and ascorbic acid plus 1/25,000 photosensitizer.

*A-N-A-BC*—The Knop's *BC* series receiving *a*-naphthalene acetic acid.

*A-BC-P*—Knop's plus 10 p. p. m. each of thiamin chloride and ascorbic acid plus *a*-naphthalene acetic acid and photosensitizer.

*A-N-A*—The control series receiving *a*-naphthalene acetic acid.

*A. Ricinus Communis.*—Five-week-old *Ricinus communis* seedlings (Group I) of the control and Knop's-*BC* series gave no visible response to the addition of 2.5 mg. of *a*-naphthalene acetic acid applied directly to the sand. Again, on the subsequent addition of 10 mg. of *a*-naphthalene acetic acid no immediate responses, such as epinasty of the leaves and bending, were noticeable. Daily observations, however, revealed definite retardation or inhibition of top growth which proved to be a permanent retardation.

Attention is directed to the antidoting effect of the combined vitamins $B_1$ and C exhibited as increased growth over the *A-N-A* series. Beneficial results from the vitamin additions are prolonged as shown by continued increase in growth through the period of flowering (Figs. 4, 5).

The noticeable response of *Ricinus communis* (Group I) suggested the advisability of the repetition of the treatment in a second group (Group II). In this group a Knop's control, a Knop's-*BC* and a Knop's-*BC-P* series were run, two plants to the pot, eight pots to each series. A general advanced rate of growth was noted in both series receiving supplementary vitamin feedings over that of the controls. The series receiving photosensitizer in addition to the vitamins exhibited a definite increase in stem

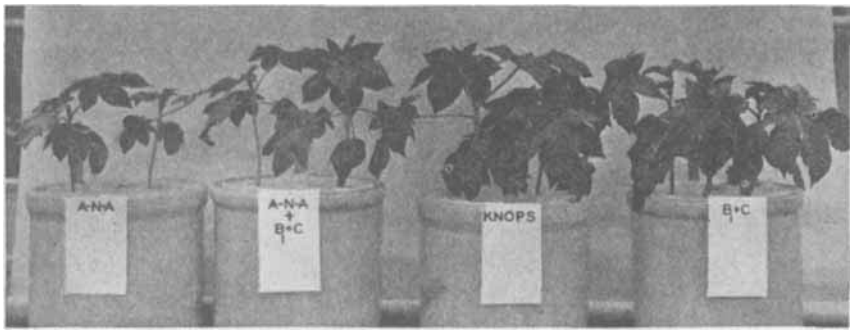


Fig. 4.—Illustrating the Antitoxic Value Against *a*-Naphthalene Acetic Acid Toxicity (Left) by Treatment with Vitamins $B_1$ and C (Second from Left); (Second from Right) Knop's Control; (Right) Vitamin Control.

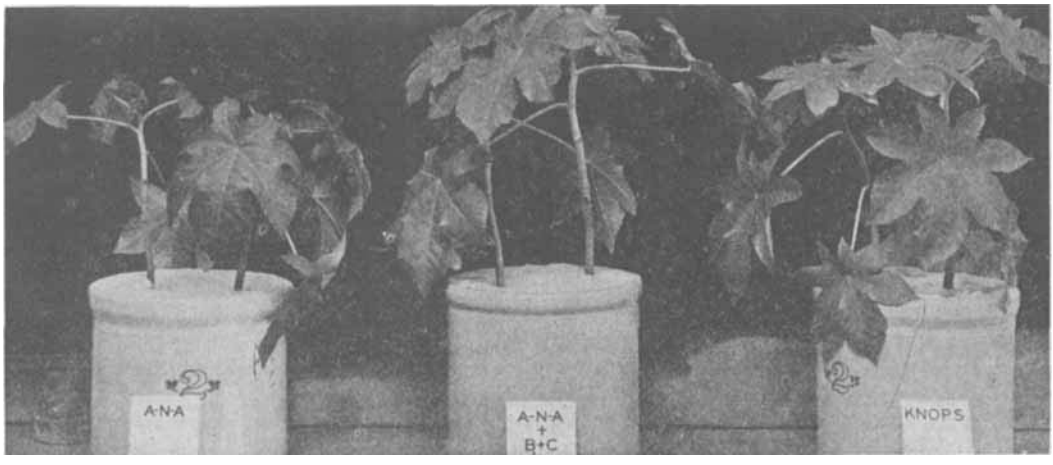


Fig. 5.—*Ricinus communis* (Group I). Toxicity of *a*-Naphthalene Acetic Acid (Left) on Top Growth and Antidoting Effect of Vitamins $B_1$ and C (Center). Knop's Solution Control (Right). Above Are the Same Plants as Shown in Fig. 4.

elongation and growth rate above those receiving Knop's plus the vitamins. Size and number of leaves were comparable in all series. Simultaneous blossoming was also noted. Difference in top growth was pronounced (Fig. 6).

*Flowering Ricinus Communis—Root Growth.*—Root studies of the two groups immediately after flowering are shown synoptically below.

Knop's Control: Abundant number of thick, fairly long primary roots. These branch from the hypocotyl region. Secondary roots are fairly coarse but very abundant branching is also evident from tap root.

Knop's-*BC:* This series shows a similar number of primary roots as compared with Knop's control, branching from the hypocotyl region. A finer system of secondary roots is in evidence and they appear functional.

*A-N-A:* The branching primary roots of this series are spotted with a white warty-like excrescence of the same appearance as that in the *A-N-A-BC* group. Primary roots are smaller than in the *A-N-A-BC* group. Secondary roots coarse. Noticeable because of its absence is the usual outstanding tap root.

All plants seem to show a large top root ratio.

*B. Atropa Belladonna.*—Since the controls and Knop's-*BC* series of Belladonna of Groups I, II and III responded fairly uniformly in each series these will be mentioned first.

The three-inch seedlings of *Atropa Belladonna* receiving Knop's-*BC* gave the most vigorous early growth responses.

Also evident in this series was the better leaf color and less anthocyanin pigmentation of the stems. The early rapid growth of the Knop's-

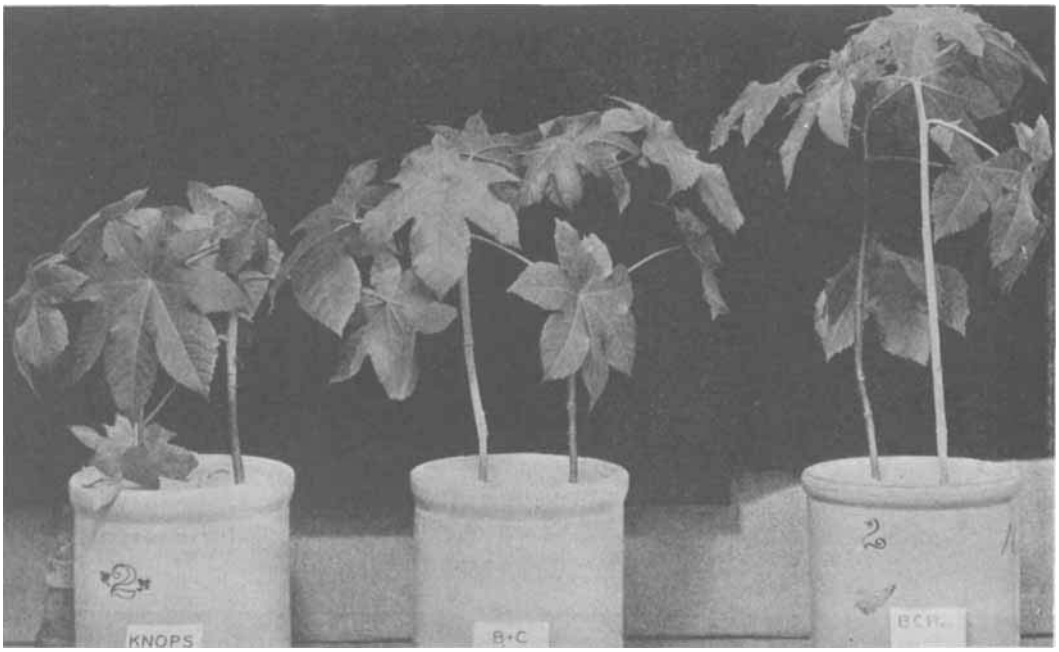


Fig. 6.—*Ricinus communis* (Group II). Top Growth Response of the Vitamins $B_1$ Plus C (Center) Over Knop's Control (Left). Increased Stem Elongation of Vitamin Treated Plants Plus Photosensitizer (Right).

*A-N-A-BC:* This series presents the heaviest root system. Secondary branching occurs close to the soil line and appears fibrous, with an abundance of such secondary roots are present toward the base of the pot. A white excrescence is evident along the primary roots of this series.

Knop's-*BC-P:* This series has the smallest roots of either group, fairly uniform primary root branching is evident from the hypocotyl region. The primary roots are approximately two-thirds the size of those of the control. There is a much finer anastomozed branching system of secondary roots. The latter are in abundance but much less fibrous than those of other series. No excrescence is evident in this system.

*BC* series seemed to cease after the plants had acquired a few leaves and a height of approximately eight inches. From this point these plants were overtaken by the controls in growth.

Typical flowering plants from the Knop's-*BC* series show a slightly improved top growth and deeper green color over plants of the same age in the control series (Figs. 7, 8).

Further growth responses were noticeable but difficult to evaluate definitely. Earlier and more universal basal branching was produced in the Knop's-*BC* group (Figs. 7, 8). Control group did exhibit some of this type branching but in relatively few plants. Blossoming occurred simultaneously in both series at twelve weeks of age. No difference


Fig. 7.—Flowering Belladonnas (Group II) of the Knop's Control Series, Age Twelve Weeks.


Fig. 8.—Flowering Belladonnas (Group II) Receiving Vitamins $B_1$ and C 10 p. p. m. in Addition to the Knop's Solution. Note Basal Branching and Deeper Green Color. Age Twelve Weeks.

in duration of period of flowering or number of blossoms was noticed.

In general, the Knop's-*BC* series gave the least evidence of chlorosis, though this did occur in both series demanding supplementary feeding of the ferric ion as 10 p. p. m. of ferric and ammonium citrate and additional nitrogen through a 0.1 per cent solution of ammonium sulfate.

Immediately after blossoming both series of plants were stripped of their leaves which were oven-dried for future alkaloidal assay.

The series of Belladonna seedlings receiving *a*-naphthalene acetic acid in addition to their regular nutrients represented a most interesting study. Each of the three series in this group received the same concentration and dosage of *a*-naphthalene acetic acid. As was noted in the Castor Bean seedlings, the initial addition of 2.5 mg. *a*-naphthalene acetic acid per pot gave no visible response. Within a period of twelve hours following the next and last addition of *a*-naphthalene acetic acid, 10 mg. per pot, a definite epinastic and bending response was noted. The toxic effect shown by defoliation was most noticeable in the control series.

First to revive from this toxic dose were the two series which received vitamins in their nutrient. The most rapid and energetic response was shown by that group receiving *A-N-A-BC* and *A-BC-P*. Greater top and root growth is very evident in the plants receiving vitamins. New shoots arose from old stems in the *A-N-A-BC* group (Fig. 9). The figure also indicates the degree of the *a*-naphthalene acetic acid toxicity.

Mention has been made of the fact that the phyto-

Fig. 9.—The Antidoting Effect of Vitamins $B_1$ and C (Center) Against *a*-Naphthalene Acetic Acid Toxicity on Roots (Left). Improved Response (Right) Through Addition of Photosensitizer Plus the Vitamins. Plants of Like Age.

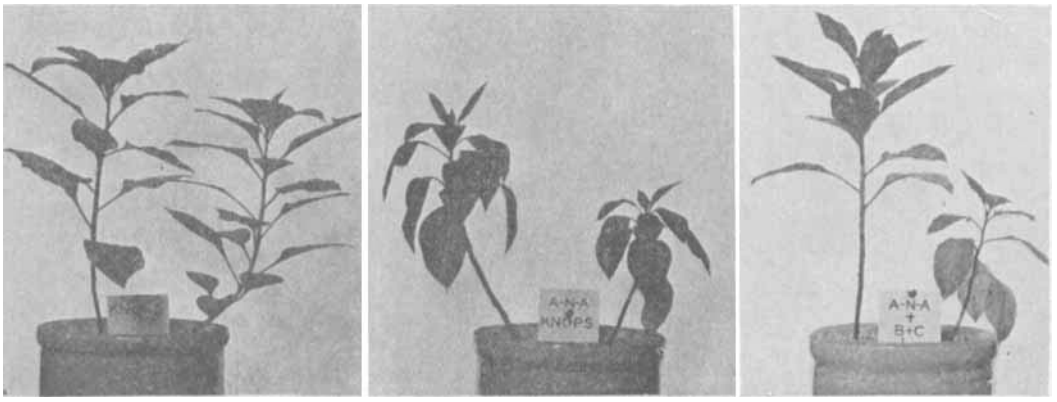


Fig. 10.—The Epinastic Response of *a*-Naphthalene Acetic Acid (Center) on Belladonna of the Knop's Control Series. Retarded or Delayed Epinastic Response in Belladonna Plants (Right) of the Same Age Receiving the Same Dosage of *a*-Naphthalene Acetic Acid, Subsequent to Knop's-BC. Untreated Control (Left).

hormones are more toxic to young than mature plants. With this thought in mind, Belladonna plants just about to flower having been treated up to this time with Knop's and Knop's-*BC*, respectively, were given a dosage of 10 mg. of *a*-naphthalene acetic acid per pot. Figure 10 shows the epinastic response which was induced in the plants of both series but which is much more pronounced in the control group.

The Knop's-*BC* plants receiving 10 mg. of *a*-naphthalene acetic acid did not show as marked epinastic responses to *a*-naphthalene acetic acid and exhibited recovery sooner than the controls with *a*-naphthalene acetic acid but without the vitamins. Lower leaves on both plants dried completely but at no time during the period of two weeks after the toxic dose was administered did either group lose all leaves. Those receiving the Knop's-*BC* were always more vigorous than the control.

## DISCUSSION

The foregoing results coincide with those of other workers in showing (1) a toxic effect of *a*-naphthalene acetic acid (7, 18, 25); (2) a beneficial effect of vitamins on plant growth (2, 3, 4, 5, 6, 11, 12, 13) and (3) variable effects of those stimulants upon seed germination (8, 11, 12, 13, 19). Treatment is beneficial in many, though not in all cases (8, 13, 19), the effect varying with

concentration and different combinations of stimulants.

The variability in germination of *Ricinus communis* and *Atropa Belladonna* in response to a given stimulant suggests wide differences between species, as also noted by other investigators (5, 13, 20), especially as to optimal concentrations of the added chemicals.

It is obvious that seeds slow to germinate, such as *Atropa Belladonna* and others, require a powder treatment of lower concentration, even though growth substances are released slowly, as shown by Grace (8). Grace, experimenting with two dusting techniques, which she generally found to be safer than treatment with solutions, used talc or a standard mercurial dust disinfectant for treatment of seeds of wheat and barley. In Grace's experiments concentrations of 5 p. p. m. of growth substances were found to be effective in many instances.

Literature dealing with the growth effects or response of plants to the water-soluble vitamins is limited. Von Hausen experimented with the absorption and germination effects of ascorbic acid by pea seeds. She found solutions of ascorbic acid in higher concentration than 0.4 per cent could not be used without a serious loss of the germinating power of the seeds. Havas (13) using vitamin C in a solution for seed treatment also noted a delay in germination in solutions above a certain concentration. Optimum growth conditions were obtained by use of vitamin $B_1$ by Robbins and Bartley (20) working with excised tomato root tips in artificial nutrient cultures. The work of Bonner (4) and Bonner and Greene (5) indicate the value of vitamin $B_1$ in the form of improved root growth. They found vitamin $B_1$ to be not so much for the initiation of the root primordia as for their development. The present investigation correlates well with the foregoing in showing the value of simultaneous administration of the two vitamins $B_1$ and C to the soil of plants in inducing increased root growth and improved top growth (Figs. 5, 6, 7, 8, 9, 10). These vitamins also display an antitoxic effect upon the toxicity resulting from *a*-naphthalene acetic acid (Figs. 5, 9, 10).

It has been demonstrated by Loehwing and Baugess (17) that the application of heteroauxin nutrient solutions directly to the soil result in stimulation of early growth. Other hormone substances, likewise, have been applied to the soil (14) and found to induce all of the responses obtained by applying the material to the aerial parts of plants in the form of a lanolin paste. Absorption of growth substances in this experiment is evidenced by (1) the inhibitory growth response by the *Ricinus communis*, (2) through the delayed or inhibited germination of the *Atropa Belladonna*, (3) the marked epinastic response of the Belladonna plants and (4) the definite antitoxic responses exhibited by supplementary feeding of the two water-soluble vitamins.

The fact that the antidoting or antitoxic value of the vitamins is not ascribable to a chemical combination of the hormone with the vitamins, thus perhaps rendering either or both inert or unavailable to the plants, is demonstrated by the increased growth of the plants in the *A-N-A-BC* series over that of the controls (Fig. 5). The epinastic response which was manifest upon the administration of 10 mg. of *a*-naphthalene acetic acid to Belladonna plants just prior to anthesis shows the comparatively low threshold which these plants have for this particular hormone. The small amount of epinasty in the vitamin-treated Belladonnas later became more severe than pictured (Fig. 10). The vitamins served to retard the epinasty of the leaves both in onset and duration. Tobacco, also a member of the *Solanaceæ* (Nightshade Family), when treated with *a*-naphthalene acetic acid (14) induced epinasty of the leaves but only with larger dosage. This serves as an illustration of the varying hormone threshold of plants in the same family. Differences in species of plant, concentration of hormones and method of hormone application are not only important but even crucial in investigations of this sort (12).

It was observed that the retarded growth rate of the *Ricinus communis* in the *a*-naphthalene acetic acid control was permanent. No marked increase in growth rate was noticed following the original re-

tardation and, though the plants continued to grow, at no time did they equal the regular controls in stature (Figs. 4, 5). If the vitamins $B_1$ and C exhibit both such stimulatory effects and antidoting properties toward other pharmaceutical and economic plants, their action is well worthy of further study. The value of earlier germination, increased growth (top and root) and early maturation of official and agricultural plants is obvious.

## CONCLUSIONS

1. Hormone and hormone plus vitamins ($B_1$ and C) as dust treatment of seeds influence both germination and growth responses of *Atropa Belladonna* and *Ricinus communis*. The effect of a given stimulant, whether beneficial or injurious, varies with its concentration, the species and age of the plants.

2. *a*-Naphthalene acetic acid applied as a solution in toxic dosage induces varying response in different plants. The toxic threshold varies with the type of plant.

3. The simultaneous administration of vitamin $B_1$ and vitamin C exhibits a combined effect on the general growth of the plants studied, which is more than a mere additive stimulus.

4. The simultaneous administration of vitamin $B_1$ and vitamin C exhibits an antitoxic or antidoting property toward plants which have previously been subjected to toxic doses of hormones such as *a*-naphthalene acetic acid. This antitoxic reaction (occurring as improved top and root growth) is not ascribable to a neutralizing effect or chemical combination between the *a*-naphthalene acetic acid and the vitamins, as evidenced by the better top growth of the *A-N-A-BC* plants over the controls.

5. The antitoxic quality of concurrent administration of the water-soluble vitamins $B_1$ and C is also of value in delaying and reducing the degree of epinastic responses of Belladonnas about to flower.

6. Photosensitizer added directly, in aqueous solution, to the soil of plants containing both vitamins $B_1$ and C, produces increased top growth.

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# A Study of the Stability of Liquid Preparations Containing Pepsin*

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This work is an endeavor to study the stability of liquid preparations containing pepsin, and to investigate the possibility of stabilizing the activity of peptic systems. Heretofore, as mentioned by Worrell (1), stability studies of pepsin preparations have been inconclusive, due primarily to the lack of a satisfactory method of assay. Worrell (1) had this objective in mind, namely, to develop a procedure which could be employed successfully in the accurate assay of pepsin preparations for activity. In the experiments conducted in this research his method of assay for liquid preparations containing pepsin has been used entirely.

During the course of making an enzyme preparation, it is often noted that there is a rather acute loss in activity of the enzyme. Similar losses are noted when the enzyme solution is permitted to stand at room temperature for a period of time. Northrop (2, 3) indicates that a major source of inactivation in the case of pepsin and trypsin is due to denaturation of the enzyme protein.

In attacking the problem of stabilizing the activity of liquid preparations containing pepsin, it was deemed fundamental that a study first be made of the influence of individual factors or variables on the maintenance of peptic activity over an extended period of time. The factors which have received special consideration include temperature, $p_H$, antioxidants (maleic acid, hydroquinone, resorcinol), preservatives (alcohol, "Merthiolate"), protective agents (acacia) and low concentrations of amino acids (tyrosine).

## EXPERIMENTAL

A number of preparations were made, each having one or more of the above variables which differentiated it from the standard. A standard preparation was designated as one having approximately five per cent of pepsin in distilled water, and which was stored at room temperature under nitrogen. Any substance added in order to study its effect on the stability of pepsin solutions was considered to be a variable. All of the samples were placed in ordinary clear glass bottles and stored in the dark.

Table I.—A Summary of Assay Results of Pepsin Solutions on Stability Tests. ("*n*" in the Number Designating the Sample Indicates Storage under Nitrogen; "*a*" Indicates Storage under Air)

| Sample | Variable | Concentration of Pepsin (%) 1st Week | 6th Week | 14th Week | % Loss in Activity |
|---|---|---|---|---|---|
| 3*n* | Storage at 5° C. | 5.4 | 5.0 | 4.9 | 9.3 |
| 4*n* | 1% maleic acid | 5.2 | 3.4 | 2.5 | 51.9 |
| 5*n* | 3% maleic acid | 4.8 | 3.0 | 1.9 | 60.4 |
| 6*n* | 5% maleic acid | 4.8 | 2.5 | 1.3 | 72.9 |
| 7*n* | 2% resorcinol | 5.5 | 4.5 | 4.6 | 16.4 |
| 8*n* | 2% hydroquinone | 5.3 | 4.2 | 2.8 | 47.2 |
| 10*n* | 10% U. S. P. Alcohol | 5.7 | 4.8 | 4.9 | 14.0 |
| 16*a* | 0.05% "Merthiolate," storage under air | 4.6 | 4.4 | 4.2 | 8.7 |
| 19*n* | 0.05% "Merthiolate" | 4.5 | 4.0 | 4.1 | 8.9 |
| 21*n* | 0.1% maleic acid, 10% U. S. P. Alcohol | 5.3 | 5.2 | 4.7 | 11.3 |
| 22*n* | 10% U. S. P. Alcohol | 5.2 | 5.0 | 4.7 | 9.6 |
| 24*n* | 0.05% "Merthiolate" | 5.4 | 4.9 | 4.5 | 16.7 |
| 25*n* | 0.05% "Merthiolate," 0.1% maleic acid | 5.7 | 4.8 | 4.5 | 21.1 |
| 26*n* | 0.05% "Merthiolate," 1% acacia | 5.3 | 4.9 | 4.6 | 13.2 |
| 27*n* | 0.05% "Merthiolate," satd. with tyrosine | 5.3 | 4.9 | 4.6 | 13.2 |

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