J Plant Growth Regul (2003) 21:459–468
DOI: 10.1007/s00344-002-0022-3

J P G R
Journal of Plant Growth Regulation



CONTRIBUTED ARTICLES

# Exposure of Petunia Seedlings to Ethylene Decreased Apical Dominance by Reducing the Ratio of Auxin to Cytokinin

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## ABSTRACT

Seedlings of *Petunia* x *hybrida* 'Orchid' treated with the ethylene-releasing compound ethephon at 0.9, 1.7, and 3.5 mM evolved ethylene at a higher rate as the concentration of ethephon increased. Regardless of the concentration of ethephon applied, ethylene evolution peaked 6 to 8 h following application. Evidence that ethephon application decreased apical dominance included an increase in the number of new nodes on the main stem and a sustained increase in the length of new and existing lateral shoots compared to the control (no ethephon). Plants treated with 3.5 mM ethephon developed mild chlorosis, whereas a concentration of 1.7 mM ethephon decreased apical dominance without phytotoxic effects. The auxin/cytokinin ratio decreased in the apical shoot section as early as 1 h after ethephon treatment. In contrast, a decrease in the ratio in the subapical shoot section was not detected until 24 h after ethephon application. Reduction in auxin/cytokinin ratio was a result of a decrease in indole-3-acetic acid (IAA) and an increase of zeatin riboside (ZR), but not isopentenyladenosine (iPA). These results suggest that exposing 'Orchid' petunia seedlings to ethylene via ethephon lowers the auxin/cytokinin ratio, thereby promoting the outgrowth of lateral shoots.

**Key words:** *Petunia* x *hybrida*; Ethephon; Indole-3-acetic acid; Isopentenyladenosine; Zeatin riboside; Lateral shoots; Plant architecture

## INTRODUCTION

*Petunia* x *hybrida* Hort. Vilm.-Andr. seedlings are modified to a short bushy architecture by the outgrowth of lateral buds and shoots (that is, reduced

Received: 12 August 1999; accepted: 18 November 2002; Online publication: 28 March 2003
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apical dominance) following the application of ethephon, an ethylene-releasing compound (Burg 1973; Haver and Schuch 2001). Similar effects have been documented in other ornamental species (de Wilde 1971; Ernest and Valdovinos 1971; Emery and others 1998). Auxins and cytokinins are generally recognized as the more important factors controlling apical dominance (Bangerth and others 2000) and their role has been confirmed through the use of the tools of molecular biology (Klee and Romano 1993; Cline 1994). Whereas it is clear that the ratio of auxin to cytokinin, not the absolute amount of either hormone, controls apical dominance and hence, plant architecture (Strafstrom 1993; Bangerth 1994; Cline 1994; Emery and others 1998), the mechanism controlling this ratio remains unresolved (Beveridge 2000). However, it is presumed that biotic and abiotic factors that alter apical dominance do so by altering the ratio of auxin/cytokinin (Hillman 1984).

Exposure of plant tissues and organs to ethylene at concentrations higher than basal ethylene levels triggers expression of competent genes (Osborne and others 1984; Bleecker 1991) that can alter the synthesis, degradation, or action of hormones (Suttle 1991; Sanyal and Bangerth 1998; Winer and others 2000). It is well documented that ethylene reduces endogenous IAA concentrations by several different mechanisms: ethylene-stimulated conjugation of IAA to inactive products (Riov and others 1982); decreased polar transport of IAA from the shoot apex, the main IAA source in young seedlings (Suttle 1991; Sanyal and Bangerth 1998); and stimulated IAA catabolism (Sagee and others 1990; Winer and others 2000). For petunia it is not known whether exposure to ethylene reduces transport of auxin from the shoot apex enough to cause a shift in auxin/cytokinin ratio sufficient to evoke the observed morphological changes or whether a commensurate increase in cytokinin concentration to the axillary buds is also involved. Decapitating bean plants reduced basipetal transport of IAA and resulted in a rapid and significant increase in cytokinins in the xylem sap and loss of apical dominance (Bangerth 1994). These responses were reversed when auxin was replaced (Li and Bangerth 1999). The source of cytokinins is presumed to be the roots (Beck 1996; Beveridge and others 1997; Bangerth and others 2000), but increased synthesis in the buds or stem has not been ruled out (King and Van Staden 1990; Cline 1994; Faiss and others 1997). We hypothesize that in petunia, ethylene released from ethephon treatment acts not only by decreasing IAA but also by increasing cytokinin concentrations, resulting in a more compact bushy plant as the ratio shifts in favor of cytokinins.

Response to ethephon is variable, from undesirable phytotoxic effects to a complete lack of response. This variability results from uneven breakdown of ethephon and the influence of the plants' nutritional and water status on basal ethylene metabolism, wounding, pathogen invasion, the target tissue, and the autocatalytic synthesis of ethylene in response to treatments (Abeles and others 1992). Ethephon releases ethylene upon contact with a solution or surface with a pH higher than 4.0 (Warner and Leopold 1969; Yang 1969; Biddle and others 1976; Denney and Martin 1994). The rate of ethylene release is dependent on ambient air temperature and relative humidity, the chemistry of the plant surface, and the rate of its metabolism within the cell (Beaudry and Kays 1988; Denney and Martin, 1994; Olien and Bukovac 1978). Any change in one or more of these factors leads to a change in the rate and quantitative amount of ethylene released from ethephon. In most cases, the majority of ethylene produced upon initial exposure to the ethylene-releasing compound is from the degradation of the applied compound and not from endogenous biosynthesis (Olien and Bukovac 1978; Lurssen 1982; Beaudry and Kays 1988). Whether or not this is the case for petunia remains to be determined. Changes in plant architecture result only when target tissues are exposed to ethylene levels higher than endogenous basal levels. The ability to attain a desired plant response to ethephon application might be improved by knowing how much ethylene is released from ethephon relative to the amount produced endogenously by the target tissue.

The goal of this study was to determine the concentration of exogenously applied ethylene required to reduce apical dominance in a commercial cultivar of petunia. To improve the reproducibility of the treatment, the amounts of ethylene derived from applied ethephon and endogenously synthesized by the plant were measured. Additionally, we quantified the degree of apical dominance expressed during vegetative growth as well as the auxin/cytokinin ratio in apical and subapical halves of the shoot of ethephon-treated and untreated petunia plants. The objective was to determine if ethylene reduces apical dominance by reducing IAA and/or increasing the cytokinin concentration of the petunia shoot. An understanding of the mechanism by which ethephon treatment reduces apical dominance might make it possible to improve the reliability of the commercial practice.

**Table 1.** Seedling Age of *Petunia* x *hybrida* 'Orchid' at Harvest

| Ethephon concentration (mM) | Experiment II Age (days) | Experiment III Age (days) |
|---|---|---|
| 0 (control) | 55 | 58 |
| 0.9 | 59 | 62 |
| 1.7 | 62 | 62 |
| 3.5 | 63 | 64 |

*Harvest of each treatment began when four or more of the eight replications were at first flower. Plants were 42 days old when treated.*

## Material and Methods

### Plant Material

Seeds of *Petunia* x *hybrida* Hort. Vilm.-Andr. 'Orchid' (Madness Series, Ball Seed Co., West Chicago, IL) were sown on Metro-Mix 220 (The Scotts Co., Marysville, OH) in 162-cell plug trays (TLC Polyform, Inc., Plymouth, MN) with a volume of 12 mL/cell for the single-leaf experiment and in 512-cell plug trays (Blackmore Company, Inc., Belleville, MI) with a volume of 4.0 mL/cell for whole plant experiments. Following germination, seedlings were watered daily with a N-P-K (20.0-4.3-16.6) water soluble fertilizer (Peters Excel, Scotts-Sierra Horticult. Prod. Co., Marysville, OH) at 50 ppm N for the first week and at 150 ppm N for the remainder of the growing period. Photosynthetically active radiation (PAR) at canopy level, measured with a PAR quantum sensor (LI-COR, Lincoln, NE), averaged 1050 $Mmol \cdot m^{-2} \cdot s^{-1}$ midday under cloudless conditions between April and August. Glasshouse temperature setpoints were 25°C maximum day and 18°C minimum night. Temperature and PAR were continuously monitored with a datalogger (Campbell 21XL, Salt Lake City, UT).

### Single-Leaf Experiment (Exp. I)

To inhibit endogenous ethylene production, 42-day-old seedlings of 'Orchid' petunia were sprayed twice to runoff with a 100 MM solution of aminoethoxyvinylglycine (AVG, Sigma Chemical Co., St. Louis, MO) containing 0.1% X-77 Spreader (Kalo Laboratories, Inc., San Francisco, CA). Fourteen hours later the most recent fully expanded leaf was excised from plants of uniform size, weighed, and sprayed to runoff with distilled water (control) or with ethephon (Florel, 3.9% a.i., Rhone Poulenc, Research Triangle Park, NC) using a hand-held sprayer. Following treatment with ethephon, the leaf was weighed immediately to determine the amount of compound applied to the leaf surface. The leaf was inserted, petiole first, into a 27.6-mL glass test tube containing 1 mL of distilled water to keep the leaf fully hydrated. The test tubes were sealed with a 20-mm rubber stopper sleeve (Wheaton, Millville, NJ) at intervals between 0 and 12 h after ethephon treatment. After sealing the test tube and allowing a 2-h incubation in the dark at 20°C, a 1 mL gas sample was withdrawn from the headspace with a needle attached to a syringe and immediately injected into a gas chromatograph equipped with a flame-ionization detector (Perkin Elmer Autosystem with a 0.6 m × 3.2 mm column of Carboxen 1000, 45-60 mesh, 165°C column temperature, with air, hydrogen and helium at 420, 45, 60 $mL \cdot min^{-1}$, respectively). Test tubes were arranged in a completely randomized design with eight single-leaf replications per treatment per sampling time.

### Whole Plant Experiments (Exp. II and III)

Plug trays of 42-day-old 'Orchid' petunia seedlings were divided in half: one-half received two applications of a 100 MM AVG solution to inhibit endogenous ethylene production containing 0.1% X-77 Spreader; the other half was sprayed with distilled water. After 14 h each of the two sets of seedlings was treated with distilled water (control) or with ethephon applied to runoff using a hand-held sprayer. Treated seedling trays were arranged in a completely randomized design.

At 0 to 12 h following the application of ethephon, whole shoots of four randomly chosen plants from each treatment were severed at the substrate level. Samples for ethylene evolution were arranged in a completely randomized design with four whole-shoot replications per treatment per sampling time. The entire shoot was weighed and placed in a 60-mL syringe sealed with a 20-mm rubber stopper sleeve. Sampling and ethylene analysis of the sample was completed as described above. To evaluate vegetative development, an additional eight plants were randomly chosen from each treatment 24 h after ethephon treatment and transplanted into 5.1 cm square pots (100 mL) with the same medium used in plug trays and arranged in a completely randomized design. The plants were watered and fertilized as described above.

In previous experiments, we observed that lateral branching increased when flowering began, so plants within a treatment were harvested when four of the eight plant replications were at first flower

(Table 1). Seven of the eight replications were used to determine changes in apical dominance. Apical dominance was assessed by counting the number of nodes produced by the main stem and measuring and counting lateral shoots with a minimum of 1 cm of growth at first flower. Average lateral shoot length or lateral index was calculated by dividing the total length of lateral shoots by the total number of lateral shoots. Root and shoot dry mass were determined after drying in a forced air oven at 65°C for 48 h. The remaining replication was used for visual documentation and was not included in the analysis.

The initial whole-plant experiment was undertaken to determine ethephon concentrations that resulted in significant reductions in apical dominance. The entire experiment was repeated to verify the results and to correlate the response to changes in the auxin to cytokinin ratio in the shoot.

### Hormone Analysis

Indole-3-acetic acid (IAA), isopentenyladenosine (iPA), and zeatin riboside (ZR) were analyzed in apical and subapical shoot samples taken 1 h and 24 h after ethephon application. Samples were only taken from plants that were not treated with AVG but were treated either with distilled water (control) or with 1.7 mM ethephon, the concentration yielding the most favorable vegetative growth response (Haver 1998). The apical portion of the shoot included the shoot apex and two fully expanded leaves, while the subapical portion included the remainder of the shoot consisting of two to three fully expanded leaves and approximately 3 cm of stem tissue. Samples consisted of twenty pooled plants from each treatment with three replications per treatment. Samples were immediately frozen in liquid nitrogen, freeze-dried, and stored at −20°C until analysis.

Each sample was divided into three 150-mg subsamples and extracted in 12 mL of cold 80% methanol in 25-mL Erlenmeyer flasks for 16 h at 4°C. The internal standard $^{14}$C-IAA (specific activity 57 mCi/mmole, American Radiolabel Chemical, Inc., St. Louis, MO.) was added to each extraction to estimate purification losses for IAA. Parallel recovery studies with tritium-labeled iPA and ZR resulted in an average recovery of 87%. Recoveries were not used to correct for loss.

Purification of the samples and analysis were performed as described previously (Boner and Bangerth 1988a, b) with some modifications (Bertling and Bangerth 1995). Following the removal of methanol under low pressure, the extracted samples (adjusted to pH > 8.5 with 0.1 M ammonium acetate) were applied to a 5-mL polyvinylpyrrolidone (PVP) column below which was attached a 4-mL [2-(diethylamino)ethyl-Sephadex®] (DEAE) column. The column was eluted with 30 mL of 0.01 M ammonium acetate, pH 8.0. Cytokinins were collected in Sep-Pak C18 cartridges (Waters Corp.) attached to the end of the DEAE column. The PVP column was removed and acidic hormones were eluted from the DEAE column with 25 mL of 1.5 M acetic acid and collected in a second Sep-Pak C18 cartridge attached to the DEAE column. Sep-Paks were washed with 5 mL distilled water and the hormones were eluted from the cartridges with 4 mL (cytokinins) or 5 mL (acidic hormones) of 50% methanol and dried under vacuum. The acidic fraction, containing IAA, was methylated with diazomethane prior to radioimmunoassay (RIA) analysis.

Polyclonal antibody production, for use in RIA, included the coupling of each hormone to bovine serum albumin and the subsequent inoculation of rabbits with the coupled conjugate to produce antisera (Cutting and others 1983, 1986a, b). Tracer compounds consisted of tritium-labeled IAA and iPA obtained commercially (Amersham Life Science, Inc., Arlington Heights, IL.), and tritium-labeled ZR prepared with tritiated sodium borohydride (Weiler 1980; McDonald and others 1981). All immunoassays were performed in triplicate using the method described by Cutting and others (1983). Hormone quantification was calculated from the bound radioactivity using the spline approximation method and was reliable from 0.01 ng/100 µL to 50 ng/ 100 µL for each of the three hormones analyzed. Samples were diluted to obtain concentrations in this range.

### Statistical Analyses

Treatment effects on vegetative growth, ethylene production and amounts of IAA, iPA and ZR were determined by general linear model analysis with the statistical software package SAS (SAS Institute, Cary, NC). Means were separated using Duncan's Multiple Range test at $p \leq 0.05$ except as noted.

## Results

### Ethylene Evolution

The rate of ethylene evolved by a single-leaf of 'Orchid' petunia, treated with the ethylene synthesis inhibitor AVG prior to exposure of the leaf to ethephon under laboratory conditions (*Exp. I*), was

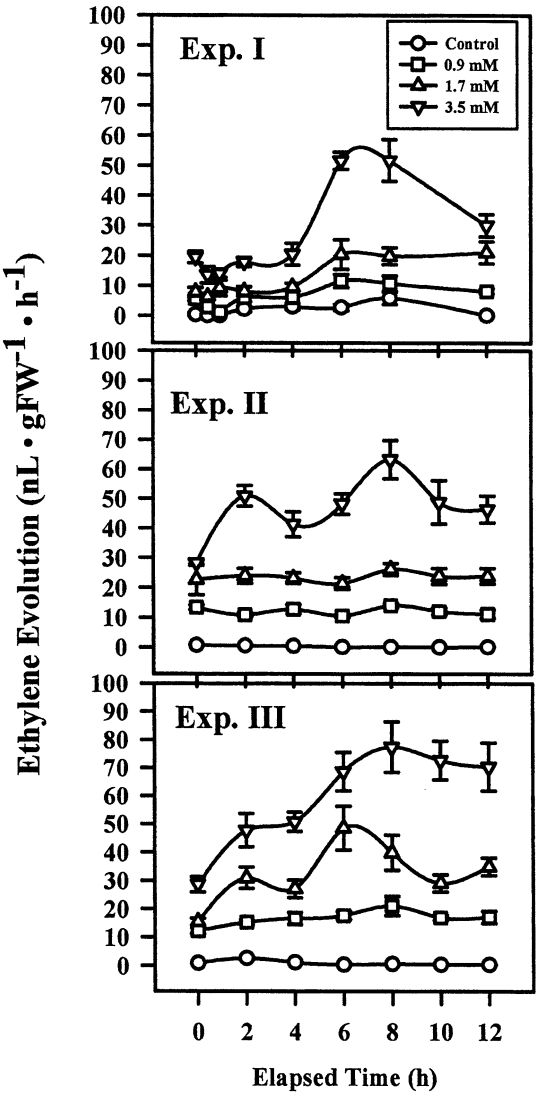


**Figure 1.** Rate of ethylene evolution of *Petunia* x *hybrida* 'Orchid' leaves under laboratory conditions (*Exp. I*) or whole shoots under greenhouse conditions (*Exp. II* and *III*) in response to treatment with ethephon at 0.9, 1.7, and 3.5 mM. Bars represent ± SE (n = 8).

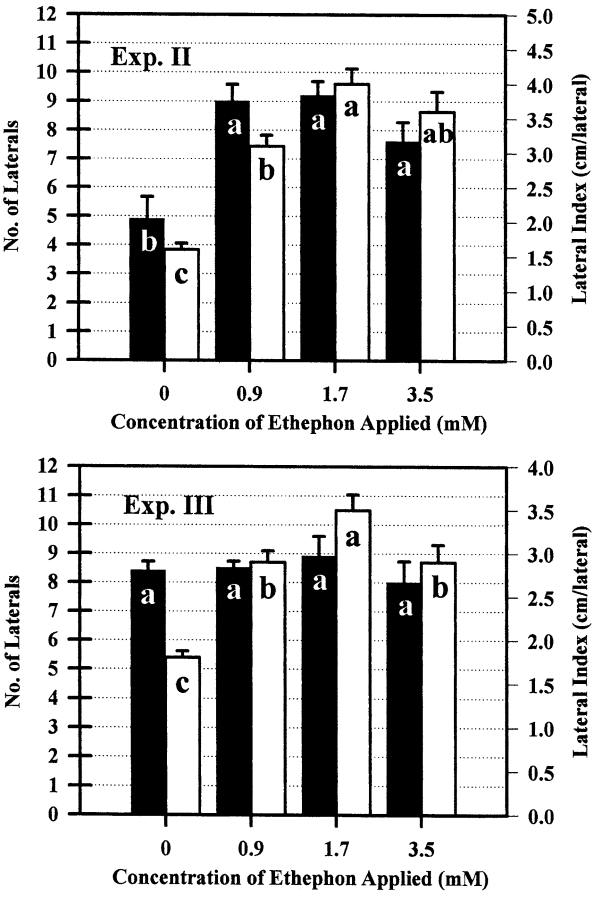


**Figure 2.** The number of lateral shoots 1 cm or longer (black bars) and the lateral index representing the average length of lateral shoots at least 1 cm long (white bars) at first flower (Table 1) of *Petunia* x *hybrida* 'Orchid' treated with ethephon at 0.9, 1.7, and 3.5 mM under greenhouse conditions (*Exps. II* and *III*). Results from AVG-treated plants and non-AVG-treated plants were not significantly different and therefore data were pooled. Means followed by different letters are significantlydifferent at $p \leq 0.05$, Duncan's Multiple Range Test Bars represent ± SE (n = 16).

similar to that obtained for a shoot from whole plants not treated with AVG prior to ethephon treatment in a greenhouse environment (*Exp. II* and *III*) (Figure 1). Shoots from whole 'Orchid' petunia plants treated with AVG evolved ethylene at rates similar to shoots from untreated control plants during the 12 h sampling period following the application of increasing concentrations of ethephon from 0 to 3.5 mM to each set of plants (data not shown). The results establish that endogenous ethylene biosynthesis in response to ethephon treatment at the concentrations used in this study is negligible in petunia and indicate that differences in the rates of ethylene evolved between *Exp. II* and *III* are solely due to differences in the rates of ethylene released from the exogenously applied ethephon. Ethylene evolution from a single-leaf or a whole petunia shoot treated with ethephon increased in proportion to the concentration applied (Figure 1). Therefore, ethylene evolution from the shoot of petunia is directly related to the concentration of ethephon applied to the plant and endogenous ethylene is eliminated as a variable influencing the response of petunia to ethephon treatment.

In all three experiments ethylene evolution reached the maximum rate within 30 min of the 0.9 mM ethephon application; this rate was sustained for 12 h (Figure 1). For the 1.7 mM ethephon treatment, ethylene evolution did not peak until 6 h

**Table 2.** Shoot and Root Dry Mass and Number of Nodes per Plant at First Flower of *Petunia* x *hybrida* 'Orchid' Treated with Three Concentrations of Ethephon

| | Experiment II | | | Experiment III | | |
|---|---|---|---|---|---|---|
| Ethephon Conc. (mM) | Node (No.) | Shoot Mass (g) | Root Mass (g) | Node (No.) | Shoot Mass (g) | Root Mass (g) |
| 0 (control) | 12 c[a] | 0.90 c | 0.20[a] | 15[c] | 1.06[b] | 0.35[b] |
| 0.9 | 13 b | 1.12 bc | 0.19[ab] | 16[c] | 1.21[b] | 0.34[b] |
| 1.7 | 16 a | 1.45 a | 0.21[a] | 19[b] | 1.46[a] | 0.44[a] |
| 3.5 | 16 a | 1.35 ab | 0.14[b] | 21[a] | 1.19[b] | 0.36[b] |

[a]*Means within a column followed by different letters are significantly different at $p \leq 0.05$, Duncan's Multiple Range.Results from AVG treated plants and non-AVG treated plants were not significantly different and therefore data were pooled (n = 14).*

after application in *Exp. I* and *III*. In *Exp. II*, ethylene reached the maximum rate immediately after spraying 1.7 mM ethephon and remained at that level for the 12-h sampling period with only minor fluctuations. Maximum rates were similar in *Exp. I* and *II*, but higher in *Exp. III* in response to 1.7 mM and 3.5 mM ethephon. In *Exp. II,* shoots treated with 3.5 mM ethephon produced bursts of ethylene with the maximum rate of 63 nL·g $FW^{-1}$·$h^{-1}$ occurring 8 h after ethephon application. Ethylene evolution also peaked 8 h after 3.5 mM ethephon was applied to shoots in *Exp. III,* but at a higher rate, 80 nL·g $FW^{-1}$·$h^{-1}$. Ethylene evolution from a single leaf under laboratory conditions (*Exp. I*) peaked approximately 6 to 8 h after application of 3.5 mM ethephon.

## Vegetative Growth Response to Ethephon

The number of new lateral shoots increased in ethephon-treated plants at all concentrations compared to controls in *Exp. II,* but no differences were observed in *Exp. III* (Figure 2). The lateral index increased in both experiments as ethephon concentration increased from 0.0 to 1.7 mM, even in *Exp. III* where the number of laterals did not increase in response to ethephon (Figure 2). The application of ethephon also increased the number of nodes produced by the main stem prior to the appearance of the first flower (Table 2). Ethephon at 1.7 and 3.5 mM increased the number of nodes by 25% in *Exp. II* and 21% to 29% in *Exp. III,* respectively, compared to the control. An increase in the number of nodes combined with an increase in the length of lateral shoots/node established a decrease in apical dominance in the two separate whole plant experiments.

Shoot dry mass increased as ethephon concentration increased from 0.0 to 1.7 mM and declined at 3.5 mM in both whole plant experiments (Table 2). The presence of mild chlorosis at 3.5 mM suggests possible phytotoxicity of 'Orchid' petunia to ethephon. Approximately twice as much root biomass was produced in *Exp. III* compared to *Exp. II,* but trends among treatments were similar. Root dry mass in both experiments declined with the 3.5 mM ethephon treatment, further indication of the sensitivity of 'Orchid' petunia to the high concentration of ethephon. In *Exp. II,* ethephon treatments ranging from 0.9 to 1.7 mM had no effect on root dry mass, whereas in *Exp. III,* root dry mass increased in response to the 1.7 mM ethephon treatment. Roots appeared healthy in both experiments, but as ethephon concentration increased, root penetration into the substrate declined (data not shown).

## Effect of Ethephon Treatment on IAA, iPA and ZR

Ethephon application at 1.7 mM significantly reduced the IAA concentration in the apical shoot section (Table 3). At both sampling times, ethephon reduced the IAA concentration 50% to 0.28 ng·-plant$^{-1}$ (data not shown).

Isopentenyladenosine concentrations in the apical shoot section were approximately 10-fold lower than ZR concentrations (data not shown) and showed no response to the 1.7 mM ethephon application, remaining unchanged over 24 h (Table 3). One hour after ethephon treatment the concentration of ZR in the apical shoot section was 850% higher than that of the control (Table 4). The substantial increase in ZR coupled with the decrease in IAA in the apical shoot section in response to ethephon application resulted in a 68% decrease in the auxin/cytokinin ratio after 1 h (Table 4). However, 23 h later (24 h after ethephon application) the concentration of ZR in ethephon-treated apical shoot sections decreased 34%, whereas the concentration of ZR in apical shoot sections from con-

**Table 3.** Summary of General Linear Model Analysis Representing P-values for IAA, iPA, ZR, and Auxin/Cytokinin ratio in Apical (A) and Subapical (SA) Shoot Sections of *Petunia* x *hybrida* 'Orchid' 1 or 24 h after Treatment with Distilled Water (control) or Ethephon at 1.7 mM

| | IAA | | iPA | | ZR | | | |
|---|---|---|---|---|---|---|---|---|
| | ($ng \cdot plant^{-1}$) | | ($ng \cdot plant^{-1}$) | | ($ng \cdot plant^{-1}$) | | Ratio | |
| Treatment | A | SA | A | SA | A | SA | A | SA |
| Concentration (C)[a] | 0.06 | NS | NS | NS | 0.009 | 0.08 | 0.0007 | NS |
| TAT[b] | NS | 0.07 | NS | NS | NS | 0.003 | 0.003 | 0.0001 |
| C x TAT[c] | NS | NS | NS | NS | 0.017 | 0.03 | 0.04 | 0.005 |

*[a]Ethephon concentration; 0 or 1.7 mM, [b]Time elapsed after treatment with ethephon, [c]Interaction between ethephon treatment and time elapsed after treatment.*
*[NS]Nonsignificant at $p \leq 0.08$.*

**Table 4.** Means Separation for Zeatin Riboside (ZR) and Auxin/Cytokinin Ratio in Apical (A) and Subapical (SA) Shoot Sections 1 or 24 h after Treatment with Distilled Water (control) or Ethephon at 1.7 mM (n = 3)

| | ZR ($ng \cdot plant^{-1}$) | | Ratio | |
|---|---|---|---|---|
| Ethephon Conc. (mM) × Time (h) | A | SA | A | SA |
| 0.0 mM × 1 h (control) | 0.16 c[a] | $0.56^{c}$ | $1.03^{a}$ | $4.05^{b}$ |
| 1.7 mM × 1 h | $1.52^{a}$ | $0.40^{c}$ | $0.33^{bc}$ | $4.91^{a}$ |
| 0.0 mM × 24 h (control) | $0.91^{b}$ | $1.75^{b}$ | $0.47^{b}$ | $2.01^{c}$ |
| 1.7 mM × 24 h | $1.01^{ab}$ | $2.74^{a}$ | $0.17^{c}$ | $1.37^{d}$ |

*[a]Means within a column followed by different letters are significantly different at $p \leq 0.08$, Duncan's Multiple Range.Each replication consisted of 20 plants pooled from each non-AVG treatment.*

trol plants increased 469% (Table 4). Thus, 24 h after treatment, the ZR concentration in ethephon-treated apical shoot sections was not significantly different from that of the control (Table 4). Despite the fact that ZR concentrations in the apical shoot sections were similar 24 h after treatment, the auxin/cytokinin ratio remained significantly low in the ethephon-treated apical shoot section compared to the control (Table 4) due to the lower IAA concentration.

Ethephon treatment had no effect on IAA concentration in the remainder of the shoot, the subapical shoot section (Table 3). Interestingly, IAA levels in the subapical shoot decreased 33% to 4.24 $ng \cdot plant^{-1}$ from the 1-h sampling to the 24-h sampling, regardless of treatment (data not shown).

As in the apical shoot section, iPA concentrations in the subapical shoot section were low, showed no response to the 1.7 mM ethephon application and remained unchanged over 24 h (Table 3). Ethephon treatment had no effect on the concentration of ZR in the subapical shoot section compared to control plants 1 h after application (Table 4). Despite a sizable increase in the concentration of ZR in the subapical sections of control plants between the 1-h and 24-h sampling times, the concentration of ZR in the subapical shoot section of ethephon-treated plants was 57% greater (Table 4).

A high auxin/cytokinin ratio in the subapical shoot section was observed in both ethephon-treated plants and the control at the 1-h sampling (Table 4). The ratio decreased after 24 h in both the control and ethephon-treated plants, resulting in a 32% lower ratio in ethephon-treated plants than in the control plants.

## Discussion

Apical dominance in petunia is considered to be ''intermediate'' due to the outgrowth of lateral buds without restriction of the shoot apex (Cline 1997). However, an ''intermediate'' response is still a function of the dominance of the shoot apex over

lateral buds. Evidence that ethephon application to *Petunia* x *hybrida* 'Orchid' reduced apical dominance included an increase in the number of nodes on the main stem accompanied by an increase in the number of new lateral shoots at these nodes and sustained elongation of these lateral shoots (*Exp. II*). Although the number of new lateral shoots was not increased by ethephon treatment in *Exp. III*, the lateral index increased in response to ethephon application as they did in *Exp. II*.

Whereas an increase in night temperature from 15.6°C to 21.1°C increases the length of lateral shoots in several petunia cultivars (Piringer and Cathey I960), it is unlikely that temperature contributed to the increased lateral shoot growth observed in *Exp. III* compared to *Exp. II*. Maximum and minimum setpoints in *Exp. II* and *III* were the same. Short-day photoperiods have been shown to increase lateral shoot development of petunia, but both experiments were conducted under long-day photoperiods (Merritt and others 1983).

Differences in lateral shoot growth observed in *Exp. II* and *III* can most likely be attributed to differences in root growth between the experiments. Overall root growth in the control plants in *Exp. III* was nearly double that of the control plants in *Exp. II*. Increased root growth may have provided the shoot with an increased supply of cytokinins. Although cytokinin levels were not measured in *Exp. II*, ZR levels in control plants increased between the 1-h and 24-h sampling times in both the apical and subapical portions of the shoot in *Exp. III* (Table 4). Most likely this increase is a snapshot of the larger increase that occurred over the course of *Exp. III*. Ethephon application further increased ZR levels compared to the controls at 1 h in the apical shoot and at 24 h in the subapical shoot, lowering the auxin/cytokinin ratio and promoting lateral shoot growth.

Bangerth and others (2000) equated lateral buds under apical dominance to dormant buds. Exogenous application of dormancy-breaking agents resulted in rapid increase in shoot cytokinin concentrations (Cutting 1991), especially ZR (Cook and others 2001). Thus it cannot be ruled out that ethephon application directly causes the accumulation of ZR in the apical and subapical shoot. Alternatively, the results of this study are consistent with an ethephon-induced reduction in IAA concentration that in turn causes accumulation of ZR by an IAA-cytokinin feedback loop (Bangerth 1994; Bangerth and others 2000). Bangerth (1994) observed that following the decapitation of bean plants, cytokinin concentrations in xylem exudate increased 25% after 16 h and 4000% after 24 h. In *Exp. III*, ZR increased 850% in the apical shoot 1 h after ethephon application compared to the control, but after 24 h, ZR levels were similar to the controls. Bangerth (1994) suggests that an IAA and cytokinin feedback loop allows the plant to respond rapidly to changes in the environment. Decreased transport of IAA out of the apex would increase transport of root-produced cytokinins in the xylem, thereby stimulating the shoot apex to resume production and transport of IAA out of the shoot, which in turn represses export of cytokinins out of the roots and into the shoots via the xylem. This response was confirmed by exogenous application of IAA to the decapitated shoot (Bangerth and others 2000). ZR, specifically, has been demonstrated to move from the roots via the xylem (Taylor and others 1990; Hammerton and others 1996). The IAA and ZR levels in *Exp. III* are consistent with this feedback loop. Ethephon application at 1.7 mM reduced IAA in the apical shoot section by 50%, a reduction that was detected at both the 1- and 24-h sampling times. Because of high variability, IAA levels were not significantly different in the subapical portion of the shoot. IAA levels, however, declined as ZR levels increased. Initially, ZR in the apical shoot increased, but after 24 h ZR decreased in the apical shoot and increased in the subapical portion of the shoot. Due to the decline in IAA accompanied by the increase in ZR, the auxin/cytokinin ratio decreased, favoring the growth of lateral shoots in the subapical shoot section.

In this study, concentrations of iPA were always low relative to ZR, consistent with rapid hydroxylation to ZR, and not affected by ethephon. The results suggest that the putative role played by the controlled hydroxylation of iP cytokinins to Z cytokinins in apical dominance (King and Van Staden 1990) does not operate in 'Orchid' petunia.

Manipulation of plant architecture through the use of ethephon to reduce apical dominance in petunia is a valuable commercial tool. Favorable results were obtained by applying ethephon at 1.7 mM. Higher concentrations resulted in phytotoxic effects such as chlorosis and reduced root growth. These adverse effects are most likely a result of exposing the shoot to sustained levels of ethylene above a critical level for an extended period of time. Although ethylene evolution peaked 6 to 8 h following application, production rates remained high even 12 h after application. All three experiments demonstrated that ethylene evolution from ethephon-treated petunia plants increases with higher ethephon concentrations and is not influenced by endogenous ethylene production by the plant. These results support the hypothesis that the majority of the ethylene released upon exposure to

ethephon is attributable to the degradation of the compound (Olien and Bukovac 1978; Lurssen 1982; and Beaudry and Kays 1988). The apparent absence in 'Orchid' petunia of an autocatalytic ethylene response to the application of ethephon at 0.9, 1.7, and 3.5 mM makes it a good choice for commercial manipulation with ethephon. Further work on additional factors that influence the breakdown of ethephon such as temperature and humidity at application time, as well as the age of the target tissue, will improve the reproducibility of the response to ethephon. With the knowledge that ethephon has an effect on the auxin/cytokinin ratio, new strategies can be developed to improve the reliability of ethephon treatment and/or promote a greater reduction in apical dominance by treating plants with cytokinin compounds prior to or following ethephon application. It might even be possible to replace ethephon treatment with the application of a cytokinin.

## ACKNOWLEDGMENTS

This paper represents a portion of the dissertation submitted by D.L. Haver in partial fulfillment of the requirements for a Ph.D. in Botany at the University of California.

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