THE EFFECTS OF 6-BENZYLAMINO PURINE ON AXILLARY BUD GROWTH
OF ILEX CRENATA THUNB. 'ROTUNDIFOLIA'

by

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in
Horticulture

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February, 1977
Blacksburg, Virginia
DEDICATION

The author wishes to dedicate this to his loving wife, Eranda, without whose support and willing sacrifices this work could not have been completed.
ACKNOWLEDGMENTS

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The author wishes to thank his Mother for her conveying a love, respect, and desire for education.
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I. INTRODUCTION

It has been well documented that apical dominance is controlled by numerous factors and their interrelationships. While conclusions from the research conducted on herbaceous materials can be applied to woody plants in general, there is a real need for experiments to be conducted with specific woody ornamental plants.

Many holly species exhibit strong apical dominance. Unless much of the new growth is pruned to stimulate axillary bud development, the dominant apex develops an upright sparsely-branched plant (Fig. 1). Pruning is therefore necessary to produce compact well branched plants.

Chemical pinching agents have been used to induce axillary bud development in some plants thereby reducing the need for manual pruning. In this way, pruning problems have been reduced in azaleas (9). Attempts to replace manual pruning with chemical pinching agents on hollies have been less successful. Applications of chemical pinching agents to hollies must be made at the time of apical bud growth since applications before bud break are ineffective and applications made during active growth damage the new shoots (23).

Many plants, when pruned either manually or with chemical pinching agents, have a tendency to resume growth from the uppermost axillary bud. To produce a well branched plant, growth of more than one axillary bud is essential. Cytokinin applications following treatment with chemical pinching agents have resulted in enhanced axillary bud growth of azaleas and photinia (35). Cytokinins have also been used to stimulate axillary
Figure 1. A typical unpruned 'Rotundifolia' holly liner following three successive flushes of growth.
bud development in roses (7) and hollies (60).

Cultivars of *Ilex crenata* constitute an important part of the woody ornamental nursery trade. Estimates based on a collective survey of production and marketing in eleven southern states place the annual sales of *Ilex crenata* sp. in Virginia to be approximately one and a quarter million dollars (46).

The objectives of this series of experiments with *Ilex crenata* 'Rotundifolia' were: 1) to determine what concentration of cytokinin is most effective in stimulating axillary bud growth, and 2) to determine the optimum phase of axillary bud development for cytokinin application.
II. LITERATURE REVIEW

It has been recognized for many years that the shoot apex in some way inhibits the growth and development of axillary buds. The phenomenon has been termed apical dominance. Even before the specific growth hormones had been isolated and named, Snow (44) theorized that a growth hormone was produced in the young developing leaves of apical buds and was transmitted in a basipetal direction where it inhibited axillary bud growth. In his work with Pisum sativum he found that this growth hormone could also be transmitted laterally across nodes of decapitated plants. When a shoot was completely defoliated, axillary buds were inhibited by the growth hormone produced in the opposite shoot (45). Working with Vicia faba, Thimann and Skoog (47) found that a growth substance was produced in apical buds and possibly in young expanding leaves. When the unknown growth substance was present, the axillary buds were inhibited. They also found that when apical buds were removed and placed on agar blocks, the growth substance diffused out of the bud into the blocks. When the agar blocks containing the growth substance were placed on decapitated plants, the substance diffused into the plants and caused axillary bud inhibition (47). They stated that the growth substance, or auxin, (because of its effects in the coleoptile test) was produced in the apex of the shoot and inhibited axillary bud development (48). Consequently, they found that auxin applied to decapitated plants repressed axillary bud development.

The response by various species to auxins applied to decapitated plants may range from complete inhibition to almost no inhibition.
Salisbury and Ross (38) point out the lack of response of Xanthium strumarium to auxin applied to decapitated plants. It must be noted that there is very little, if any, apical dominance displayed by this specie in the natural state. Apical dominance can be classed as (a) inhibition of branching, (b) regulation of the rate of growth of certain branches, or (c) the control of branch angles (22). In each case, auxin can be implicated in contributing to apical dominance. Wickson and Thimann (55), using IAA-^{14}C on decapitated plants, showed that IAA moved into axillary buds and was responsible for their inhibition.

Inhibition of growing buds was found to be directly proportional to content of radioactivity recorded. Titman and Wetmore (49) demonstrated that the apical bud caused an inhibition of the normal elongation in short lateral shoots of Cercidiphyllum. Verner (51) reported that indolebutyric acid applied to decapitated apple seedlings caused wide crotch angle formation of all scaffold limbs.

One of the early theories of the mode of action of apical dominance was the "nutritive theory" or "nutrient flow theory". Went (53) suggested that since the apical bud was the first to be formed, it commanded a preferential nutrient supply. While nutrient flow is still considered to be important in apical dominance, it is considered in terms of "auxin-directed" transport. Wareing and Phillips (52) stated that ^{32}P-phosphate and ^{14}C-sucrose can be used to demonstrate that nutrients move to and accumulate in regions of high exogenous auxin concentration. Booth et al. (5) found that 6-11 hours after IAA application to Solanum andigena, increased nutrient accumulation could be measured in the area of application. This response could be due to
stimulation of growth in the immediate treatment area; however, due to the short time involved, the response is probably not growth but a stimulation of active uptake and enhanced transport to the treated area. Van Overbeek (50) suggested that high auxin content affected transport by inhibiting protoplasmic streaming in peas. Davies and Wareing (12) found that auxin stimulated $^{32}$P-transport acropetally in *Populus robusta* by enhancement of protoplasmic streaming.

High auxin levels have been implicated in the inhibition of vascular tissue formation joining axillary buds to the central vascular system of the shoot thereby reducing the capacity of axillary buds to obtain a supply of nutrients. Van Overbeek (50) suggested that inactivity of axillary buds was due to the impedance of transport through the vascular system. He explained that axillary buds could be inhibited while the apical bud could continue to grow due to the much wider vascular bundles of the main shoot. Gregory and Veale (13) stated that restricted nutrient flow could have been the result of arrested development of vascular tissues to axillary buds. By controlling the development of vascular tissues to axillary buds, auxin led to the deprivation of axillary shoots (13).

McIntyre (24) suggested that the vascular tissues to axillary buds are limited by auxin supply in the axillary bud rather than suppressed by it. Thus, upon decapitation increased nutrients are available to axillary buds. Metabolism and growth are stimulated resulting in increased auxin production in the axillary bud. This auxin in turn further enhances nutrient flow resulting in increased vascular tissues to axillary buds. Panigrahi and Audus (31) using uracil-2-$^{14}$C applied
to cotyledons of decapitated *Vicia faba* showed that movement of nutrients into axillary buds preceded growth and may be its cause. Then using 2,3,5-triiodobenzoic acid to block IAA movement, they demonstrated that IAA suppresses movement of nutrients into axillary buds.

While auxin can be shown to be an important factor in apical dominance, it is only one of the factors responsible for this phenomenon. Jacobs and Case (18) found that auxin alone, regardless of concentration, was not capable of replacing the apical bud in its inhibition of axillary buds of Alaska pea for more than a few days. In another study with *Phaseolus vulgaris* similar results were found. When applied to decapitated plants, IAA was found to be ineffective in maintaining apical dominance after only five days (11). Likewise, Shein and Jackson (42) found that IAA alone reduced axillary bud growth on decapitated pea plants but could not reestablish apical dominance. Apparently, other factors are important in apical dominance.

Cytokinins have received widespread interest in their involvement in axillary bud development. Cytokinins have been shown to stimulate axillary bud development in various plants (1,37,39,40). Carpenter and Rodriguez (7) demonstrated that cytokinins caused axillary bud development in greenhouse roses. In woody ornamentals cytokinins have been effective in stimulating axillary bud growth when applied to intact plants (60) or following chemical pinching agents (35). Cytokinins have been effective in overcoming apical dominance in apple trees in both a dormant (21,57) and actively growing state (6,10,21,56).

Wickson and Thimann (54) showed that kinetin allowed the develop-
ment of axillary buds in the presence of an intact apex, and concluded that apical dominance may be due to a balance between auxin and kinetin. Sachs and Thimann (36) found that kinetin applied directly to axillary buds of intact *Helianthus annuus* plants released them from apical dominance, and suggested that the development of axillary buds depends primarily on the balance between endogenous auxin and a kinetin factor at the local site. Supraoptimal levels of applied cytokinins reduced growth in tobacco (14) and in hollies (39).

Clearly, cytokinins can be shown to be involved in the phenomenon of apical dominance. However, Shein and Jackson (42) conducted extensive studies with *Phaseolus vulgaris* and found that kinetin alone was not effective in releasing axillary buds from apical dominance. Sachs and Thimann (36,37) observed that the initial release of axillary buds by cytokinin resulted in only transitory growth, and subsequent applications of auxin or gibberellin were needed for further elongation and development. Ali and Fletcher (1) reported that growth of buds released by cytokinins was further enhanced by applications of gibberellic acid and indicated that these hormones have a sequential role in releasing buds from apical dominance. Simultaneous application of both hormones to *Solanum andigena* completely inhibited axillary bud growth (59). By using $[^{14}C]$ benzylamino purine it was demonstrated that cytokinin accumulates in the axillary buds of cuttings prior to their growth as leafy shoots (59).

While it has been demonstrated that direct applications of cytokinins to intact plants cause axillary bud outgrowth, it is not known whether the cytokinins required for normal axillary bud growth have to
be supplied from the roots or whether they are synthesized within the bud tissues (34). From the root exudate of decapitated sunflower plants Kende (20) isolated kinetin-like factors capable of preventing chlorophyll degradation and of stimulating cell division. Carr and Burrows (8) have also isolated substances with kinetin-like activity from root exudates. Cytokinin has been isolated from xylem sap of Perilla frutescens and was found to increase considerably during fruit development (4). Woolley and Wareing (59) demonstrated the importance of roots as the source of cytokinin. The presence of roots, treatment with 6-benzylamino purine (BA) and 6-(4-hydroxy-3-methyl but-2-enyl) amino purine (zeatin) all lead to formation of vigorous leafy shoots (59).

Gibberellins play an indirect role in apical dominance. They do not cause apical dominance as does auxin, and they do not release axillary buds from inhibition as do cytokinins (52). Shein and Jackson (42) found that gibberellic acid (GA₃) alone seldom increased growth of axillary buds and in some cases caused inhibition due to enhanced apical growth. Gibberellin exerts its effect only subsequent to the release of axillary buds by cytokinins (36,54,56).

The effects of auxins, cytokinins, and gibberellins and their interactions on apical dominance may be related in part to hormone-directed transport of both inorganic nutrients from the roots and assimilates from the leaves. Seth and Wareing (41) found that kinetin and GA₃ alone had little effect on phloem transport in decapitated peas and beans but when applied in conjunction with IAA they enhanced the effect of the latter. Mullins (28) found that applications of mixtures of IAA, GA₃, and cytokinins to French beans increased incorporation of
$^{14}$C-leucine into protein. Thus, the enhanced accumulation of $^{14}$C-photosynthate by treated internodes may have resulted from an increase in demand for substrate (28). Jeffcoat and Harris (19) found that both IAA and GA$_3$ are active in hormone-directed transport in carnations. Mothes et al. (26) found that kinetin application to tobacco leaves caused accumulation of amino acids against concentration gradients. They also found that $\alpha$-aminoisobutyric acid and other amino acids were accumulated in kinetin treated tissues even though they were not incorporated into protein. Osborne and Black (30) found that the basipetal transport of $^{14}$C-labeled BA was enhanced when applied in conjunction with IAA and suggested that transport of the two growth hormones may be interdependent.

When GA$_3$ and IAA are applied to decapitated plants there appears to be a synergistic effect enhancing apical dominance farther down the developing shoots (18,33,42). Earlier workers also found that inhibition increases with distance from the apical bud (44,53). However, in the natural state development of axillary buds on older shoots is more frequent, indicating the inhibiting effect of the apical bud diminishes with distance from the apical bud. The reason may not be the distance of the axillary buds from the apex, but their proximity to the root system and its endogenous source of cytokinin supply. Smith and Wareing (43) demonstrated with willow shoots that axillary buds most proximal to the root system become dominant. They also showed that this dominance was independent of nutrients supplied to the roots.

Variable responses to applied growth-regulating substances on axillary bud inhibition may be due partly to environmentally induced
differences in nutritional status of the experimental plants (25). Beever and Woolhouse (4) reported that after 35 inductive cycles of short day treatment the cytokinin content in the sap of short day plants was five times greater than that of control plants. McIntyre (25) found that reduction in light intensity markedly inhibited bud growth of *Phaseolus vulgaris*. Itai and Vaadia (17) suggested that cytokinins are primary factors in a control system that facilitates root-shoot communication and demonstrated that as a result of osmotic stress in the nutrient medium, cytokinin export from tobacco roots was suppressed. They also stated that water stress through enhanced evaporative demands reduced cytokinin content in sunflower leaves by involving a chemical transformation of the cytokinin molecule (16). McIntyre (25) found that reduction in water stress was accompanied by significant increases in bud growth in French beans. Likewise, as levels of nitrogen applications were increased, axillary bud growth increased (25).

Individual growth factors should be studied under well defined environmental conditions involving as many of the interacting factors as practical. Cytokinins have been shown to play an important role in apical dominance.
III. MATERIALS AND METHODS

Unbranched 'Rotundifolia' holly cuttings 10 cm long were rooted in 6 cm diameter rose pots under intermittent mist. A commercial mix of peat moss, weblite, and vermiculite (2:2:1 v/v/v) was used as a propagation medium and as a subsequent potting mix. After rooting for eight weeks the liners were removed from the mist and fertilized weekly with 25 ml 20-20-20 soluble fertilizer per plant at a rate of 500 ppm N. Plants were also fertilized once the first week with 25 ml soluble trace elements (7 g/l) and Sequestrene 330 Fe chelate. For all experiments long day conditions were maintained by providing incandescent light at approximately 162 lux from 11 PM until 2 AM. Due to the difficulty in controlling greenhouse conditions, daytime temperatures ranged from 26-30°C. Night temperature was maintained at 21°C.

Solutions of 6-benzylamino purine (BA) were prepared by dissolving BA crystals in a few drops of 1 N KOH before adding 10 ml 80% ethanol and diluting with distilled water. Fresh solutions were prepared on each treatment date to avoid precipitation. Tween 80 (polyoxyethylene sorbitan monooleate) at 0.05% was used as a surfactant. Foliar applications to plants were made using a hand-operated aerosol sprayer. Applications were repeated 24 hours later.

A. Cytokinin concentration on rooted cuttings: The purpose of this

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1 Weblite Corporation, Roanoke, Va.
3 Ciba-Geigy Corp., Greensboro, N.C.
4 Sigma Chemical Corporation, St. Louis, Mo.
experiment was to determine the optimum concentration of cytokinin application to stimulate axillary bud development on rooted cuttings. Solutions of BA were prepared as described earlier. Shell product SD 8339 6-(benzylamino)-9-(2-tetrahydropyranyl)-9H-purine (PBA) was diluted to appropriate concentrations with distilled water containing Tween 80 (0.05%). Unbranched rooted cuttings 10 cm long were treated with either BA or PBA at 400, 600, or 800 ppm. Intact controls and decapitated controls were also sprayed with distilled water containing Tween 80 (0.05%). All plants were sprayed to runoff using approximately 1 ml solution per plant. A randomized complete block design of four replicates with six plants per treatment was used. Two months later, following a flush of growth, plants were evaluated for response to cytokinin treatment.

B. BA concentration on individual shoots: This study was designed to determine the optimum concentration of BA application required to stimulate axillary bud growth on individual shoots of larger holly plants. Liners 20 cm tall with approximately 25 shoots each were used. Two individual shoots per plant were treated with BA at 0, 600, 800, 1000, or 1200 ppm. To insure localized applications to individual shoots only, the small end of a funnel was lowered over the shoot and the sprayer was directed into the face of the funnel. Each shoot was sprayed with 1 ml solution. At the time of application terminal buds were swelling just prior to a flush of growth. A randomized complete block design of five replicates with three shoots per treatment was used.

C. BA concentration on whole plants: This experiment was conducted to
determine the optimum concentration of BA application to stimulate axillary bud development on entire plants. A secondary purpose of this experiment was to determine the effect of BA concentration on mineral nutrient content and soluble carbohydrate content of treated shoots. Liners 20 cm tall with approximately 25 shoots each were treated with 5 ml solution of BA at 0, 600, 800, 1000, or 1200 ppm. A randomized complete block design of three replicates containing six plants per treatment was used.

Eighteen days later when stem thickening and red pigmentation was observed on treated plants, terminal stem sections 4 cm long were taken from three plants per treatment of each replicate to determine mineral nutrients and soluble carbohydrate contents. Plant tissue samples were oven-dried for 48 hr. at 70°C. Samples were then ground in a Wiley mill using a 20 mesh screen and total N determinations were made using a modified micro-Kjeldahl method (32). Phosphorus was determined colorimetrically with a Bausch and Lomb Spectronic 20 at 640 nm (29). Atomic absorption spectrophotometry was used to determine K, Mg, and Ca contents (15). A modified autoanalyzer system with a potassium ferricyanide reagent and a sodium phosphate buffer were used to determine soluble carbohydrate content (58).

Two months after treatment and following a flush of growth, remaining plants were evaluated for response to BA treatment.

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6 Perkin-Elmer Corp., Norwalk, Conn.
7 Technicon Instrument Corp., Tarrytown, N.Y.
D. BA concentration on pruned plants: This experiment was conducted to determine the optimum concentration of BA application to stimulate axillary bud development on pruned plants. Secondary observations on the effects of BA application on leaf size and relative cell size were recorded. Liners 20 cm tall with approximately 10 shoots were pruned back to 12 cm above the soil line. Two weeks later, when axillary bud swelling was apparent, plants were treated with 5 ml solution of BA at 0, 600, 800, 1000, or 1200 ppm. A randomized complete block design of five replicates with three plants per treatment was used.

Two months later, following a flush of growth, responses to BA treatment were recorded. At this time leaves were also collected from the fourth node of shoots of the new flush of growth following BA treatment. Thirty leaves per treatment were studied. Average leaf area in cm\(^2\) was determined using an automatic area meter\(^8\).

Individual leaves were then placed in test tubes and covered with 5 ml of a 1:1 (v/v) mixture of glacial acetic acid and hydrogen peroxide (35%). Test tubes were held in a boiling water bath under an exhaust hood for 30 minutes or longer if necessary. (Trimming edges of the leaf facilitated digestion.) When the upper and lower epidermal layers were bleached and separated, the liquid was decanted and the leaves were washed several times with distilled water. Epidermal layers remain intact and can be stored several months in distilled water under refrigeration.

Upper epidermal layers were studied using a microscope at 440 X.

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\(^8\) Hayashi Denko Co., Ltd., Tokyo, Japan
An eyepiece micrometer disc ruled in squares was inserted in the eye-piece. To relate cell size to actual measurement, a stage micrometer ruled in .1 and .01 mm increments was observed through the eyepiece micrometer.

E. BA treatment at various phases of development: The effectiveness of BA 800 ppm treatment to stimulate axillary bud growth on unbranched rooted cuttings when applied at various phases of bud development was studied (Fig. 2). The following four phases were:

Phase I  - one week after removal from the mist and prior to any apparent axillary bud swelling
Phase II  - when buds averaged 3 mm in length
Phase III - axillary shoots were 2.5 cm long
Phase IV - plants had completed the initial flush of growth and terminal buds were swelling prior to a second flush of growth.

Each rooted cutting was treated with 1 ml BA solution at 800 ppm. A randomized complete block design of four replicates with six plants per treatment was used.

After plants were evaluated for initial response to cytokinin treatment at various phases of growth, half of the plants in each treatment were pruned back to 12 cm above the soil line. The purpose of this pruning was to determine if cytokinin response in respect to increased shoot number would persist following pruning and subsequent regrowth. Two months later plants were evaluated.

F. Additive effect of multiple BA treatments: The purpose of this experiment was to determine if there was an additive effect of treating
Figure 2. Phases of bud development of 'Rotundifolia' holly liners from left to right: Phase I - no apparent axillary bud swelling, Phase II - axillary buds averaged 3 mm long, Phase III - axillary shoots were 2.5 cm long, Phase IV (not shown) - at the end of the initial flush of growth, buds swelling prior to secondary growth.
rooted cuttings with BA 800 ppm at both Phase II and at Phase IV. Separate groups of plants were treated at Phase II, Phase II and IV combined, and at Phase IV with intact controls at each treatment date. A randomized complete block design of five replicates with four plants per treatment was used. Six weeks after Phase IV treatment, plants were evaluated for response to BA treatment. All plants were oven-dried at 70°C for two weeks to determine total dry weight.
IV. RESULTS AND DISCUSSION

A. Cytokinin concentration on rooted cuttings: The greatest number of axillary buds stimulated to grow were on plants treated with BA at 800 ppm, BA at 400 ppm, and PBA at 600 and 800 ppm (Table 1). Decapitated controls produced fewer shoots per plant than any other treatment (Fig. 3). This is an indication that the common practice of decapitating holly liners reduces the total number of shoots per plant by removing the terminal bud as well as four or five adjacent axillary buds. Decapitation also resulted in growth from the uppermost remaining buds while buds on cytokinin-treated plants and intact controls developed from nodes near the soil line first and proceeded up the stem. This development of basal buds was similar to responses in willows where buds adjacent to the roots developed first (43). Treatment with BA at 800 ppm resulted in well branched liners with actively growing basal shoots not controlled by the apical shoot. However, apical shoots of control plants soon became dominant and suppressed further development of basal shoots. Plants treated with BA at 400 ppm and PBA at 800 ppm also had a tendency to develop a strong apical shoot similar to intact controls. Consequently, it was concluded that in this experiment the most desirable plants (based on total shoot number and uniform growth of all shoots) were plants treated with BA at 800 ppm.

B. BA concentration on individual shoots: There was no promotion of axillary bud growth on treated shoots except where there was an obvious suppression of the apical bud by treatment with BA at 1000 ppm or 1200 ppm (Table 2). These higher levels of BA seemed to act as chemical
Table 1. Growth responses of 'Rotundifolia' holly to applications of various concentrations of cytokinins.²

<table>
<thead>
<tr>
<th>Cytokinin concn. (ppm)²</th>
<th>Total no. shoots/plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact Control</td>
<td>5.2 d</td>
</tr>
<tr>
<td>Decapitated Control</td>
<td>4.0 e</td>
</tr>
<tr>
<td>BA 400</td>
<td>6.2 ab</td>
</tr>
<tr>
<td>BA 600</td>
<td>5.6 bcd</td>
</tr>
<tr>
<td>BA 800</td>
<td>6.4 a</td>
</tr>
<tr>
<td>PBA 400</td>
<td>5.4 cd</td>
</tr>
<tr>
<td>PBA 600</td>
<td>5.9 abcd</td>
</tr>
<tr>
<td>PBA 800</td>
<td>6.0 abc</td>
</tr>
</tbody>
</table>

² Means of 24 plants per treatment; mean separation by Duncan's multiple range test at 5% level

² Applications repeated after 24 hours
Figure 3. Growth patterns of 'Rotundifolia' holly liners following BA treatment. Left to right; Intact control, Decapitated control, BA at 800 ppm.
Table 2. Growth responses of individual 'Rotundifolia' holly shoots to various concentrations of BA.\textsuperscript{z}

<table>
<thead>
<tr>
<th>BA concn. (ppm)\textsuperscript{Y}</th>
<th>No. new shoots per treated stem</th>
<th>No. axillary breaks on apical shoots</th>
<th>Visual observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0 b</td>
<td>0.0 c</td>
<td>no stem thickening</td>
</tr>
<tr>
<td>600</td>
<td>0.0 b</td>
<td>2.8 b</td>
<td>slight stem thickening</td>
</tr>
<tr>
<td>800</td>
<td>0.0 b</td>
<td>5.3 a</td>
<td>heavy stem thickening</td>
</tr>
<tr>
<td>1000</td>
<td>2.1 a</td>
<td>0.0 c</td>
<td>slight stem thickening</td>
</tr>
<tr>
<td>1200</td>
<td>1.6 a</td>
<td>0.0 c</td>
<td>no stem thickening</td>
</tr>
</tbody>
</table>

\textsuperscript{z} Means of 15 shoots per treatment; mean separation within columns by Duncan's multiple range test at 5\% level

\textsuperscript{Y} Applications repeated after 24 hours
pinching agents thereby promoting the growth of axillary buds below the apex as mechanical pruning would (Fig. 4). Supraoptimal levels of applied cytokinins have been shown to curtail sharply the extent of cell division in tobacco plants (14) and to reduce the numbers of new shoots on hollies (39). The apical buds of control plants and of plants treated with BA at 600 and 800 ppm remained dominant and grew normally. Shoots produced by the apical buds on plants treated with BA at 600 or 800 ppm developed numerous axillary bud breaks (Table 2; Fig. 5). No axillary bud growth occurred on control plants.

The initial response observed on individual shoots two weeks after treatment was a thickening of the terminal 4-5 cm of shoots treated with BA at 800 ppm (Fig. 6). This stem thickening was evident to a lesser degree in stems treated with BA at 600 ppm and completely lacking in control plants. Since the affected stem sections were treated after a flush of growth was completed, it is proposed that the thickening was not due to stimulation of cell division but to cell expansion due to accumulation of nutrients and assimilates by hormone-directed transport. This is in agreement with other workers who have found that applied growth regulators affected hormone-directed transport of nutrients (31) and assimilates (19,28,41).

In conjunction with thickening of stem sections a red pigmentation was also observed. The most prominent pigmentation was noted on stem sections which exhibited the greatest thickening (i.e. BA 800 ppm treated stems). The development of a red pigmentation in holly stems treated with BA is similar in appearance to red pigments developed in grape leaves and inflorescence following treatment with BA and PBA (27).
Figure 4. Slight swelling and inhibition of 'Rotundifolia' holly shoots treated with BA at 1000 ppm, left, and BA at 1200 ppm, right.
Figure 5. Axillary shoot development on apical shoots of 'Rotundifolia' holly stems treated with BA at 800 ppm.
Figure 6. Stem thickening of 'Rotundifolia' holly shoots treated with BA at 800 ppm.
Bachelard (2) also reported the formation of anthocyanin in red maple cuttings following root formation. Bamberger and Mayer (3) reported the induction of "nitrogenous anthocyanins" in Amaranthus seedlings following treatment with kinetin and suggested that the kinetin in some way affected nitrogen metabolism. As the new flush of growth following BA treatment of 'Rotundifolia' liners proceeded, the red pigmentation faded. This is in agreement with the transitory appearance of cytokinin-induced pigments mentioned earlier (2,27). The appearance and subsequent disappearance of the red pigments may be another indication of hormone-directed transport.

Enhanced axillary breaks on apical shoots growing from stems treated with BA at 600 or 800 ppm may have been the result of localized accumulation of nutrients and carbohydrates releasing axillary buds from correlative inhibition.

C. **BA concentration on whole plants:** Treatment with BA at various concentrations did not enhance the total number of new shoots per plant (data not shown). It is proposed that BA treatments were not applied at the optimal phase of bud development for uptake by the plant. Lewis and Haun (23) demonstrated that the phase of bud development is critical to plant response to plant growth regulators. The destruction of apical buds of plants treated with BA at 1200 ppm observed in the preceding experiment did not occur indicating that treatment may have slightly preceded the optimum time for BA application.

When stem sections were analyzed for total nitrogen content, percent nitrogen of sections treated with BA at 1200 ppm was greater than all other treatments (Table 3). There were no differences in P, K, Mg
Table 3. Mineral nutrient and soluble carbohydrate content of 'Rotundifolium' holly shoot sections as affected by BA application.

<table>
<thead>
<tr>
<th>BA concn. (ppm)</th>
<th>Nutrient content (% dry wt.)</th>
<th>Total soluble carbohydrates (% dry wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>P</td>
</tr>
<tr>
<td>Control</td>
<td>2.64 b</td>
<td>.26</td>
</tr>
<tr>
<td>600</td>
<td>2.62 b</td>
<td>.24</td>
</tr>
<tr>
<td>800</td>
<td>2.74 b</td>
<td>.18</td>
</tr>
<tr>
<td>1000</td>
<td>2.74 b</td>
<td>.18</td>
</tr>
<tr>
<td>1200</td>
<td>2.93 a</td>
<td>.21</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

\(^2\) Means of nine plants per treatment; mean separation within columns by Duncan's multiple range test at 5% level; n.s. = not significant

\(^Y\) Applications repeated after 24 hours
and Ca content among treatments. Differences in soluble carbohydrate content were relatively small and cannot be related to growth.

D. BA concentration on pruned plants: There were no differences in total numbers of shoots per plant nor average shoot length among treatments. It is likely that harsh pruning and fertilization can account for maximum axillary bud breaks even on control plants.

While there was no stimulation of axillary bud development by BA treatment, there was a noticeable suppression of leaf size on stems which grew following BA treatment. Cytokinins have been observed to cause a suppression of leaf size in tobacco (40) and in hollies (60). In this experiment there was a 20% suppression of average leaf size on plants treated with BA at 600, 800, or 1000 ppm and a 46% suppression of average leaf size on plants treated with BA at 1200 ppm (Table 4).

While workers have reported a suppression in leaf size as a response to cytokinin treatment, the effect on cell size and cell numbers was not investigated (40,60). When leaves in this experiment were partially digested and the upper epidermal layers observed under the microscope, it was calculated that BA treatment resulted in cells up to 24% larger than cells of control plants (Fig. 7). When average leaf area and numbers of cells per grid were used to calculate the average number of upper epidermal cells per leaf, it was found that BA application at 1200 ppm resulted in a 60% decrease in cell numbers (Table 4). This is in agreement with work with tobacco pith cells which indicated that supraoptimal levels of applied cytokinin inhibited cell division (14). Hagen and Marcus (14) reported up to 85% inhibition of the
Table 4. Effects of BA concentration on leaf size and upper epidermal cell numbers of 'Rotundifolia' holly.

<table>
<thead>
<tr>
<th>BA concn. (ppm)</th>
<th>Avg. leaf area (cm²)</th>
<th>Relative cell size (cells/grid)</th>
<th>No. cells per leaf (in 1000's)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.32 a</td>
<td>41 a</td>
<td>195.8 a</td>
</tr>
<tr>
<td>600</td>
<td>1.85 b</td>
<td>38 b</td>
<td>145.8 b</td>
</tr>
<tr>
<td>800</td>
<td>1.86 b</td>
<td>37 bc</td>
<td>141.6 b</td>
</tr>
<tr>
<td>1000</td>
<td>1.90 b</td>
<td>35 c</td>
<td>134.6 b</td>
</tr>
<tr>
<td>1200</td>
<td>1.23 c</td>
<td>31 d</td>
<td>79.0 c</td>
</tr>
</tbody>
</table>

*Means of 30 leaves per treatment; mean separation within columns by Duncan's multiple range test at 5% level

Y Applications repeated after 24 hours
Figure 7. Relative sizes of upper epidermal cells of leaves from 'Rotundifolia' holly (left to right) treated with BA at 0 ppm, 1000 ppm, and 1200 ppm (magnification = 750 X).
increase in cell number of cultured tobacco pith cells. The results of this experiment could suggest that as cell division is curtailed by BA application, available nutrients and assimilates are utilized in greater cell expansion.

E. BA treatment at various phases of development: Application of BA at 800 ppm enhanced axillary bud growth when applications were made either at Phase II or at Phase IV (Table 5). Enhanced shoot growth on plants treated at Phase II developed from axillary buds on the single stem liner. Treatment at Phase IV stimulated a secondary flush of growth on the shoots which grew during the initial flush of growth. While the total number of shoots per plant for Phase II and Phase IV treated plants were the same, the distribution of the shoots was critical to the quality of plant form (Fig. 8). Phase II plants were shorter, compact and completely lacked the secondary flush of growth which characterized Phase IV plants and occurred sporadically in control plants.

Apical buds of Phase II plants and Phase IV plants were similar in physiological development in that Phase IV plants were about to start a second flush of growth. This is a further indication that the physiological stage of bud development is critical to the effectiveness of applications of growth regulators and may be linked to cytokinin penetration of expanding bud scales as in tea crabapples (6).

Following pruning and regrowth, no differences in the total numbers of shoots per plant of unpruned plants was observed over all treatments (Table 5). The trend of Phase II and Phase IV plants having more shoots than all other treatments remained the same. Pruning
Table 5. The effects of BA treatment at 800 ppm and pruning on growth of 'Rotundifolia' holly.

<table>
<thead>
<tr>
<th>Phase of treatment</th>
<th>Number of initial shoots</th>
<th>Total no. shoots</th>
<th>Final height (cm)</th>
<th>Total dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unpruned</td>
<td>Pruned</td>
<td>Unpruned</td>
<td>Pruned</td>
</tr>
<tr>
<td>Intact Control</td>
<td>6.2 b</td>
<td>9.0</td>
<td>18.3</td>
<td>32.0</td>
</tr>
<tr>
<td>Decapitated Control</td>
<td>4.9 c</td>
<td>7.3</td>
<td>18.0</td>
<td>35.0</td>
</tr>
<tr>
<td>Phase I</td>
<td>6.7 b</td>
<td>6.9</td>
<td>18.5</td>
<td>35.4</td>
</tr>
<tr>
<td>Phase II</td>
<td>7.9 a</td>
<td>9.4</td>
<td>20.6</td>
<td>27.0</td>
</tr>
<tr>
<td>Phase III</td>
<td>6.5 b</td>
<td>7.3</td>
<td>18.3</td>
<td>30.3</td>
</tr>
<tr>
<td>Phase IV</td>
<td>8.6 a</td>
<td>9.9</td>
<td>20.6</td>
<td>32.7</td>
</tr>
</tbody>
</table>

n.s. n.s. n.s. n.s. n.s. n.s.

z Mean separation within columns by Duncan's multiple range test at 5% level; n.s. = not significant

y Applications repeated after 24 hours

x Means of 24 plants per treatment

w Means of 12 plants per treatment
Figure 8. Growth of 'Rotundifolia' holly liners following BA treatment. Left to right; Phase II, Phase IV, Control. (Arrows indicate secondary shoots.)
more than doubled the total number of shoots per plant of all treatment phases including controls. In both pruned and unpruned plants, plants from treatments with fewer initial shoots developed more secondary breaks. This accounts for the loss of difference between phases. Pruning reduced the final plant height of all treatments by an average of 20% and reduced the total dry weight by 13%. These reductions can be related solely to removal of top growth rather than to a suppression in the secondary flush of growth.

F. Additive effect of multiple BA treatments: Plants with combined treatment at Phase II and again at Phase IV developed more shoots per plant than all other treatments (Table 6). Although not significant at the .05 level, plants treated with BA at Phase II had 14% more shoots per plant than controls and plants treated at Phase IV had 33% more shoots per plant than controls. Combined treatment at Phase II and again at Phase IV resulted in a 68% increase in total number of shoots per plant over controls. This additive effect can be accounted for by the fact that the effectiveness of cytokinins to stimulate axillary bud growth on hollies is enhanced when applied at or near bud break. Only one shoot of Phase II treated plants (the rooted cutting) was available for cytokinin absorption. Phase IV plants contained several axillary shoots stimulated to grow on the liner by high nutrition maintained throughout the experiment. Plants treated at Phase II and again at Phase IV contained the greatest number of shoots available for cytokinin penetration on the second treatment date.

Combined treatment at Phase II and again at Phase IV also resulted in an 18% suppression in final plant height and a 12% suppression in
Table 6. The effects of BA treatment at various phases of bud development on growth of 'Rotundifolia' holly.\(^z\)

<table>
<thead>
<tr>
<th></th>
<th>BA 800 ppm(^Y)</th>
<th>Total no. shoots/plant</th>
<th>Final height (cm)</th>
<th>Total dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase II</td>
<td>8.4 b</td>
<td>25.7 a</td>
<td>3.3 ab</td>
<td></td>
</tr>
<tr>
<td>Phase II Control</td>
<td>7.4 bc</td>
<td>25.4 a</td>
<td>3.6 a</td>
<td></td>
</tr>
<tr>
<td>Phase II + IV</td>
<td>11.6 a</td>
<td>20.9 b</td>
<td>3.1 b</td>
<td></td>
</tr>
<tr>
<td>Phase II + IV Control</td>
<td>6.9 c</td>
<td>25.4 a</td>
<td>3.5 a</td>
<td></td>
</tr>
<tr>
<td>Phase IV</td>
<td>8.5 b</td>
<td>21.6 b</td>
<td>3.0 b</td>
<td></td>
</tr>
<tr>
<td>Phase IV Control</td>
<td>6.4 bc</td>
<td>24.4 a</td>
<td>3.3 ab</td>
<td></td>
</tr>
</tbody>
</table>

\(^z\) Means of 20 plants per treatment; mean separation within columns by Duncan's multiple range test at 5% level

\(^Y\) Applications repeated after 24 hours
total dry weight per plant (Table 6). This suppressed shoot growth of cytokinin-induced shoots is in agreement with earlier work with hollies (60), roses (7), and apples (56). One explanation for a suppression in growth of BA-induced shoots in this study is that the greater number of actively growing shoots on such small plants are in direct competition for available nutrients and assimilates. Therefore, plants with greater numbers of shoots did not attain the final height of plants with fewer actively growing shoots. A second possibility is suggested by reports that axillary buds released by cytokinin applications usually fail to reach self-sufficiency in auxin and gibberellin (1,34,36,37). The suppression in dry weight could be due to an obvious suppression in average leaf size of BA treated plants since the leaves represent a large portion of the total dry weight of small holly liners.
V. SUMMARY AND CONCLUSIONS

Applications of BA at 800 ppm to unbranched rooted cuttings of 'Rotundifolia' holly enhanced axillary shoot development resulting in well branched liners. Applications must be made as buds are swelling prior to a flush of growth. Applications to the same plants preceding two successive growth cycles had an additive effect resulting in a 68% increase in number of shoots per plant over controls.

Applications of BA at 1000 ppm and 1200 ppm to individual shoots of larger plants inhibited apical bud growth. Applications of BA at 1200 ppm to whole plants resulted in growth of leaves which were 46% smaller than leaves of control plants. These suppressed leaves contained 60% fewer upper epidermal cells than leaves of control plants and average epidermal cell size was 24% larger than those of control plants.

While applications of BA have been effective in stimulating axillary shoot growth on 'Rotundifolia' holly, it must be recognized that even under the controlled conditions of these experiments responses to BA treatment have been variable. It must also be recognized that the timing for effective BA application is critical and may be limited to several days preceding an active flush of growth. Nevertheless, nurserymen might benefit by treating unbranched rooted liners prior to the initial flush of growth and subsequent movement to the field. Further research under field conditions should be conducted before recommendations for the use of BA in the nursery industry can be made.
VI. LITERATURE CITED


37. -------------. 1967. The role of auxins and cytokinins in the release of buds from dominance. Amer. J. Bot. 54:136-144.


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THE EFFECTS OF 6-BENZYLAMINO PURINE ON AXILLARY BUD GROWTH
OF ILEX CRENATA THUNB. 'ROTUNDIFOLIA'

by
David W. Bradshaw

(ABSTRACT)

The effects of 6-benzylamino purine (BA) concentration and timing of BA application at various phases of bud development of Ilex crenata Thunb. 'Rotundifolia' were studied.

Foliar applications of BA at 800 ppm to unbranched rooted cuttings enhanced axillary bud development resulting in well branched liners. Axillary bud development of rooted cuttings was enhanced when applications were made when buds were swelling preceding a flush of growth. Applications made prior to bud swelling or made during a flush of growth were ineffective in stimulating additional axillary bud development. Combined applications of BA at 800 ppm to liners preceding two successive flushes of growth resulted in a 68% increase in total numbers of shoots per plant over control plants. Applications of BA at 600 ppm and 800 ppm to individual shoots resulted in swelling of the terminal 4-5 cm section of treated stems and development of a red pigment followed by growth of numerous axillary shoots on the stem produced by the apical bud. Applications of BA at 1000 ppm and 1200 ppm to individual shoots acted as chemical pinching agents inhibiting apical buds. Leaves on shoots which developed from liners treated with BA at 1200 ppm were 46% smaller than those on control plants. These suppressed leaves con-
tained 60% fewer upper epidermal cells than leaves of control plants, and average epidermal cell size was 24% larger than those of control plants.