Use of Plant Growth Regulators to Enhance Branching of *Clematis* spp.

by

Sadie Erica Puglisi

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Approved:

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Joyce Latimer, Chairman   Holly Scoggins, Committee Member

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Greg Eaton, Committee Member

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Abstract

Clematis spp. L. is a twining vine covered in showy blooms. Typical growth of hybrids is from the main leader, producing a thin, unbranched plant with one cyme. Apical dominance is released by cutting back the vine during production. Cutting back, or pinching, of a plant is labor intensive and compromises bloom for vegetative growth at time of sales. The purpose of this project was to eliminate manual pinching by treating young plants with chemical plant growth regulators (PGRs) that enhance branching without removal of the apical meristem. The first project evaluated the use of Atrimmec (dikegulac sodium), Fascination (BA+GA$_{4+7}$), Florel (ethephon), and Dropp 50 (thidiazuron) on *Clematis* cultivars Ernest Markham, and Hagley Hybrid, and *Clematis viticella* 'Polish Spirit.' Plants treated with 800 mg·L^{-1} Atrimmec, or 800 or 1200 mg·L^{-1} Fascination experienced an increase in branch numbers. The second experiment manipulated the ratio of the components of Fascination, 6-BA and GA$_{4+7,}$ to reduce phytotoxicity experienced in the first experiment. The optimal ratio to enhance branching was 1:1, which is the stock solution for Fascination. All ratios produced

phytotoxic symptoms. A third experiment tested lower rates of thidiazuron and added

CPPU (forchlorfenuron) to the list of PGRs to test. The last experiment took the most

effective PGR treatments, Atrimmec at 800 mg·L^{-1}, and Fascination at 800 or 1200 mg·L^{-1}, and compared them to the current production practices of pinching. Large flowering

cultivars of clematis were used, including 'Comotesse de Bouchard,' 'Ernest Markham,'

and 'Hagley Hybrid.' Atrimmec increased branch numbers and suppressed leader lengths

without a mechanical pinch. Results from Fascination varied by cultivar.

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Statement of Purpose

Vigorous growth of vines is a beneficial quality due to consumer's tendency to purchase

plants that are fully-grown and produce quick results in the landscape. However, the

growth of many *Clematis* cultivars is primarily from the main leader, producing a long,

thin plant. Retailers prefer to sell a well-branched plant that fills the container in which it

is displayed and producers prefer a well-branched, compact plant for easy handling and

shipping (personal communication, Becky Moss, Riverbend Nursery, Riner VA). To

achieve this, the grower needs to increase the lateral branching of the plants, and decrease

the length of the leaders. Under current production practices, growth stimulation of the

axillary buds is achieved by pinching, or cutting back, the apical meristem. This activity

is tedious, labor intensive, and delays flowering. If a worker is making $9.25 an hour, an

average of $887.71 is spent by greenhouse operators to cut back 92.9 m^2 of *Vinca minor*

'Bowles', a plant with a similar growth habit as clematis (personal communication,

Becky Moss, Riverbend Nursery, River VA). On the other hand the average cost of 25

seconds is needed to spray 1 m^2 of PGR with a hand held CO_2 sprayer. At a pay rate of

$9.25/hour that's $64.24 per 92.9 m^2 for labor. A PGR would have to cost $823.47 a

quart to equal the cost of a manual pinch. The trade formulation of ethephon, Florel,

costs $19 a quart. The cost of using a chemical branch enhancing plant growth regulators (PGR) would vary depending on the cost of the product and the method of application. However, the cost of PGR use would be less than that of manual pinching.

  Growers who are buying liners in the spring for summer sales typically have to make the choice between pinching to increase lateral branching or selling plants that have fewer branches but that are in bloom. Those who choose to start clematis in the winter generally apply two or more pinches before May sales. Some species are pinched up to once a week until the sale date (personal communication, Johnny Patterson, Lancaster Farms, Suffolk VA). Clematis that are grown through the summer and fall, then overwintered, do not require such frequent pinching. However, this lengthy production schedule means the clematis plants are taking up valuable space that could be used for plants that can be grown and sold in less time, which, therefore, make more money for growers compared to clematis.

The purpose of this project was to test the effects of five plant growth regulators (PGRs), namely dikegulac (Atrimmec, PBI Gordon, Kansas City, Mo.); BA+GA$_{4+7}$ (Fascination, Valent USA, Germantown, Tenn.); ethephon (Florel, Monterey, Fresno, CA); thidiazuron (Dropp 50 WP, Uniroyal, Middlebury, Conn.) and florclorfenuron (CPPU, Valent USA), on growth of various clematis hybrids. By evaluating leader length, lateral bud break, and time from treatment to bloom, we determined the PGR's potential to decrease labor

cost, decrease production time, and create a plant that is well branched and blooming at the time of sale.

Description of Plant Material

Clematis L. is the only woody-stemmed vine in the family Ranunculaceae. This family encompasses about 50 to 52 genera and 2,000 species (Walters and Keil, 1996). Alternate, opposite or whorled leaves characterize Ranunculaceae. Plants are synoecious (perfect flowers), or, rarely, dioecious. Inflorescences vary; flowers are perfect and regular. Plants have four sepals, often petaloid, and five carpels, which are distinct. Ovaries of the carpels are superior with one locule and one to many ovules; each carpel has one style. Fruits in this family include achenes or follicles. The preceding description is of a typical plant in the Ranunculaceae family, there being exceptions to every rule.

Clematis vines have the following specific characterizations of this family (Grey-Wilson, 2000); opposite leaves, occasionally whorled, simple or compound. The inflorescence forms a three-flowered, dichasial cyme (forming pairs of flowers arising from the axils of opposite bracts on the pedicels of the preceding flower). The terminal flower opens first,

followed by the lateral flowers. Flowers are radially symmetrical and usually

hermaphroditic. Sepals make the showy color display clematis are known for, and they

are typically four in number, although sometimes five to eight sepals can be present.

Petals are absent on clematis, however staminodes (modified anthers) can often be as

showy as petals giving the plant a double look. The fruit of clematis is an achene.

Ruth Gooch (1996) publishes the following history of clematis in the book <u>Clematis; The</u>

<u>Complete Guide.</u>   The name comes from the Greek word 'Klema' which means 'vine-

like.' Clematis was first written about in the 1500's. The first clematis to come into

Britain was *C. viticella* then named the 'Purple Virgin's Bower' in honor of Queen

Elizabeth I, who was known as the 'Virgin Queen.' Clematis continued to be referred to

as the 'Virgins Bower' from that point. Clematis was a popular plant until the

appearance of clematis wilt, a fungus that infects the plant causing death with symptoms

similar to drought. A renewed interest in clematis in the 20th century is credited to Ernest

Markham, head gardener at Gravetye Manor in Sussex, for the owner William Robinson.

Markham and a handful of other well-known plantsmen can be credited for a multitude of

the popular varieties still grown today.

Clematis hybrids are divided into three taxonomic groups based on pruning requirements

(Gooch, 1996):

      Group 1, Floribundas: Characterized by minimal pruning requirements. This

group includes the *Clematis* species *armandii, cirrhosa, alpina, macropetala,* and *montana*. Plants in this group flower on old wood so pruning should be done in the late winter as the buds begin to swell. Those branches or shoots that do not have buds present should be cut back to ground level or to the first living node.

Group 2, Patens: Characterized by 'light pruning.' These plants first bloom early in the spring or late in the winter. This group includes the popular large flowering hybrids 'Nelly Moser', 'Mrs. Norman Thompson' and 'Marie Boisselot.' Double flowered clematis are typically part of this group as well. After the first bloom any branch or shoot without buds should be taken off. A second bloom will follow late summer. Following the second bloom, the top 30 cm of the plant should be removed, as it will die back, followed by removal of any shoots or branches that did not bloom in the present season. This group also blooms on old growth so pruning when viable buds can be seen is important to ensure blooming wood is retained.

Group 3, Jackmanii: Characterized by 'hard pruning.' This group includes the *Clematis* species *texensis, viticella, orientalis* and *tanguticas* and hybrids that bloom late in the season. These plants bloom in late fall or early winter, on new growth.   After flowering plants should be pruned to the second node from the soil, or 47 cm from the ground, to promote new shoots and branches and ensure

an abundance of blooms the following year.

Clematis used in this research project are representatives of the following species which (Gooch, 1996):

Clematis cirrhosa L.: These plants are often evergreen and benefit from a protected site. Hybrids in this subgroup include 'Comtesse de Bouchard' and 'Ernest Markham.' 'Comtesse de Bouchard' is a small flowering hybrid, pink in color, with tepals that curve slightly downward. This cultivar was hybridized by Morel in 1906. This plant grows 2 to 2.5 meters long and flowers early to late summer and belongs to pruning group three. 'Ernest Markham' was one of the many seedlings given to Jackman's Nursery, England, after Markham's death in 1937. The nurseryman named this brilliant magenta flower after it's founder. 'Ernest Markham' grows up to 5 meters tall and blooms early to late summer or mid summer to early autumn. In warm climates, this plant could be pruned as group three, milder regions as group two.

Clematis florida Thunb.: This group contains the hybrid 'Hagley Hybrid' raised by Percy Picton, head gardener of Hagley Hall, and introduced by Jim Fisk in 1956. This plant grows 2 to 2.5 m tall and blooms in early summer to early autumn. The blooms of this cultivar of medium size, pink and have ruffly edges. 'Hagley Hybrid' should be pruned according to guidelines of group three.

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Clematis viticella L.: This is the only plant used in this project that is not a hybrid. The cultivar used was 'Polish Spirit.' This is an easy to grow species that flowers on the current season's growth. The plants will grow 2.5 to 3 m before flowering, and should then be cut back as a group three plant. This species is characterized by an abundance of small purple flowers.

Clematis Production

Clematis vines are typically propagated by the use of cuttings, seed, or division of roots. Raymond Evison compiled guidelines for clematis production in the proceedings of the International Plant Propagators' Society (1977). Evison states seed production is used mainly for the species, and vegetative material is used for cultivars. Cuttings are taken from young plants 6 weeks after the plant has been potted in a 7-cm pot. Stock plants are not used as they produce cutting material that is too large in diameter for commercial production. Cuttings contain two fully matured nodes with mature leaves. Cutting material remains covered so as not to expose it to sunlight. Fungicide drenches are used to prevent fungal infection. Cuttings are inserted in media containing one part loam, one part peat, two parts grit and two parts fine screening sand. Cuttings are inserted until one node is just below the surface of the compost. Cuttings should be spaced no less than a centimeter from each other. Remaining foliage creates a microclimate of moist, warm conditions suitable for *Botrytis* growth. Well-spaced cuttings reduce such a suitable

climate for disease spread. Cutting trays are placed on propagation beds at ground level

with adequate drainage. Electric heating cables keep the beds at 24°C. Air temperature

is between 16°C and 32°C. Direct sunlight is avoided until cuttings are fully rooted.

Misting is important during the day, but cutting surfaces should be dry by nightfall.

Misting is not a sufficient means of irrigation; cuttings should be checked and spot

watered each morning. Fungicide is applied as a drench every 2 weeks to prevent fungal

infection. Cuttings root within 21 days and are allowed to grow on for 5 more weeks.

Plant roots are trimmed to 7.5 cm from the tip of the plant to the bottom of the root

system before being transplanted into 7 cm pots. Media used for the pots include 65%

peat, 15% loam and 20% grit and sand. Pots are placed in a polythene tunnel-house for 3

weeks. Such a house provides high humidity levels and hot temperatures. Houses are

vented when temperatures exceed 32°C. Potted plants are used for new cuttings 6 weeks

after they have been transplanted, then allowed to grow for 6 more weeks before being

sold.

Few wholesale producers start clematis from cuttings. A majority of the wholesale

market purchases size 50 (4.7 cm) plugs and transplants them into trade gallons (3.71 L).

Media used varies by producer. After new growth is seen in the trade gallons, plants are

pinched to the first node above the soil line. Two weeks later they are pinched to the

second node above the soil line. From this point, plants are pinched according to lateral

growth. Those that have a suitable number of branches will be allowed to grow after the

second pinch. Plants with very little lateral branching can be pinched as often as once a

week. Plants are trained on trellises that can vary from a thin bamboo stake to an

elaborate trellis. Plants are sold 8 to 10 weeks after transplant.

Plant Growth Regulators

A natural plant hormone is defined as an organic compound synthesized in one part of a

plant that, in very small concentrations, is translocated to another location, where it

causes a physiological response (Galston et al., 1980; Salisbury and Ross, 1991).

Response systems must be comprised of three components to be defined as hormonal

(Salisbury and Ross, 1991): 1) first a hormone must be present in sufficient quantity in

the proper cell; 2) the hormone must be recognized and bound tightly by each of the

target cells; and 3) lastly, a receptor protein must cause a metabolic change that triggers

amplification of the hormonal signal or messenger.   Therefore, an exogenous application

of hormone may lead, not simply to a response by a single tissue, but also may be

accompanied by a change in hormone concentration, and frequency and availability of a

receptor protein which could amplify the hormonal signal (Salisbury and Ross, 1991).

This last part is key to the objectives of this research, as we are not only looking for an

increase in branch number, but outgrowth of those induced branches and effects on

bloom time. This research is based on our speculation that axillary buds are present on

the vines, but held dormant by apical dominance. To release the vines from apical

dominance would release the axillary buds from dormancy. This introduction will briefly summarize the hormones involved in apical dominance.

<u>Auxin</u>

Auxin, or indoleacetic acid (IAA), is synthesized from the amino acid tryptophan or indole in the leaf primordia of young leaves and developing seeds (Salisbury, 1991). Auxin is synthesized in meristematic tissue and transported to the roots via the phloem. Auxin is responsible for cell enlargement and cell division, and is the main hormone responsible for apical dominance. Early research indicates auxin is responsible for inhibitory states of lateral buds (Galston et al., 1980). Plants experiencing apical dominance that are decapitated experience new lateral growth. When the tops of plants are replaced, or agar blocks containing auxin replaces the tops of plants, lateral growth is once again inhibited. This response confirms that auxin is the hormone responsible for apical dominance and inhibition of lateral shoot development. However, in the case of auxin replacement by an agar block, 1000 times the amount of auxin is necessary to inhibit buds than is naturally needed for inhibition. This raises questions as to the solitary role of auxin involved in lateral bud dormancy. Experiments tracking auxin with ^{14}C-labeled indole-3-acetic acid (IAA) showed the hormone moving down the stems of plants, but not entering the area in which the lateral buds lay dormant (Salisbury and Ross, 1991).

Furthermore, auxin applied directly to lateral buds does not inhibit growth, and in some cases, encourages it. A review by Chatfield et al. (2000) discusses the theory that a secondary messenger triggered by auxin may be responsible for lateral inhibition. The most likely candidate for synergism is ethylene. When excess levels of auxin are synthesized, ethylene is produced. However, early studies show preventing the synthesis of ethylene decreases bud inhibition (Galston, 1995). The review by Chatfield et al. (2000) summarized the following experiments. An ethylene biosynthesis inhibitor, aminoethoxyvinylglycine (AVG), was applied to excised *Arabidopsis* buds. Bud growth continued, though at a reduced rate. Likewise, using the ethylene insensitive mutant *etr1*, bud outgrowth was slower compared to wild type *Arabidopsis*. In both cases, apically applied auxin, 1-naphthalene acetic acid (NAA), still inhibited lateral bud growth. Ethylene is not considered a secondary messenger for auxin inhibition of lateral buds. Similar studies with abscisic acid insensitive mutants *abi1-1* and *abi1-2* of *Arabidopsis* showed similar response to NAA as the wild type plants, ruling out abscisic acid as a messenger. Combinations of apically and basally applied NAA and the cytokinin benzyladenine (BA) were applied to excised *Arabidopsis* buds. Results indicate that 1μM BA released inhibition of growth from 1μM NAA for most buds. Some buds did remain inhibited, but for a shortened duration. Application of BA must be basal to reduce inhibition from auxin. A combination of BA and NAA applied apically had a synergistic inhibition of outgrowth greater than either hormone alone that was harder to overcome with basal BA. In conclusion, Chatfield et al. (2000) suggested that the

concept of a second hormonal messenger to auxin inhibition was false and that other parameters such as nutrient source-sink relations should be investigated.

Cytokinin

Synthesis of cytokinins, like zeatin and benzyladenine, is by biochemical modification of adenine (Davies, 1995). Synthesis occurs in root tips and the hormone is translocated in the xylem to the shoots. Cytokinins, in the presence of auxin, are responsible for cell division in *in vitro* propagation. Exogenous application of cytokinin, or high levels in transgenic plants with genes for enhanced synthesis of cytokinin, experience an increase in lateral bud growth (Davies, 1995). Direct application of cytokinin to axillary buds increased bud growth for a number of days. However, an application of auxin or gibberellins is needed to sustain growth. Exogenous applications of benzyladenine (BA), a synthetic form of cytokinin, increased axillary shoot growth in *Euphorbia lathyris* L. (Preece, 1989), *Dieffenbachi* Lodd. 'Welkeri' (Wilson and Nell, 1983), and *Anthurium* Andre. (Imamura and Higaki, 1988). Benzyladenine increased lateral branch development and decreased the height of *Peperomia obtusifolia*L. (Henny, 1985) creating a more compact, shorter plant.

Ethylene

Ethylene is a gas that is synthesized from methionine in response to stress (Davies, 1995). Transport is by diffusion from the site of synthesis, however, an intermediate, 1-aminocyclopropane-1-carboxylic acid (ACC), may be transported further from the wound

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site, producing effects similar to ethylene. Common responses to the hormone include release from dormancy, shoot and root growth and differentiation and adventitious root formation.

Gibberellin

Gibberellins are synthesized from mevalonic acid in young tissues of shoots and developing seeds (Davies, 1995). Transport is via both the xylem and the phloem. The effects of gibberellins vary by plant species (Salisbury, 1969). Some plant species respond with an increase in height due to an increase in cell length. Other plant species respond to gibberellins by increasing cell number as well as an increase in size, most likely cell length. Gibberellins prevent the development of lateral buds when applied to decapitated shoots of several species (Salisbury, 1969). Exogenous applications of GA_{4+7} inhibited lateral bud break of *Euphorbia lathyris,* and increased plant height, quadratically (Preece, 1989).

However, gibberellins have also been noted to increase lateral branching in plants. GA_3 was applied to English ivy (*Hedra helix* L.) at various rates to pruned and intact plants (Lewnes and Moser, 1976). Pruned plants experienced an increase in bud break on primary lateral shoots. Intact plants responded differently, as GA did not affect those buds that developed prior to treatment. Lateral growth occurred only on bud initiation that took place subsequent to GA treatment. Imamura and Higaki (1988) experienced a slight linear decrease in the number of shoots produced from pinched juvenile *Anthurium*

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plants with an increase in GA concentration. However, a linear increase in shoots was

observed with an increase of GA concentration on pinched plants. Similar results

occurred with mature *Anthurium,* however, applications of 500 ppm GA resulted in an

increase in lateral shoots without pinching. In conclusion, the effect of gibberellins on

lateral branch development varies with species and gibberellin type and is dependent on

the amount of apical influence.

Description of Each Plant Growth Regulator

The following chemical PGRs were used in this project. Trade names belong to the

respective companies listed.

Atrimmec

 Dikegulac (Atrimmec, PBI Gordon, Kansas City, Mo.), 2,3:4,6-bio-O- (1-

methylethylidene) – O-(2)-hexulofuranosonic acid, is a monosaccharide that was first

reported in 1975 as a growth retardant (Nickell, 1982). Gibberellin 3 (GA_3) counteracts

the affects of dikegulac on *Avea fatua* and pea (*Pisum sativum* L.) leading to the belief

that dikegulac either antagonizes the biosynthesis or mode of action of GA_3 (Bocion and

de Silva, 1977). Gressel et al. (1976) further studied this belief and stated that gibberellin

stimulates precursor incorporation into DNA and that dikegulac suppresses this

stimulation, which is reversible with an application of GA. Dikegulac also suppresses the

incorporation of uridine into rRNA precursors and mature rRNA (Gressel et al., 1976).

To test for translocation, one leaf of chrysanthemum was painted with 3% dikegulac (Bocion and de Silva, 1977). Chlorosis was seen in the subtending axillary branch 29 days after treatment, indicating translocation in the phloem. In the same experiment, translocation was also expressed by stunting of the main shoot indicating the chemical had traveled to the apical meristem and inhibited growth in some way. Penetration of dikegulac through leaves is dependent on plant age (Bocion and de Silva, 1977). Treatment of the upper most leaf of chrysanthemum produced the most effects on the apex, followed by the middle and lower leaves, respectively. Increased absorption in juvenile leaves may be correlated with the high photosynthetic capacity of young leaves.

Fascination

BA+GA$_{4+7}$ (Fascination, Valent USA, Germantown Tenn.), N-(phenylmethyl)-1H-purine 6-amine, is an equal combination of 6-benzyladenine and gibberellin 4 + gibberellin 7. This chemical is a straight application of hormones; therefore, the mode of action is that of the hormone applied. The affects of GA$_{4+7}$ and BA have been previously discussed.

Florel

Ethephon (Florel, Monterey, Fresno, CA.), 2-chloroethylphosphonic acid, is an ethylene-releasing compound synthesized in 1946 (Neumann, 1988). Typical uses include defoliation and thinning of fruits. The ethephon compound has a central phosphorus

atom that is attacked by water or hydroxyl ions, which leads to the simultaneous

elimination of chlorine and the liberation of ethylene (Neumann, 1988).   A pH above 4.5

causes the molecules to convert to dianionic form, thereby becoming susceptible to

hydrolysis. Therefore, an increase in pH causes an increase in ethylene release.

Translocation of ethephon appears to be in a source to sink direction via the phloem

(Neumann, 1988). Translocation rates vary with leaf age and location, decreasing with

age. Ethephon penetration varies widely among species. Leaf penetration is temperature

dependent, increasing 55 times between 15°C and 35°C (Nuemann, 1988). Ethephon

penetrates 20 to 25 times more on the abaxial surface of leaves compared to the adaxial

surface. Penetration through fruit ranges from slight to 20%.

Dropp 50 and CPPU

Thidiazuron (Dropp 50, Uniroyal Chemical, Middlebury, Conn.), N-phenyl-*N*'- 1,2,3,-

thiadiazol-5-ylurea, is a synthetic diphenylurea (DPU) type cytokinin that is thought to

encourage the synthesis and /or accumulation of purine type cytokinins (Thomas and

Katterman, 1986). Forchlorfenuron (CPPU, Valent USA, Germantown, Tenn.), N-

(2_chloro-4-pyridyl)-N'-phenylurea, is a synthetic phenylurea cytokinin. Both of these

cytokinins are used to induce shoot development in tissue culture. Dropp 50 also is used

commercially as a cotton defoliant.

Previous use of Chemicals as Branch Enhancers

Dikegulac

Dikegulac has been used for years to overcome apical dominance and increase axillary

shoot production in azalea (de Silva et al., 1976; Bocion and de Silva, 1977). Slight

chlorosis and leaf deformation was noticeable 2 weeks after treatment in both studies.

Similar chlorotic effects were observed with the application of dikegulac on *Salvia*

farinacea Benth. (Banko and Stefani, 1996). Dikegulac has been reported to increase

shoot number while decreasing shoot length of *Lonicera x heckrotti* Rehd. (Bruner et al.,

2000)*, Hedra helix* (Al-Juboory and Williams, 1990)*, Hypericum* C. (Thomas et al.,

1992)*,* and *Vinca minor* L. (Foley and Keever, 1993)*.* Bruner et al. (2000) experienced a

linear or quadratic decrease in shoot elongation of pruned and non-pruned plants with an

increase in chemical concentration. Eight weeks after treatment, low and high rates of

dikegulac resulted in similar elongation results. Foley and Keever (1993) also

experienced a decrease in primary runner length of *H. helix* with an increase in dikegulac

rates. Runner numbers increased with increasing concentration from 250 ppm until 1000

ppm, after which runner numbers were suppressed. An initial suppression of runner

numbers was experienced at all rates, but eventually dissipated.

BA+GA$_{4+7}$

This BA+GA$_{4+7}$ product, under the name Promalin (Abbott, Abbott Park, Ill.), increased

lateral bud break and increased shoot length in *E. lathyris* (Preece, 1989), *Hypericum*

(Thomas et al., 1992)*,* and *Portulaca grandiflora* Hook. (Banko and Stefani, 1997).

Foley and Keever (1993) noticed an increase in primary runner numbers and an increase in runner length of *V. minor* through week eight of the experiment with an increase of Promalin concentration. A second experiment was conducted to observe different rates and the affects of a second application. In this experiment, differences in the primary and secondary shoots occurred. Primary runner numbers increased linearly or quadratically throughout the experiment as rates increased. However, primary runner lengths were not affected (Foley and Keever, 1993). Secondary runner numbers and lengths increased cubically and quadratically, respectively, as Promalin rate increased. This is in contradiction to the study done on portulaca, where both secondary and primary shoot lengths increased (Banko and Stefani, 1997). Promalin produced a linear increase in height of geranium (*Pelargonium x hortorum* L.H. Baily) as rates increased (Foley and Keever, 1992). Axillary shoot number did not differ from the control at the two highest rates in this experiment. The number of axillary shoots did increase at the 300 ppm concentration. Phytotoxicity may have contributed to the lack of increase in axillary shoot number.

Ethephon

The effects of ethephon vary by species. Multiple applications of ethephon at 500 mg·L^{-1} increased branching and decreased internode lengths of zonal geraniums (Konjoian, 1997). At a concentration of 750 ppm, ethephon retarded the main shoot length of portulaca, but had no effect on axillary shoot breaks (Banko and Stefani, 1997).

Ethephon applications on rose cultivars 'Mercedes' and 'Sonia' increased basal shoots, but height remained similar to that of the controls (Grzesik and Rudnicki, 1989). Ethephon treatments on *Salvia farinacea* 'Victoria Blue' produced a more compact plant that was full in appearance (Banko and Stefani, 1996). Treatments of 500 ppm suppressed the main shoot length of salvia, but allowed prolific growth of the lateral shoots to produce fuller plants. The full appearance is a reflection of the increased lateral shoot to main shoot length ratio. Applications of 1,000 ppm produced the same response, but the plants were too short for the market. Similar effects appeared when ethephon was applied to geraniums (Foley and Keever, 1992). A single application of 500 ppm ethephon produced a decrease in axillary shoot length, a decrease in axillary shoot caliper, and an increase in axillary shoot number. Ethephon increased the amount of terminal cuttings, but did not increase the number of single node cuttings.

Thidiazuron

Thidiazuron (TD) has not been studied for production use; it has only been used in tissue culture and as a cotton defoliant. Studies have been done to observe the effects of TD on adventitious shoot formation. In a study by Goldfarb et al. (1991), thidiazuron was applied as a pulse to Douglas fir seedlings. The cotyledons of the seedlings were removed and grown on agar blocks. Adventitious shoots were counted and compared to those plant pulsed with BA. BA at 800 µM gave the greatest number of shoots, however, the effects of TD were similar to BA. The optimal rate of thidiazuron was that of the lowest concentration, 200 µM. The number of buds decreased with increasing

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concentration of TD. Lower concentrations of TD were found to be optimal when using

the chemical for tissue culture (Thomas and Katterman, 1986). General growth and cell

division stimulation becomes saturated at low levels of TD, making the chemical more

effective than purine type cytokinins (Thomas and Katterman, 1986).

Forchlorfenuron

CPPU has not been used in commercial production as a foliar spray. CPPU is currently

used in tissue culture to induce shoot development. CPPU has previously been shown to

release *in vitro* cultured *Rosa hybrida* from apical dominance and stimulate axillary bud

break (Toteva et al., 2000).

Literature Cited

Al-Juboory, K H., and D.J. Williams.1991. Effects of two growth regulators on lateral branching and shoot growth of English ivy (*Hedera helix*). PGRSA Quarterly 19:35-40.

Banko, T.J., and M.A. Stefani. 1977. Growth regulators enhance branching of portulaca. Proc. SNA Res. Conf . 42:288-289.

Banko, T.J., and M.A. Stefani. 1996. Chemical growth control of *Salvia farinacea* 'Victoria Blue'. Proc. SNA Res. Conf. 41:214-215.

Bocion, P.F., and W.H. de Silva.1977. Some effects of dikegulac on the physiology of whole plants and tissues: Interactions with plant hormones. pp198-198. Proc. Intl. Conf. on Plant Growth Substances, Lausanne, Switzerland.

Bruner, L.L., G.J. Keever, R.A. Kessler, and C.H. Gilliam. 2000. Growth regulation effects of *Lonicera x heckrotti* (Goldflame Honeysuckle). Proc. SNA Conf . 45:263-264.

Chatfield, S.P., P. Stirnberg, B.G. Forde, and O. Leyser. 2000. The hormonal regulation of axillary bud growth in *Arabidopsis*. Plant J. 24:(2)159-169.

Davies, P.J. 1995. Section of Plant Biology. In: Cornell University. Plant hormones: Physiology, biochemistry and molecular biology. Kluwer Academic publishers, New York, NY.

Evison, R.J. 1977. Propagation of Clematis. Proc. from the Intl. Plant Prop. Society

Foley, J T. and G.J. Keever. 1993. Chemically induced branching of *Vinca minor*. J Environ. Hort. 11(4):149-152.

Foley, J.T., and G.J. Keever. 1992. Chemical promotion of axillary shoot development of geranium stock plants. J. Environ. Hort. 10(2): 90-94.

Galston, A., P. Davies and R. Lisatter. 1980. The life of a green plant. 2nd ed. Prentice Hall, Englewood Cliffs, N.J.

Goldfarb, B., G.T. Howe, L.M. Bailey, S.H. Strauss, and J.B. Zaerr. 1990. A liquid cytokinin pulse induces adventitious shoot formation from douglas fir cotyledons. Plant Cell Reports 10:(3)156-160.

Gooch, R. 1996. Clematis: The complete guide. Crowood, Ramsbury, Wiltshire.

Grey-Wilson, C. 2000. Clematis, the genus: a comprehensive guide. Timber Press, Portland, Orgon.

Gressel, J., A Kadouri, D. Atsmon, and N. Cohen. 1976. Effects of dikegulac sodium on nucleic acid syntheses. Collected abstracts of the paper demonstrations:The 9th international conference of plant growth substances. pp.1-2.

Grzesik, M. and R.M. Rudnicki. 1989. Effect of growth regulators on growth and the branching of roses "Sonia" and "Mercedes". Acta Hort. 251:409-413.

Henny, R.J. 1985. BA induces lateral ranching of *Peperomia obtusifolia*. HortScience 20(1):115-116.

Imamura, J.S. and T. Higaki. 1988. Effect of GA_3 and BA on lateral shoot production on *Anthurium*. HortScience 23(2):353-354.

Kapchina-Toteva, N., H.J. van Telgen, and E. Yakimova. 2000. Role of phenylurea cytokinin CPPU in apical dominance release in *in vitro* cultured *Rosa hybrida* L. J. of Plant Growth Regulat. 19:232-237.

Konjoian, P. 1997. Florel use on perennials. KFES Newsnotes. 97-1.

Lewnes, M.A., and B.C. Moser. 1976. Growth regulator effects on apical dominance of English ivy. HortScience 11(5):484-485.

Neumann, P.M. 1988. Plant growth and leaf applied chemicals. CRC, FL.

Nickell, L.G. 1982. Plant growth regulators. Springer Verlag, N.Y.

Preece, J.E. 1990. Growth stimulation of *Euphorbia lathyris* L. by GA_{4+7} and BA. J Plant Growth Regulat. 9:85-88.

Salisbury, F.B. and C. Ross. 1969. Plant Physiology. Wadsworth, CA.

Salisbury, F.B. and C.W. Ross. 1992. Plant development, p.357-408. In: Plant physiology. 4th ed. Wadsworth, Belmont, CA.

Thomas, J.C. and F.R. Katterman. 1986. Cytokinin activity induced by thidiazuron. Plant Physiol. 81:681-683.

Thomas, P.A., J.G. Latimer, D. Zhang, and S. Hartzler. 1992. Effects of plant growth regulators on lateral branching and shoot growth of *Hypericum*. Proc. SNA Res. Conf. 37:261-263.

Walters, D.R., and D.J. Kiel. 1996. Vascular plant taxonomy 4th ed. Kendall Hunt, Dubuque, Iowa.

Wilson, M.R. and T.A. Nell. 1983. Foliar applications of BA increase branching of 'Welkeri' dieffenbachia. HortScience 18(4):447-448.

Plant Growth Regulators to Enhance Branching of

Clematis *spp.*

Sadie Puglisi[1]; Joyce Latimer[2] and Holly Scoggins[3]

Department of Horticulture, 301 Saunders Hall, Virginia Polytechnic Institute & State

University, Blacksburg, VA 24061-0327

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[1]Research Assistant. This paper is based on a portion of a thesis submitted by the senior

  author in partial fulfillment of the requirements for the M.S. degree.

[2]Professor.

[3]Assistant Professor.

Developmental Physiology

The Use of Plant Growth Regulators to Enhance Branching of *Clematis* spp.

Keywords: Atrimmec, Fascination, Florel, Dropp 50, CPPU, dikegulac, BA, GA_{4+7}, ethephon, thidiazuron, forchlorfenuron

Abstract

The purpose of this experiment was to test the effects of five plant growth regulators (PGRs) on growth of *Clematis* L. large flowering hybrids, Ernest Markham and Hagley Hybrid, and *Clematis viticella* 'Polish Spirit.' Foliar sprays of dikegulac (Atrimmec), BA+GA_{4+7} (Fascination), ethephon (Florel), thidiazuron (Dropp 50 WP) and forchlorfenuron (CPPU), were applied to actively growing plants. Data collected on the two large flowering hybrids included a count of branches, a measure of leader lengths, and the number of weeks to bloom. Mean branch number and mean leader lengths were compared within a chemical between cultivars and between rates. Leader lengths were measured on 'Polish Spirit' and compared between rates within each chemical. Results indicate clematis growers could use 800 mg·L^{-1} dikegulac or either 800 or 1200 mg·L^{-1} BA+GA_{4+7} to increase branch number, and 1,000 mg·L^{-1} ethephon or 800 mg·L^{-1} dikegulac to effectively suppress leader lengths. Plants treated with BA+GA_{4+7} expressed bud blast, leaf curl and tip dieback until 4 weeks after treatment (WAT). A second experiment was performed on 'Ernest Markham' to optimize the ratio of BA:GA_{4+7} in an attempt to reduce phytotoxicity. Data taken were similar to the first

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experiment. Plants treated with any of the chemical ratios expressed an equal amount of phytotoxicity. A ratio BA:GA_{4+7} of 1:1 (Fascination) produced the most branches; with an increase in leader length. In a third experiment thidiazuron at the rates of 500 to 2000 mg·L^{-1} linearly decreased leader length throughout the 8 week test but increases in branch numbers were significant only at 2 and 4 WAT. Forchlorfenuron at rates of 5 to 25 mg·L^{-1} had no significant effect on branch number or leader length. Chemical names used: dikegulac; 2,3:4,6-bio-O- (1- methylethylidene) – O-(2)-hexulofuranosonic acid; BA; N-(phenylmethyl)-1H-purine 6-amine; ethephon; 2-chloroethylphosphonic acid; thidiazuron; N-phenyl-*N*'- 1,2,3,-thiadiazol-5-ylurea; and forchlorfenuron; N-(2_chloro-4-pyridyl)-N'-phenylurea

Introduction

Clematis vines are a popular garden plant cherished for their showy blooms and twining habit. However, the growth of many clematis hybrids is primarily from the main leader, producing a long, thin plant. This is a disadvantage for the retail market because consumers want well-branched, full plants. Producers prefer a well-branched, compact plant for easy handling and shipping (personal communication, Becky Moss, Riverbend Nursery, Riner VA). To achieve this, the grower needs to increase the lateral branching of the plants, and decrease length of the leaders. Pinching the apical meristem, or "cutting back" the plant stimulates axillary buds. This activity is tedious, labor intensive and delays flowering. Growers who are buying liners in the spring for summer sales typically have to make the choice between pinching to increase lateral branching or selling a plant with fewer branches that is in bloom. Those who choose to start clematis in the winter generally apply two or more pinches before May sales. Some species are pinched as often as once a week until the sale date (personal communication, John Patterson, Lancaster Farms). Clematis that are grown through the summer and fall, then over wintered, have more time to grow into a large, well-branched plants and therefore do not require such frequent pinching. However, keeping a plant in the nursery for a long period of time costs more to produce than one that can be sold in only 8 weeks, reducing profit margins.

There are no chemical branch-enhancing plant growth regulators (PGRs) that are currently labeled for use on clematis. Use of chemical PGRs may induce branching by releasing dormant buds from apical dominance without the necessity of a manual pinch. PGRs would be an economical alternative to a manual pinch. The cost of cutting back a flat of *Vinca minor* L. 'Bowles', a vine with a similar growth rate, is $2.31 a flat (average worker pay of $9.25/hour, personal communication, Becky Moss, Riverbend Nursery, Riner VA.). Cost of PGR application would be directly related to the chemical used and method of application, however, costs would be less than manual pinching. An added benefit of PGRs may be a suppression of leader length for easier shipping and handling by the producer. Although PGRs have not been tested on *Clematis spp.* they have been effective in increasing branch numbers on various other perennials. Dikegulac (Atrimmec) has been reported to increase shoot number while decreasing shoot length of *Lonicera x heckrotti* Rehd. (Bruner et al., 2000)*, Hedra helix* L. (Al-Juboory and Williams, 1990)*, Hypericum calycinum* C. (Thomas et al., 1992)*, and *Vinca minor* L. (Foley and Keever, 1993). Bruner et al. (2000) observed shoot lengths linearly or quadratically decreased with an increase in dikegulac concentration of pruned and non-pruned plants. Foley and Keever (1993) experienced a decrease of primary runner length of *Vinca minor* with an increase in dikegulac rates beginning 4 WAT and persisting through the experiment, 10 WAT. Under the trade name Promalin (Abbott, Abbott Park, Ill.), BA+GA$_{4+7}$ (Fascination), increased lateral bud break and increased shoot length in

Euphorbia lathyris L. (Preece, 1989), *H. calycinum* (Thomas et al., 1992)*, and *Portulaca grandiflora* Hook. 'Sundial' (Banko and Stefani, 1997). Ethephon (Florel) treatments of 500 mg·L^{-1} suppressed the main shoot length of *Salvia farinacea* Benth. 'Victoria Blue' and allowed the lateral shoots to grow prolifically, thereby producing fuller plants (Banko and Stefani, 1996). Ethephon, applied to geraniums (*Pelargonium x hortorum* L.H. Baily 'Hollywood Star') produced a decrease in axillary shoot length, and an increase in axillary shoot number (Foley and Keever, 1992).

The purpose of the first experiment was to test the effects of four plant growth regulators, dikegulac, BA+GA$_{4+7}$, ethephon, and thidiazuron, on growth of various clematis cultivars. A second experiment was conducted to find optimal ratios of the BA and GA$_{4+7}$ components of Fascination to reduce phytotoxicity. In a third experiment, thidiazuron was evaluated again, at lower rates, and forchlorfenuron was tested. By evaluating lateral bud break, leader length, and time from treatment to bloom, we determined potential of PGRs to decrease labor, decrease production time, and create a plant that is well branched and blooming at the time of sale.

Materials and Methods

Experiment one

Clematis viticella 'Polish Spirit' and large flowering hybrids Ernest Markham and

Hagley Hybrid arrived well rooted in 4.7-cm pots on 4 Apr. 2001 (Yoder/Green Leaf

Perennials, Lancaster, Penn.). Plants were transplanted into trade gallon pots (3.71 L)

using a medium of 6 bark fines : 2 Canadian sphagnum peat moss : 1 perlite (by volume)

(Scotts Sierra Perennial Mix, The Scotts Co., Marysville, Ohio). Greenhouse

temperatures were set at 14°C nights and 22°C days. Plants were given a constant liquid

feed of 200 ppm N using 20N-4.4P-16.6K (Peter's 20-10-20 Fertilizer, The Scotts Co.) at

every irrigation, throughout the experiment. Three of the plant growth regulators were

applied once by foliar spray on 2 May, when new growth was seen on shoots. Rates for

each PGR were based on previous experiments on various plants previously stated. Rates

were increased from those used on herbaceous plants, or decreased from rates used on

woody shrubs. Thidiazuron and forchlorfenuron rates were based on comparisons to BA

sprays and recommendations from chemical company representatives. A wide range of

rates was used to include amounts that may not affect the plant, to amounts that may be

toxic. Chemicals were not compared to one another.

The following treatments were applied: Dikegulac (Atrimmec, PBI Gordon, Kansas City,

Mo.) with the active ingredient applied at 0, 800, 1600, 2400, or 3200 $mg·L^{-1}$, $BA+GA_{4+7}$

[6-benzyladenine (BA): Gibberellins A_4A_7 (GA_{4+7}) 1.8:1.8% a.i. w/w, Valent U.S.A

Corp., Germantown, Tenn.] applied at 0, 400, 800, 1200, or 1600 $mg·L^{-1}$ a.i., (a

surfactant, Tween 20, was added to $BA+GA_{4+7}$, and applied to controls, at a rate of 0.10

$ml·L^{-1}$); Thidiazuron (Dropp 50, Uniroyal, Middlebury, Conn.) was applied at 0, 500,

1000, 1500, or 2000 mg·L^{-1} a.i. On 5 May, ethephon (Florel, Monterey, Fresno, Calif.)

was applied at 0, 500, or 1000 mg·L^{-1} a.i. Plants treated with ethephon were the smallest

of the plants received. These plants were treated 3 days after other PGR application to

ensure sufficient growth was available for chemical uptake. Likewise, data were taken

on plants treated with ethephon 3 days after the data were taken on the other plants. All

chemical treatments were applied at a volume of 210 ml·m^{-2} using a hand-held CO_2

pressurized sprayer (R&D Sprayers Inc., Model AS, Opelousas, LA) with an 8002VS

nozzle.

Data and Statistics:

Data were collected beginning 2 weeks after treatment (WAT) and were collected again

once every 2 weeks up to 10 WAT corresponding with a normal spring production cycle.

Data collected included branch and shoot numbers on the two large flowering hybrids.

Branch numbers were not counted for 'Polish Spirit' because this cultivar has sufficient

branch numbers. Branches were considered to be any axillary shoots ≥2.5 cm in length.

Shoots were considered to be leaders coming directly from the soil line. Plant leader

lengths were measured from the rim of the pot to the actively growing meristem, flower

or bud, of the three longest leaders for the large flowering hybrids. The five longest

leaders of 'Polish Spirit' were measured. Plants treated with BA+GA$_{4+7}$, ethephon, and

thidiazuron were measured until 8 WAT. Branches of plants treated with dikegulac were

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measured until 10 WAT; leader lengths were measured until 8 WAT. For wholesale production purposes, 8 WAT would be the shipping date, or the sale date for retail growers, as plants would be about 48 cm tall at this time. Shoots were harvested and dried for 4 days at 65ºC. Weights were measured in grams. Bloom time was analyzed by percent of 'Hagley Hybrid' in bloom or past bloom stages. Hagley Hybrid was chosen to track bloom trends as it had the most number of plants in bloom.

The experiment was set up as a complete block design with six single plant replications of each of the five rates for each chemical and cultivar. Each chemical was assigned a separate bench in the same greenhouse, for a total number of four benches (appendix one). Six blocks were set up lengthwise on the benches. Each block contained one plant of each cultivar at each rate. For the two large flowering hybrids, mean branch numbers per rate were determined to be the average of the six blocks for one given rate. Mean leader lengths were the average of the three or five longest leaders per pot, then the average of the six blocks per rate. An analysis of variance was done to determine if branch numbers and leader lengths differed between cultivars and between rates within a chemical, using LS-means (SAS Institute, Cary, NC.). Main effects in the ANOVA were cultivar as a categorical variable and PGR rate as a continuous variable. Interactions between these two parameters indicate the rate applied produces different growth results depending on plant variety. Therefore, recommendations as to the rate of each PGR would vary by cultivar. For this project, cultivars are analyzed separately if interactions

between rate and cultivar significant. This is done to produce recommendations according to cultivar. If no interaction was present, data presented are the mean branch number or leader lengths of 'Ernest Markham' and 'Hagley Hybrid' combined. Means graphed are the average of 12 replications of each rate for a given PGR. If no interaction is present, both cultivars are reacting to the rate of PGR in a similar manner, even if one cultivar reacts to a greater or lesser extent to the PGR, and therefore, can be treated with the same recommended rates. 'Polish Spirit' leader lengths were not compared to the two large flowering hybrids as it has a distinctly different growth habit.   Rate effect on branch number or leader length was compared using a contrast analysis to test if effects were linear or quadratic (SAS Institute). Rate recommendations are based on linear or curvi-linear functions of the contrast analysis. A linear effect implies a greater effect from increasing PGR rate (more branches, shorter leaders etc.). A quadratic trend would imply the chemical became toxic to the plant or the growth response became saturated at high rates. Statistics were analyzed for each measurement week separately. Data are presented by week to show the trends the chemical produces at different stages of the production cycle. Sale date is considered to be 8 WAT.

BA:GA ratio

Clematis 'Ernest Markham' (Roseville Farms, Apopka, Fla.) was potted into trade gallon pots (3.7 L) from 4.7-cm plugs, using the previously described media on 21 Aug. 2001.

Cultural practices were the same as the previous experiment.

Fascination is a 1:1 ratio of the two hormones, BA, and GA_{4+7}. To test their effects the ratio of these hormones was adjusted by decreasing the amount of gibberellin by mixing BA and GA_{4+7} (components supplied by Valent USA) and decreasing the amount of gibberellin in half each time. BA and GA_{4+7} were combined at a ratio (v/v): 1:1, which is the stock formula for Fascination, and applied at four rates, 0, 800, 1200, or 1600 mg·L^{-1} a.i. Further solutions were mixed at the following ratios; 1:0.8, 1:0.4, 1:0.2 (Accel, BA+GA_{4+7} at 1.8:0.36% a.i. w/w, Valent), 1:0.1, 1:0.05, and 1:0 and applied at the corresponding rates. Each of the seven ratios was applied on 4 Sept. 2001. Tween 20 was added to each solution at 0.10 ml·L^{-1}.

Data measurements taken for BA:GA screening were the same as the first experiment from 2 to 8 WAT. The experiment was set up as a complete block design with six single plant replications of each rate for a given ratio. Mean branch numbers for each rate were calculated between the six blocks for each ratio and compared between rates. In the greenhouse, plants of two ratios were placed on each greenhouse bench, for a total of 4 benches (Appendix 2). The last bench had plants of only one ratio, BA:GA_{4+7} 1:0. The two ratios on a bench were mixed in together so each block had one rate of each ratio for a total of eight plants. Blocks were set lengthwise on the benches so block six was closest to the cooling pad and block one was closest to the fan. Statistical analysis and

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data measurements were similar to that of the first experiment in that ratios were not compared to each other, only rates within a ratio. Dry weights were measured.

Cytokinin evaluation:

In addition to the BA:GA screening, the cytokinins thidiazuron and forchlorfenuron (CPPU, Valent) were evaluated. Forchlorfenuron is a direct application of a cytokinin that is used in tissue culture to stimulate shoot growth. Forchlorfenuron was used at the recommendation of the chemical company representatives due to its similarity to thidiazuron. Rates were based on representative's recommendations. *Clematis* 'Ernest Markham' (Roseville Farms, Apopka, Fla.) was potted into trade gallon pots (3.7 L) from 4.7-cm plugs, using the previously described media on 21 Aug. 2001. Cultural practices were as previously described. The following treatments were applied once on 4 Sept. 2001: forchlorfenuron was applied at 0, 5, 10, 15, 20, or 25 mg·L^{-1} thidiazuron was applied at rates of 0, 50, 100, 150, 200, or 250 mg·L^{-1}.

The experiment was set up in the same manner as the two previous projects. Plants treated with florchlorfenuron were on a separate bench than those treated with thidiazuron (Appendix 2). Six blocks containing one plant of each rate were set lengthwise on the benches as previously described. Data measurements and statistics were the same as the previous projects from 2 to 8 WAT. Data were taken on the same

days as those in the BA:GA screening and dry weights were measured.

Results

Experiment one

Dikegulac: At the end of the experiment, 10 WAT, there was a significant difference in

the number of branches between cultivars for plants treated with dikegulac (Table 1)

(*P<0.0007*); 'Hagley Hybrid' had a greater number of branches compared to 'Ernest

Markham' (HH 8.1 vs. EM 6.5). There were no significant differences in height effects

between cultivars from 4 to 8 WAT (Table 2). There was no significant interaction

between cultivar and chemical treatments. Data presented are the means of the two

cultivars per rate. All treated plants had a significant increase in branch numbers at 8

WAT (Figure 1). Greatest number of branches occurred with 800 mg·L^{-1} of dikegulac.

Adventitious axillary buds that appeared post treatment grew close together in a cluster as

opposed to opposite one another on the axis of leaf primordia.

There was a linear suppression of leader length at increasing rates of dikegulac for all

plants from 2 WAT until measurements ended at 8 WAT (Figure 2, Table 2). Effects

were similar on 'Polish Spirit' (P<0.0001 at 8 WAT, data not presented). At all data

weeks the shortest plants were those treated with dikegulac at 3200 mg·L^{-1}. Dry weight of

plants decreased linearly as dikegulac rates increased (*P< 0.0001,* Table 3) (data not

presented).

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Dikegulac sodium delayed blooming, at 4 WAT 33% of the control plants were in bloom, but none of the plants treated with dikegulac were blooming (Table 4). At 6 WAT control plants had 50% of plants in bloom, or with at least one flower past bloom and 16% of plants treated with 800 mg·L^{-1} were in bloom. At 8 WAT control plants had 50% of plants in bloom or with at least one past bloom. The majority of plants treated with dikegulac began to bloom at 8 WAT. Plants blocked for each rate had at least one plant in bloom, and up to 4 plants in bloom (Table 4). By 10 WAT, plants treated with dikegulac were blooming at the same rate as controls.

Overall results indicate that plants treated with dikegulac had an increase in mean branch number, suppression of mean leader lengths, and a 2 to 4-week delay in bloom time.

BA+GA$_{4+7}$: Treatment effects on branching varied between cultivars from 4 to 8 WAT (Table 1). 'Hagley Hybrid' had more branches than 'Ernest Markham' (HH 8.9 vs. EM 5.6; *P<0.0008* at 8 WAT). Treatment effects on leader lengths were not significantly different between cultivars (Table 2). There was no significant interaction between PGR and cultivar. Data presented are the mean of the two cultivars. BA+GA$_{4+7}$ increased branch numbers from 2 until 8 WAT (Table 1, Figure 3). At the end of the experiment, 8 WAT, 800 and 1600 mg·L^{-1} had the most branches.

BA+GA$_{4+7}$ treatments resulted in a linear suppression of mean leader lengths with an increase of chemical concentration at 4 WAT and 6 WAT (Figure 4). Effects of BA+GA$_{4+7}$ on leader lengths of 'Polish Spirit' were similar to effects on the previous cultivars; leader lengths were suppressed until the end of the experiment (8 WAT, data not presented). However, data were not statistically significant. Dry weights of the large flowering hybrids treated with BA+GA$_{4+7}$ were not significantly different from untreated plants (Table 3).

BA+GA$_{4+7}$ also appeared to delay flowering (Table 4). At 6 WAT, 83% of control plants were blooming. The percent of treated plants blooming ranged from 16% to 83%. At 8 WAT all treated plants and control plants were in bloom and had at least one flower past bloom.

Phytotoxic symptoms occurred on all cultivars treated with BA+GA$_{4+7}$. Symptoms included leaf curl and puckering, lateral branch abortion, and death of the terminal (flower bud or growing point). Symptoms seemed to be more severe with 1200 and 1600 mg·L^{-1}, compared to 400 or 800 mg·L^{-1}. Plants appeared to grow out of phytotoxic symptoms by 4 WAT.

Overall results indicate BA+GA$_{4+7}$ increases branch number, decreases leader lengths, and delays flowering slightly.

Thidiazuron: Branching in response to thidiazuron differed between cultivars from 4 to 8 WAT (Table 1). As seen with dikegulac and BA+GA$_{4+7}$, 'Hagley Hybrid' had more branches than 'Ernest Markham' (HH 8.5 vs. EM 5.4; P<0.0008 at 8 WAT). Cultivar differences were significant for leader length effects at 2, 6, and 8 WAT (Table 2). 'Hagley Hybrid' had shorter leader lengths than 'Ernest Markham' (HH 79.8 cm vs. EM 126 cm; *P<0.0001* at 8 WAT). There were no interaction effects between rate and cultivar. Data presented are the mean of both cultivars. Thidiazuron increased branch numbers linearly at 2 WAT with 2000 mg·L^{-1} resulting in the highest mean number of branches (Figure 5). However, these differences were no longer significant after 4 WAT (Table 1). Leader lengths of 'Hagley Hybrid' and 'Ernest Markham' were not significantly affected by rates of thidiazuron (Table 2). Thidiazuron had a quadratic effect on the leader lengths of 'Polish Spirit' at 4, 6, and 8 WAT (*P<0.0001, P<0.0392,* and *P<0.0059,* respectively). Representative 'Polish Spirit' data from 8 WAT are shown in Figure 6. Treatments had a linear decrease in dry weights of the large flowering hybrids with an increase in concentration (Table 3, data not shown). Thidiazuron did not delay bloom time (Table 4).

Thidiazuron did not affect leader lengths but increased early branching. However, branch number was no longer greater than controls at time of sale, 8 WAT.

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Ethephon: A significant PGR rate x cultivar interaction effect occurred with the use of ethephon (Table 1). The interaction was significant at 6 WAT (*P<0.0002*), and 8 WAT (*P<0.0046*). Significant interactions close to sale date may imply cultivars should be treated separately for optimal results. Therefore, data were analyzed separately for each cultivar. Ethephon did not have a significant effect on the branch number of 'Ernest Markham.' 'Hagley Hybrid' experienced a suppression of branch numbers (Figure 7). Suppression effects were linearly significant at 6 WAT and 8 WAT (*P<0.0001* and *P<0.0040*, respectively).   Mean leader lengths of 'Ernest Markham' were linearly suppressed from 2 WAT until the end of the experiment (*P<0.0001* at 8 WAT) (Figure 8a). 'Hagley Hybrid' also experienced a linear suppression of leader length with increased ethephon concentration (*P<0.0015* at 8 WAT) (Figure 8b). Ethephon linearly decreased the mean leader length of 'Polish Spirit' (Figure 9). Dry weights of 'Ernest Markham' and 'Hagley Hybrid' decreased linearly with an increase in chemical concentration (Table 3, data not presented).

Plants blocked for ethephon treatment did not bloom until 6 WAT (Table 4). At this time, 33% of control plants were in bloom. Plants treated with ethephon did not bloom by the end of the experiment (8 WAT).

Ethephon significantly reduced leader length with increasing rates, and reduced branch numbers of 'Hagley Hybrid.'

BA:GA ratio

The 1 BA:1 GA treatment (equivalent to Fascination) was the only treatment that resulted in a significant increase in branch number by rate throughout the experiment (Table 5). This effect was quadratic from 2 until 8 WAT (Figure 10). Plants treated with 1BA:0.8GA had a significant increase in branch number of 'Ernest Markham' by rate at 6 and 8 WAT (data not presented). Ratios of 1BA:0.4GA and 1BA:0.1GA did not have any significant effects on branch number (Table 6). The ratios 1BA:0.2GA, 1BA:0.09GA and straight BA each had significant effects on branch number for one, sporadic data week.

Plants treated with 1BA: $1GA_{4+7}$ had a linear increase in mean leader length with increasing rate (Table 7, Figure 11). Straight BA had a linear suppression of mean leader length with an increase in chemical concentration (Table 8, Figure 12). Plants treated with Fascination had a significantly quadratic response of dry weight by rate (P< 0.0147), data not presented.

Phytotoxic symptoms occurred in all chemicals at all rates except with the controls, which were treated only with the surfactant (Tween 20). Symptoms were similar to those in previous experiments and occurred with the same severity. Plants expressed leaf puckering and curling, death of the growing point (or flower bud), and brown leaves. As

in previous experiments, plants grew out of phytotoxic symptoms 6 WAT. Phytotoxicity

from the 1BA:0.4GA, 1BA:0.1GA, and straight BA was expressed as a twisting or

contortion of the flower bud. This mutant flower bud occurred at all rates, except the

controls.

Cytokinin evaluation:

Thidiazuron: No significant effects were found with these lower rates of thidiazuron on

'Ernest Markham' (data not presented). Branch numbers remained similar across the 8

weeks of the experiment. No trend in leader length was apparent at these rates. Dry

weights of plants are similar throughout treatment rates.

Forchlorfenuron: No significant effects were seen from the use of forchlorfenuron on

'Ernest Markham' (data not presented). Branch numbers were not consistently affected

throughout the experiment. Mean leader lengths remained similar across the weeks.

Discussion

Experiment one

Dikegulac: Effects of chemical rate on branch number in our experiment were not

significant until 6 WAT (Table 1). This was expected as previous studies with dikegulac

found an initial delay in effect. Al-Juboory and Williams (1991) had an 8-week delay,

and Foley and Keever (1993) had a 6 and 11-week delay in separate experiments.

Adventitious buds broke at the axis of the buds released from dormancy, creating clusters of new growth. Branch clusters occurred on plant growth present at the time of treatment, as well as growth that occurred subsequent to treatment. Clusters could be due to an increase in the cytokinin to auxin ratio, as is the case in the common bacterial disease, "witches broom" (Galston et al., 1980). Clematis did not express leaf chlorosis, a symptom common in other reports of dikegulac use (Bocion and de Silva, 1977; Banko and Stefani, 1996). Response of branch number to rate was not linear; an application of 800 mg·L^{-1} produced the highest number of branches (Figure 1). Leader lengths were suppressed from 2 WAT until the end of the experiment at 10 WAT. Length suppression may be due to the suppression of GA synthesis or the suppression of GA stimulation of DNA precursors (Gressel et al., 1976). Quick response to this chemical is advantageous in keeping leaders from becoming entangled. Long lasting suppression effects will mean easier plants to handle at time of shipping. Bloom time of plants treated with dikegulac lagged behind those of the controls (Table 4). However, by sale time, 8 WAT, all plants had at least one flower in bloom.

Based on these data, the optimal amount of active ingredient is 800 mg·L^{-1} dikegulac. This concentration is an adequate rate for production of clematis vines as it is the optimal rate for branch stimulation and higher rates may lead to stunting of plants. Atrimmec at 800 mg·L^{-1} could be used by clematis producers to enhance branching and suppress leader lengths, without excessive delays in bloom time. Since 800 mg·L^{-1} was the lowest

rate tested in this study, lower rates also may be effective.

BA+GA$_{4+7}$: Treatments with BA+GA$_{4+7}$ had obvious visible effects just 2 WAT, with abundant lateral branches. The same new branches turned black or gray and withered at 2 WAT. Other phytotoxic symptoms included the withering of growth tips, including those that had flower buds. Although rates were statistically linear, rate concentrations of 800 mg·L^{-1} and 1600 mg·L^{-1} had similar branch numbers at 6 and 8 WAT (Figure 3). Use of this chemical at the lesser rate may reduce phytotoxicity with increased branching equal to that of higher rates.

Leader lengths were expected to increase due to the application of gibberellins. BA+GA$_{4+7}$ has been shown to increase leader length and branch number in previous studies (Preece, 1989; Thomas et al., 1992; Banko and Stefani, 1997). The cause of leader length suppression of clematis is likely due to the phytotoxic die back of the apical region of the plants. Linear effects on leader length (Figure 3) could infer that phytotoxicity effects increased with increasing rates. This situation is similar to the case of phytotoxicity from straight BA applications on florist azaleas *(Rhododendron* L. 'Gloria' and 'Prize') (Bell et al., 1997); these authors suggested the cause of shoot length suppression was spray burn of the foliage and stems. Clematis plants began to overcome phytotoxic affects at 4 WAT and overcame significant suppression of height at 8 WAT. At 8 WAT, earlier phytotoxic symptoms were still present on older leaves. Newer

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growth was of good condition. Producers may or may not determine phytotoxic

symptoms to be detrimental to sales. All plants were blooming at the time of sale, 8

WAT (Table 4). BA+GA$_{4+7}$ could be used by growers to increase branch numbers

without suppression of leader length or bloom time by time of sale.

Ethephon did not significantly increase the branch numbers of either hybrid in this study

(Table 1). Previous research has found ethephon increased branch number on clematis

(Peter Konjoian, personal communication). Research on *Portulaca grandiflora* 'Sundial'

and *Salvia farinacea* 'Victoria Blue' demonstrated a suppression of growth of the main

terminal (Banko and Stefani, 1997 and 1996, respectively). Ethephon did not increase

branch number in either study. Ethephon significantly increased axillary shoot number

on *Pelargonium x hortorum* 'Hollywood Star' (Foley and Keever, 1992). One could

generalize that effects of ethephon on branch numbers is species dependent, and not

effective for purposes of this particular research.

All plants treated with ethephon had a linear suppression of leader length (Figures 7 and

8). Ethephon has been shown decrease internode lengths on zonal geraniums

(*Pelargonium x hortorum*) and tomato plants (*Lycopersicon*) (Konjoian, 1997).

Bloom time could not be determined as plants assigned to ethephon treatments were too

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small to bloom during the time period of this project. Our overall recommendation is that Florel at 500 mg·L^{-1} could be used to suppress leader length of *Clematis spp.*

BA:GA ratio

The purpose of this project was to further explore the bud blast and tip dieback experienced in experiment one. The ratio project was based on the premise that the cytokinin (6-BA) and gibberellin (GA $_{4+7}$) mixture of Fascination was combined to break axillary buds from dormancy and enhance branch elongation, respectively. BA has been found to increase axillary branching on numerous ornamentals (Preece, 1990; Wilson and Nell, 1983; Bell et al., 1997; Imamura and Higaki, 1988). Gibberellins are known for stimulation of cell division and increasing plant height (Galston et al., 1980). The hypothesis provoking this experiment was that the GA was causing new buds to grow with such vigor the plant could not sustain the new growth, and therefore aborted them. Phytotoxicity in the form of axillary branch abortion, tip dieback, and leaf curl and puckering persisted throughout the ratio screening, regardless of the decrease in the amount of GA. Such results point to BA as the cause of the phytotoxicity. Phytotoxic necrosis at 2 WAT was experienced at high rates of BA on older leaves of 'Welkeri' *Dieffenbachia* Lodd. (Wilson and Nell, 1983). Imamura and Higaki (1988) found abnormal white leaves on *Anthurium* Andre. plants treated with BA, as well as dieback on shoots that developed subsequent to the BA application. Twisting and contorting of flower buds cannot be explained at this point.

Foliar sprays of BA alone did not increase branching: our results suggest a synergistic

effect between BA and GA that releases buds from dormancy. The ratio of 1BA:1GA

was the only ratio that had significant effects throughout the experiment on both branch

number and leader lengths (Table 6). Other reports found optimal ratios of 50 to 200

mg·L^{-1} BA: 100 mg·L^{-1} GA for English ivy (Al-Juboory and Williams, 1991). Optimal

ratios for *Euphorbia lathyris* were 300 mg·L^{-1} BA: 0 to 3 mg·L^{-1} GA (Preece, 1990).

These ratios have little or no gibberellin in them. The authors reported an inhibition of

branching with increased GA applications. Further investigation of lower BA rates is

necessary to explore reasons for BA+GA$_{4+7}$ phytotoxicity. Florist azaleas treated with

straight BA experienced symptoms such as foliar burn, extreme defoliation and stem

injury, that were not present on plants sprayed with BA+GA$_{4+7}$ (Promalin, Abbott,

Abbott Park, Ill.), suggesting that GA may reduce the phytotoxicity of BA (Bell et al.,

1997). We made similar observations of clematis plants at 2 WAT. The least amount of

phytotoxic symptoms was seen on plants treated with BA+GA$_{4+7}$, a 1:1 ratio. Symptoms

appeared worse as the amount of GA in the ratio decreased, regardless of rate of

application.

Though the data in Figure 9 shows the highest number of branches with 1200 mg·L^{-1}

BA+GA$_{4+7}$, the trend line depicts only slight increases from 800 mg·L^{-1}. Due to the

linear increase in leader length from BA+GA$_{4+7}$, growers will prefer to use lower

concentrations of this chemical to keep plant height manageable. Lower concentrations

are also more economical. Further studies of BA+GA$_{4+7}$ at both rates will be conducted

to find the most optimal compromise between leader length and branch number.

Recommendations from this ratio experiment would be an application of BA+GA$_{4+7}$ at

either 800 or 1200 mg·L^{-1} to increase axillary branch numbers and leader length.

Applications of BA can be used to suppress leader length in a linear fashion.

Cytokinin evaluation

Thidiazuron: Preliminary trials with thidiazuron found no significant effects on final

branch number of clematis. Leader length trials did not have significance effects on large

flowering cultivars, and quadratic suppression for 'Polish Spirit.' Lack of significant

results prompted us to test thidiazuron again at lower rates. Thidiazuron has been shown

to be as effective in tissue culture as BA, at a lower concentration (Goldfarb et al., 1991).

Lower rates of thidiazuron were used in the second experiment on the hypothesis that the

saturation point for this chemical was exceeded in the preliminary screening. Neither

branch nor leader lengths were affected by these lower rates used. Lack of positive

results with foliar applications may be due to a lack of translocation or perhaps the

chemical is conjugated within the leaf cells to become inactive.

Forchlorfenuron: This chemical was tested due to its similarity to thidiazuron. Reasons

for nonsignificant effects may be similar to those of thidiazuron.

Results from this project indicate that foliar sprays of dikegulac at 800 mg·L^{-1} and

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Fascination at either 800 or 1200 mg·L^{-1} can be used to increase branching of *Clematis spp*. Dikegulac has additional benefits to growers by suppressing leader lengths for a more compact plant for easier handling. BA+GA$_{4+7}$ increases leader lengths of clematis, which may be beneficial to the retail market for larger plants. The BA in BA+GA$_{4+7}$ appears to cause phytotoxicity in the form of leaf puckering and curl, tip dieback, and bud blast. Growers would need to determine if intensity of phytotoxicity from BA+GA$_{4+7}$ results in an unsaleable plant.

Literature Cited

Al-Juboory, K.H., and D.J. Williams.1991. Effects of two growth regulators on lateral Branching and shoot growth of English ivy (*Hedera helix*). PGRSA Quarterly 19:35-40.

Banko, T.J., and M.A. Stefani. 1997. Growth regulators enhance branching of portulaca. Proc. SNA Res. Conf. 42:288-289.

Banko, T.J., and M.A. Stefani. 1996. Chemical growth control of *Salvia farinacea* 'Victoria Blue'. Proc. SNA Res. Conf. 41:214-215.

Bell, M.L., R.A. Larson, and D.A. Bailey. 1997. Vegetative growth responses of florist azaleas to dikegulac, $GA_{4+7,}$ and benzylamino purine. HortScience 32(4):690-693.

Bocion, P.F., and W.H. de Silva.1977. Some effects of dikegulac on the physiology of whole plants and tissues: Interactions with plant hormones. 198-198. Proc. Intl. Conf. on Plant Growth Substances, Lausanne, Switzerland.

Bruner, L.L., G.J. Keever, R.A. Kessler, and C.H. Gilliam. 2000. Growth regulation effects of *Lonicera x heckrotti* (Goldflame Honeysuckle). Proc. SNA Res. Conf . 45:263-264.

Foley, J.T. and G.J. Keever. 1993. Chemically induced branching of *Vinca minor*. J Environ. Hort. 11(4):149-152.

Foley, J.T., and G.J. Keever.1992. Chemical promotion of axillary shoot development of geranium stock plants. J. Environ. Hort. 10(2): 90-94.

Galston, A., P. Davies and R. LiSatter. 1980. The life of a green plant. 2nd ed. Prentice Hall, Englewood Cliffs, N.J.

Gressel, J., A Kadouri, D. Atsmon, and N. Cohen. 1976. Effects of dikegulac sodium on nucleic acid syntheses. Collected abstracts of the paper demonstrations:The 9th international conference of plant growth substances. pp.1-2.

Imamura, J.S. and T. Higaki. 1988. Effect of GA_3 and BA on lateral shoot production on *Anthurium*. HortScience 23(2):353-354.

Konjoian, P. 1977. Florel use on perennials. KFES Newsnotes. 97-1.

Preece, J.E. 1990. Growth stimulation of *Euphorbia lathyris* L. by GA_{4+7} and BA. J Plant Growth Regulat. 9:85-88

Thomas, J.C. and F.R. Katterman. 1986. Cytokinin activity induced by thidiazuron. Plant Physiol. 81:681-683.

Thomas, P.A., J.G. Latimer, D. Zhang, and S. Hartzler. 1992. Effects of plant growth regulators on lateral branching and shoot growth of Hypericum. Proc. SNA Res. Conf. 37:261-263.

Walters, D.R., and D.J. Keil. 1996. Vascular plant taxonomy. 4th ed. Kendall Hunt, Dubupue, Iowa.

Wilson, M.R. and T.A. Nell. 1983. Foliar applications of BA increase branching of 'Welkeri' dieffenbachia. HortScience 18(4): 447-448.

Appendix 1

Cooling Pad

Ethephon	Thidiazuron	BA+GA$_{4+7}$	Dikegulac
3 cultivars x 3 rates	3 cultivars x 5 rates	3 cultivars x 5 rates	3 cultivars x 5 rates
3 cultivars x 3 rates	3 cultivars x 5 rates	3 cultivars x 5 rates	3 cultivars x 5 rates
3 cultivars x 3 rates	3 cultivars x 5 rates	3 cultivars x 5 rates	3 cultivars x 5 rates
3 cultivars x 3 rates	3 cultivars x 5 rates	3 cultivars x 5 rates	3 cultivars x 5 rates
3 cultivars x 3 rates	3 cultivars x 5 rates	3 cultivars x 5 rates	3 cultivars x 5 rates
3 cultivars x 3 rates	3 cultivars x 5 rates	3 cultivars x 5 rates	3 cultivars x 5 rates

Fan

Appendix 2

Cooling Pad

2 ratios x 4 rates		2 ratios x 4 rates		2 ratios x 4 rates
2 ratios x 4 rates		2 ratios x 4 rates		2 ratios x 4 rates
2 ratios x 4 rates		2 ratios x 4 rates		2 ratios x 4 rates
2 ratios x 4 rates		2 ratios x 4 rates		2 ratios x 4 rates
2 ratios x 4 rates		2 ratios x 4 rates		2 ratios x 4 rates
2 ratios x 4 rates		2 ratios x 4 rates		2 ratios x 4 rates

1 ratio x 4 rates		forchlorfenuron		thidiazuron
1 ratio x 4 rates		5 rates		5 rates
1 ratio x 4 rates		5 rates		5 rates
1 ratio x 4 rates		5 rates		5 rates
1 ratio x 4 rates		5 rates		5 rates
1 ratio x 4 rates		5 rates		5 rates

Fan

Table 1

Table 1; Exp.1; ANOVA and contrast analysis of branch numbers of *Clematis* 'Ernest Markham' and 'Hagley Hybrid' as affected by PGRs dikegulac, BA+GA$_{4+7}$, thidiazuron, and ethephon at 2,4,6,8,or 10 weeks after treatment (WAT).

	Branch ANOVA[z]																	
	0 WAT			2 WAT			4 WAT			6 WAT			8 WAT			10WAT		
	df	SS	P value	df	SS	P value	df	SS	P value	df	SS	P value	df	SS	P value	df	SS	P value
Dikegulac																		
Cultivar	1	0.417	0.6336	1	45.1	0.0006	1	0.52	0.7997	1	68.11	0.0007	1	118.6	0.0007	1	103	0.0007
cv x rate	4	5	0.6021	4	2.59	0.9386	4	42.7	0.2752	4	49.32	0.233	4	52	0.2333	1	2.501	0.5749
Rate	4	1.67	0.9199	4	7.88	0.6656	4	41.6	0.288	4	54.38	0.0295	4	106.4	0.0295	4	58.7	0.132
Linear	1	0.408	0.637	1	0.581	0.6764	1	0.2288	0.8668	1	9.25	0.3633	1	5.798	0.4253	4	69.5	0.0824
Quadratic	1	1	0.4598	1	2.186	0.4194	1	36.66	0.0393	1	29.85	0.1062	1	6.2996	0.4061	1	2.256	0.5941
BA+GA																		
Cultivar	1	6.01	0.1953	1	40.1	0.081	1	120.4	0.0075	1	112.1	0.0027	1	153.61	0.0008			
cv x rate	4	18.6	0.2723	4	86.9	0.1601	4	101.5	0.1772	4	50.43	0.354	4	31.7	0.6128			
Rate	4	12.2	0.4845	4	176.4	0.0141	4	156.1	0.0524	4	88.2	0.114	4	77.5	0.1785			
Linear	1	0.675	0.6618	1	86.7	0.0117	1	104.5	0.0122	1	54.675	0.0319	1	64.02	0.0241			
Quadratic	1	0.72	0.6514	1	72.02	0.209	1	21.4	0.2434	1	31.72	0.0986	1	1.4	0.7314			
Thidiazuron																		
Cultivar	1	0.02	0.9444	1	14.0	0.2416	1	72.6	0.0250	1	93.8	0.0003	1	144	0.0008			
cv x rate	4	13.7	0.4100	4	32.2	0.5258	4	18.5	0.8471	4	8.17	0.8543	4	43.4	0.4350			
Rate	4	16.7	0.3111	4	106	0.0440	4	96.2	0.1494	4	10.2	0.7953	4	29.2	0.6303			
Linear	1	3.33	0.3263	1	81.7	0.0063	1	72.1	0.0256	1	5.21	0.3617	1	25.2	0.1411			
Quadratic	1	9.52	0.1004	1	3.72	0.5441	1	5.72	0.5186	1	5.00	0.3711	1	1.34	0.7314			
Ethephon																		
Cultivar	1	0.25	0.6362	1	0	1	1	0	1	1	11.67	0.047	1	27.3	0.0091			
cv x rate	4	4.67	0.1386	2	7.5	0.1784	2	18.5	0.0921	2	69.4	0.0002	2	46.1	0.0046			
Rate	4	12.2	0.4845	4	176.4	0.0141	4	156.1	0.0524	4	88.2	0.114	4	77.5	0.1785			
Linear	1	0.667	0.4415	1	12.24	0.022	1	12.04	0.0762	1	82.96	0.0001	1	35	0.0037			
Quadratic	1	2	0.1877	1	5.02	0.1305	1	1.125	0.5769	1	1.03	0.5384	1	0.4091	0.7314			

[z] Mean branch numbers are sum of 12 replications of 5 rates for each of the four PGRs

Table 2

Table 2; Exp 1; ANOVA and contrast analysis of leader lengths of *Clematis* 'Ernest Markham' and 'Hagley Hybrid' as affected by PGRs dikegulac, BA+GA$_{4+7}$, thidiazuron, and ethephon at 2, 4, 6, 8 weeks after treatment (WAT)

| | Leader lengths ANOVA[z] | | | | | | | | | | | |
| | 2 WAT | | | 4 WAT | | | 6 WAT | | | 8 WAT | | |
Treatment	df	SS	P value	df	SS	P value	df	SS	P value	df	SS	P value
Dikegulac												
Cultivar	1	2066.2	0.0001	1	582.8	0.0534	1	448.7	0.306	1	1714	0.0795
cv x rate	4	511.2	0.2196	4	1487.3	0.055	4	836.07	0.736	4	4933	0.0721
Rate	4	4152.4	0.0001	4	22422	0.0001	4	27355.3	0.0001	4	21251.3	0.0001
Linear	1	3566.04	0.0001	1	20349.3	0.0001	1		0.0001	1	19140	0.0001
Quadratic	1	395.2	0.0369	1	1461.6	0.003	1		0.1833	1	216	0.5269
BA+GA												
Cultivar	1	753.38	0.0389	1	248.68	0.3802	1	913.1	0.2206	1	3449.9	0.0613
cv x rate	4	389.55	0.6749	4	3058.08	0.0626	4	6464.02	0.0408	4	8990.7	0.0641
Rate	4	924.12	0.2531	4	2877.96	0.0762	4	7892.3	0.018	4	6551.44	0.1559
Linear	1	376.09	0.1397	1	1867.1	0.0192	1	6712.9	0.0016	1	2392.4	0.1168
Quadratic	1	297.97	0.1875	1	469.8	0.2295	1	616.4	0.3128	1	1915.06	0.1594
Thidiazuron												
Cultivar	1	2305.3	0.0006	1	536.05	0.1758	1	18555.97	0.0001	1	32764	0.0001
cv x rate	4	169.9	0.9078	4	1728.16	0.211	4	1439.66	0.8109	4	775.46	0.9548
Rate	4	880.5	0.2849	4	6765.31	0.0006	4	7455.83	0.1038	4	8607.69	0.1384
Linear	1	663.78	0.0539	1	1	0.0001	1	4106.44	0.0392	1	7571.156	0.0145
Quadratic	1	10.98	0.8002	1	1	0.786	1	1178.64	0.2613	1	190.75	0.6885
Ethephon												
Cultivar	1	119.78	0.2938	1	272.19	0.2067	1	130.7	0.442	1	391.9	0.2382
cv x rate	2	66.52	0.7295	2	46	0.8683	2	1561.8	0.0453	2	8067.5	0.0001
Rate	2	6494	0.0001	2	32533	0.0001	2	53662.4	0.0001	2	51369.6	0.0001
Linear	1	5465.2	0.0001	1	24457.74	0.0001	1	39930.1	0.0001	1	41911.7	0.0001
Quadratic	1	1028.8	0.0043	1	8075.3	0.0001	1	8346.7	0.0001	1	12156.3	0.0001

[z] Mean leader lengths are sum of 6 replications of 4 rates.

Table 3

Table 3; Exp 1. ; ANOVA and contrast analysis of dry weights of Clematis 'Ernest Markham' and 'Hagley Hybrid' as affected by PGRs dikegulac, BA+GA4+7, thidiazuron, and ethephon.

	df	Dry weight ANOVA[z] SS	P value
Dikegulac			
Model	14	288.6	0.0006
Error	43	244.1	
Corrected total	57	532.7	
R^2	0.54		
Rep	5	85.4	0.0204
Cultivar	1	4.69	0.3686
Rate	4	167.1	0.0001
cv x rate	4	31.4	0.2563
Linear	1	145.9	0.0001
Quadratic	1	2.295	0.5282
BA+GA			
Model	14	39.6	0.5422
Error	45	138.3	
Corrected total	59	178	
R^2	0.22		
Rep	5	21.4	0.246
Cultivar	1	0.267	0.7697
Rate	4	11.23	0.4639
cv x rate	4	6.84	0.6955
Linear	1	8.27	
Quadratic	1	2.31	0.3905
Thidiazuron			
Model	14	73.2	0.0526
Error	45	123.8	
Corrected total	59	197	
R^2	0.37		
rep	5	4.7	0.8839
Cultivar	1	19.8	0.01
Rate	4	46.7	0.0054
cv x rate	4	1.95	0.9489
Table 3		37.3	0.0006
		4.11	0.2276
ethephon			
Model	10	388.9	0.0001
Error	25	119.3	
Corrected total	35	508.2	
R^2	0.77		
Rep	5	48.8	0.1065

Cultivar	1	1.14	0.5928
Rate	2	334	0.0001
cv x rate	2	4.36	0.6379
Linear	1	266.7	0.0001
Quadratic	1	67.67	0.0009

[z] Mean leader lengths are sum of 6 replications of 4 rates.

Table 4

Table 4: Exp. 1. Percent of *Clematis* **'Hagley Hybrid' in bloom or with at least one flower past bloom. Percents are based on 6 plants per rate.**

PGR	Rate (mg·L^{-1})	Percent plants in bloom			
		4 WAT	**6 WAT**	**8 WAT**	**10 WAT**
Dikegulac					
	0	33	50	50	50
	800	0	16	50	66
	1600	0	0	66	66
	2400	0	0	16	16
	3200	0	0	33	50
BA+GA					
	0	0	83	100	
	400	16	33	100	
	800	0	33	100	
	1200	16	50	100	
	1600	16	16	100	
Thidiazuron					
	0	50	83	83	
	500	16	50	100	
	1000	16	66	66	
	1500	16	83	83	
	2000	0	66	83	
Ethephon					
	0	0	33	33	
	500	0	0	0	
	1000	0	0	0	

Table 5

Table 5: Exp 2; ANOVA and contrast analysis of branch numbers of *Clematis* 'Ernest Markham' treated with 1 BA: 1 GA$_{4+7}$. Branches were counted at 2, 4, 6, and 8 weeks after treatement (WAT).

		Branch ANOVA									
	2 WAT			4 WAT			6 WAT			8 WAT		
PGR	df	SS	P value	df	SS	P value	df	SS	P value	df	SS	P value
1 BA: 1 GA$_{4+7}$												
Rate	3	96.6	0.0067	3	112	0.0052	3	57.1	0.0114	3	69	0.024
Linear	1	12.4	0.1453	1	18.4	0.0924	1	16.8	0.048	1	13.3	0.1392
Quadratic	1	81.2	0.0015	1	95.1	0.001	1	40	0.0047	1	48.2	0.0096

[z] Mean leader lengths are sum of 6 replications of 4 rates.

Table 6

Table 6:Exp. 2; Significance levels of a foliar application of different ratios of BA and GA$_{4+7}$ to *Clematis* 'Ernest Markham' at 8 WAT.

BA:GA$_{4+7}$		Branches	Leader length (cm)
1:1	Rate effect	0.024	0.0006
	Contrast	0.010Q^z	0.0003L
1:0.8	Rate effect	0.021	NS
	Contrast	0.113Q	NS
1:0.4	Rate effect	NS	NS
	Contrast	NS	NS
1:0.2	Rate effect	0.0102	NS
	Contrast	0.0023L	NS
	Rate effect	NS	NS
1:0.1	Contrast	NS	0.0081L
	Rate effect	NS	NS
1:0.05	Contrast	NS	NS
	Rate effect	0.0197	0.0156
1:0	Contrast	0.0045L	0.0092L

^{z} L= linear

Q= quadratic

Table 7

Table 7:Exp. 2 ANOVA and contrast analysis of leader lengths of Clematis 'Ernest Markham' 1 BA: 1 GA4+7.

	Leader Length (cm) ANOVA[z]											
	2 WAT			4 WAT			6 WAT			8 WAT		
PGR	df	SS	P value	df	SS	P value	df	SS	P value	df	SS	P value
1 BA: 1 GA$_{4+7}$												
Rate	3	1081	0.1148	3	5471	0.0001	3	3770	0.0125	3	5649	0.0006
Linear	1	570	0.0738	1	4180	0.0001	1	3002	0.0033	1	4121	0.0003
Quadratic	1	433	0.1144	1	1097	0.0112	1	633	0.1233	1	1193	0.0224

[z] Mean leader lengths are sum of 6 replications of 4 rates.

Table 8:Exp. 2 ANOVA and contrast analysis of leader lengths of Clematis 'Ernest Markham' 1 BA: O GA4+7.

	Leader Length (cm) ANOVA[z]											
	2 WAT			4 WAT			6 WAT			8 WAT		
PGR	df	SS	P value	df	SS	P value	df	SS	P value	df	SS	P value
1 BA : 0 GA$_{4+7}$												
Rate	3	2237	0.0062	3	1826	0.016	3	1718	0.0073	3	1820	0.0156
Linear	1	1468	0.0034	1	1128	0.0098	1	1160	0.0035	1	1131	0.0092
Quadratic	1	768	0.0237	1	659	0.0386	1	689	0.0172	1	688	0.0342

[z] Mean leader lengths are sum of 6 replications of 4 rates.

Figure Legends

Figure 1: Exp. 1; Effects of Dikegulac sodium treatments on number of branches of *Clematis* 'Ernest Markham' and 'Hagley Hybrid' at 8 WAT. Points are least square mean branch number of six plants per rate per cultivar. Bars represent ± standard deviation.

Figure 2: Exp. 1; Effects of dikegulac treatments on *Clematis* 'Ernest Markham' and 'Hagley Hybrid'. Leader length is the mean of the three longest leaders (cm) per plant. Points are least square mean of six plants per rate per cultivar, the mean of both cultivars. Data presented are from 8 WAT. Bars represent ± standard deviation. Effects are linear *(P<0.0001)* Y= -0.016X + 99.88; R^2= 0.90.

Figure 3: Exp. 1; Effects of BA+GA$_{4+7}$ treatments on number of branches of *Clematis* 'Ernest Markham' and 'Hagley Hybrid' at 8 WAT. Points are least square mean branch number of six plants per rate per cultivar. Bars represent ± standard deviation.

Figure 4: Exp. 1; Suppression of mean leader lengths of *Clematis* 'Hagley Hybrid' and 'Ernest Markham' by treatments of BA+GA$_{4+7}$ at 4 and 6 WAT (*P<0.0762,* and *P<0.018* respectively). Leader lengths are the mean of the three longest leaders per pot. Points are the least square means of six plants per rate, the mean of both cultivars. Bars represent ± standard deviation. Y=-0.00986X+77.31; R^2= 0.65 (4 WAT) ; Y=-0.0186X + 97.47;

R^2= 0.85 (6 WAT).

Figure 5: Exp. 1; Results of thidiazuron treatments on branch numbers of *Clematis* cultivars 'Ernest Markham' and 'Hagley Hybrid' at 2 WAT. Branches counted are ≥2.5 cm in length. Points are least square mean branch number of both cultivars, of six plants per rate. Bars represent ± standard deviation. Effects were linear *(P<0.0063)*

$Y= 0.00165X + 4.3; R^2= 0.77.$

Figure 6: Exp. 1; Quadratic response of *Clematis viticella* 'Polish Spirit' leader length to increasing rates of thidiazuron at 8 WAT. Points are least square mean of six plants per rate. Bars represent ± standard deviation. $Y= 7.9286x^2 - 48.871x + 130.18; R^2 = 0.9023.$

Figure 7: Exp. 1; Results of ethephon treatments on branch numbers of Clematis 'Hagley Hybrid' at 6 and 8 WAT. Branches counted are ≥2.5 cm in length. Points are least square mean branch number of six plants per rate. Ethephon treatments were single foliar sprays at 0, 500, or 1,000 mg·L^{-1}. $R^2=0.95$ and 0.99 for 6 and 8 WAT, respectively.

Figure 8: Exp. 1; Suppression of mean leader lengths of two *Clematis* cultivars, a) 'Ernest Markham' and b) 'Hagley Hybrid', by treatment with ethephon. Points are the least square means of six plants per rate. Ethephon treatments were single foliar sprays at 0, 500 or 1,000 mg·L^{-1}. $R^2=0.80.$

Figure 9: Exp. 1; Suppression of leader lengths of *Clematis viticella* 'Polish Spirit' by ethephon. Leader lengths are mean of five longest leaders (cm) per plant. Points are least square mean of six plants per rate. Bars represent ± standard deviation. P<0.0001 at 8

WAT. Y=-0.1037+ 110.3; R^2=0.85.

Figure 10: Exp. 2; Effects of increasing rates of BA+GA$_{4+7}$ on branch number of *Clematis* 'Ernest Markham' at 8 WAT. Branches are ≥2.5 cm in length; data are the mean of six plants per rate. Bars represent ± standard deviation. Effects are quadratic (P<0.0096) Y=-0.000004X^2+0.0005426X+6.2453125; R^2=0.27.

Figure 11: Exp. 2; Effects of increasing rates of BA+GA$_{4+7}$ on mean leader lengths of *Clematis* 'Ernest Markham'. Data are mean of three longest leaders per plant, mean of six plants per rate. Bars represent ± standard deviation. Effects are linear at 8 WAT (*P<0.0003*) Y= 0.0242*X* + 24.14; R^2=0.88.

Figure 12: Exp. 2; Effects of increasing rates of benzyladenine on leader length of *Clematis* 'Ernest Markham' by date. Data are the lengths of the three longest leaders per plant, mean of six plants per rate. Bars represent ± standard deviation. Effects are linear at 8 WAT (P<0.0092) Y= -0.011X + 55.33; R^2= 0.77.
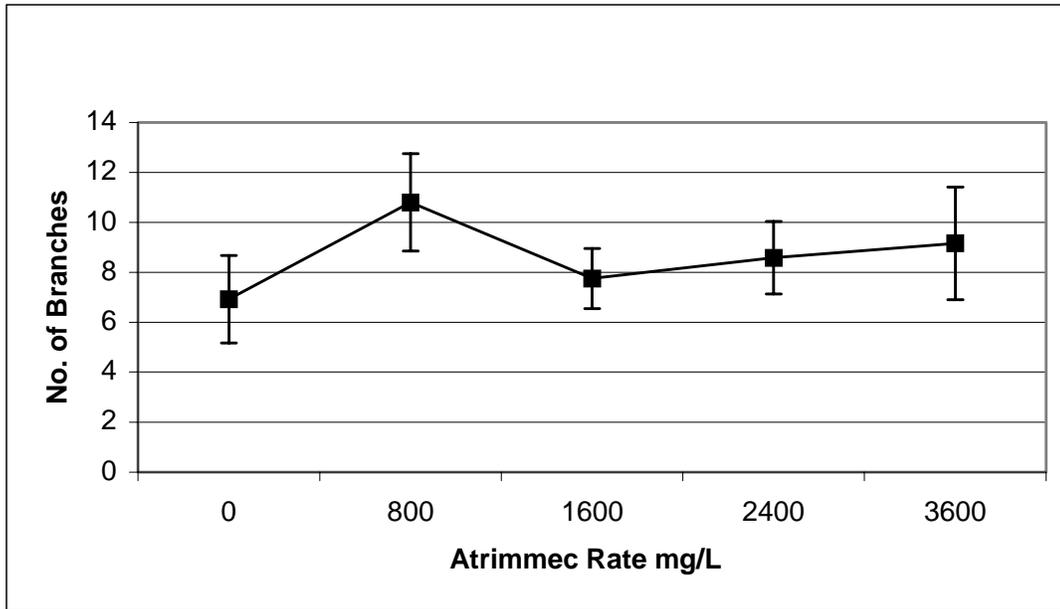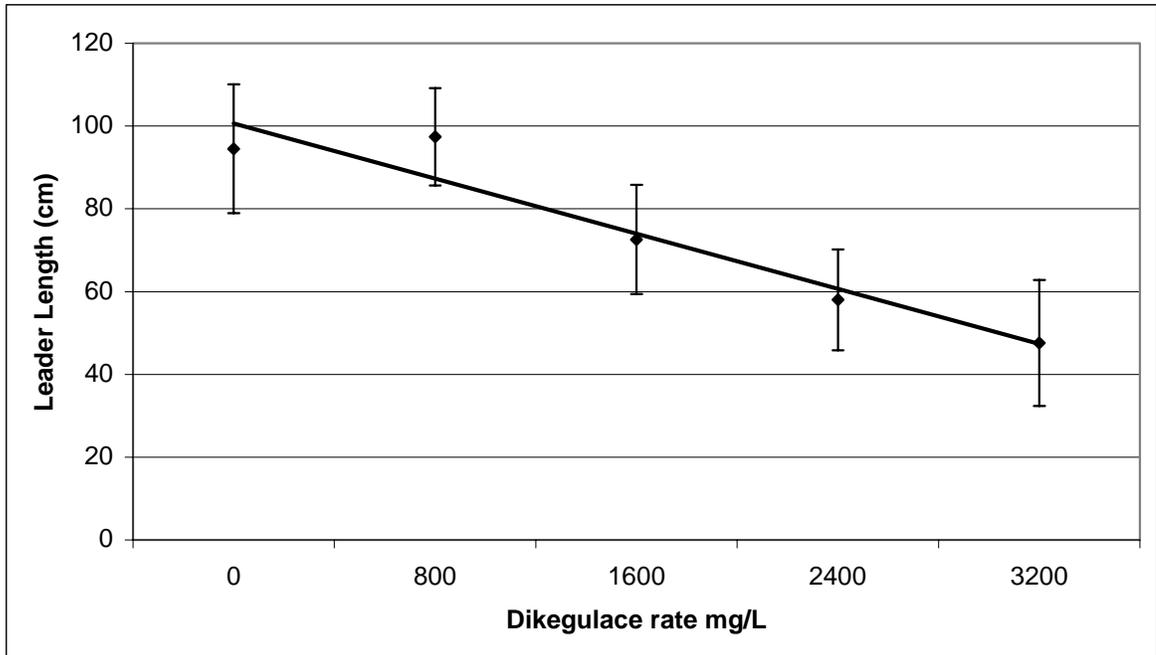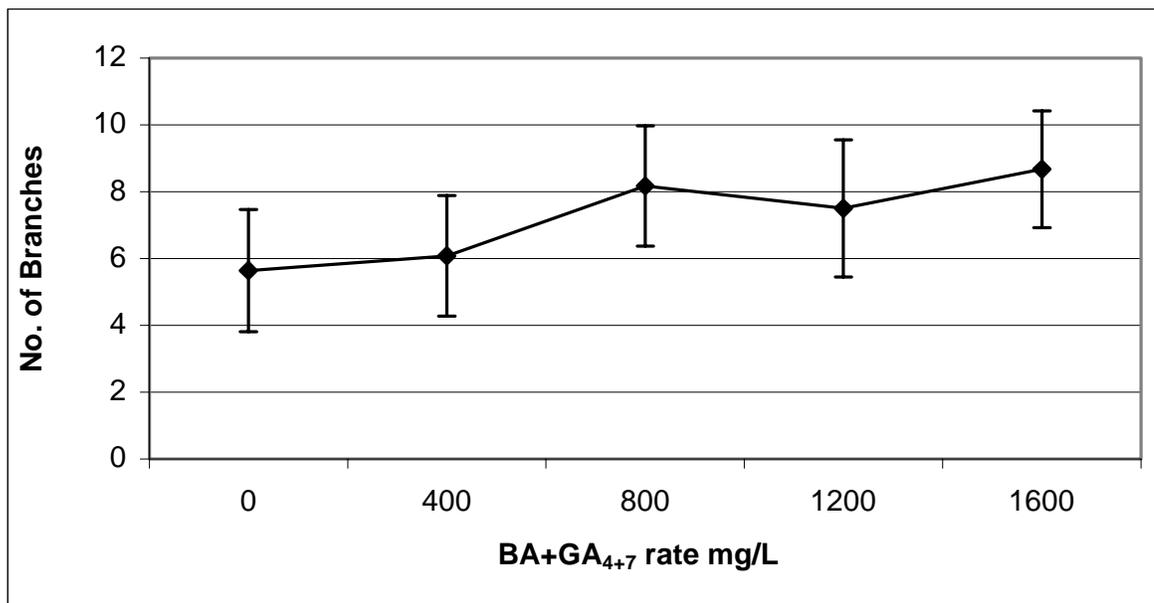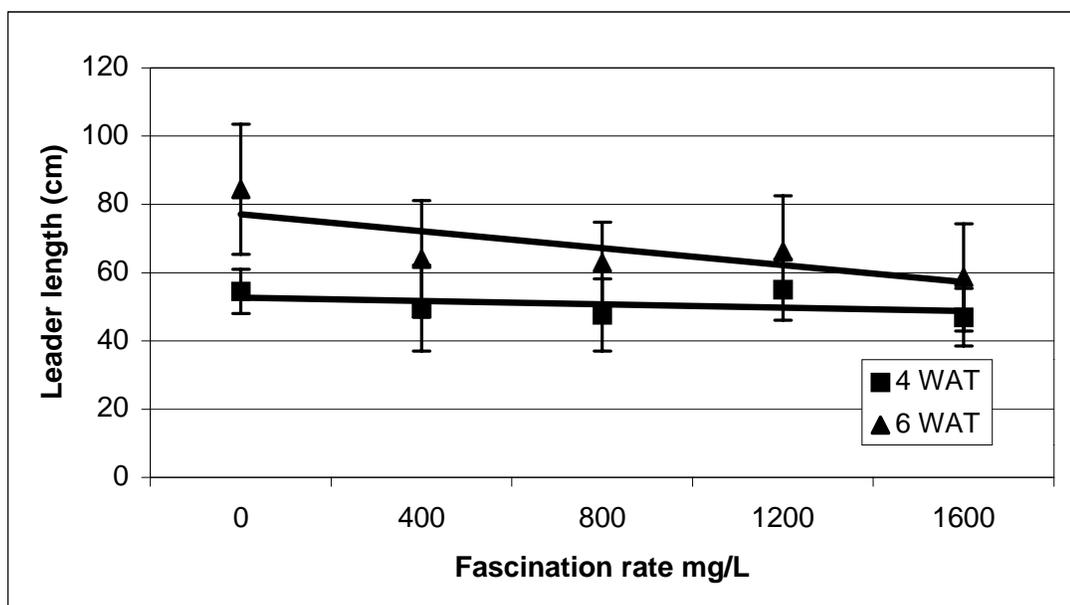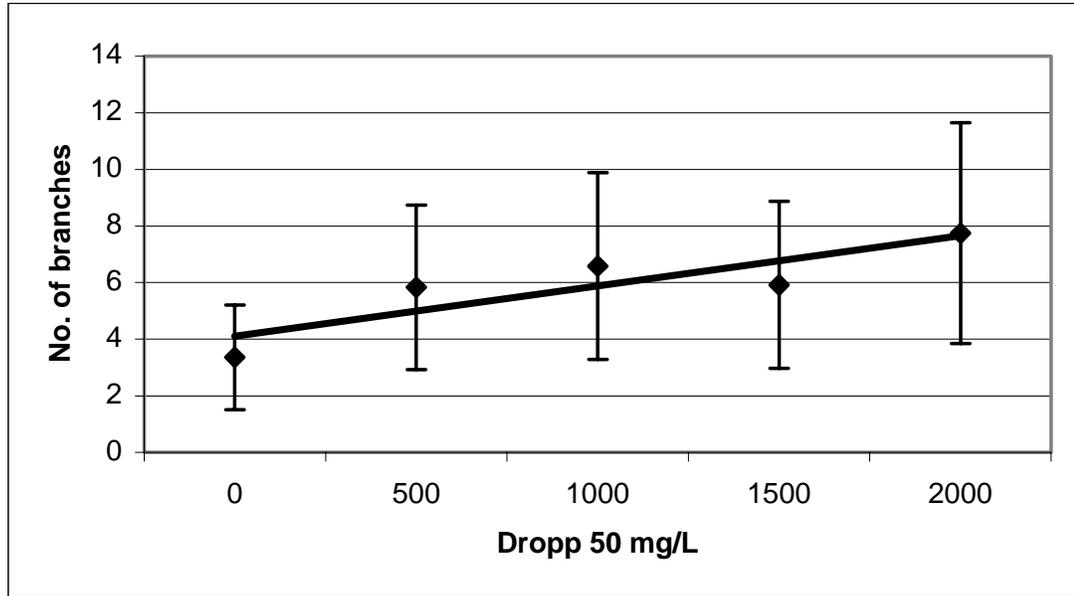
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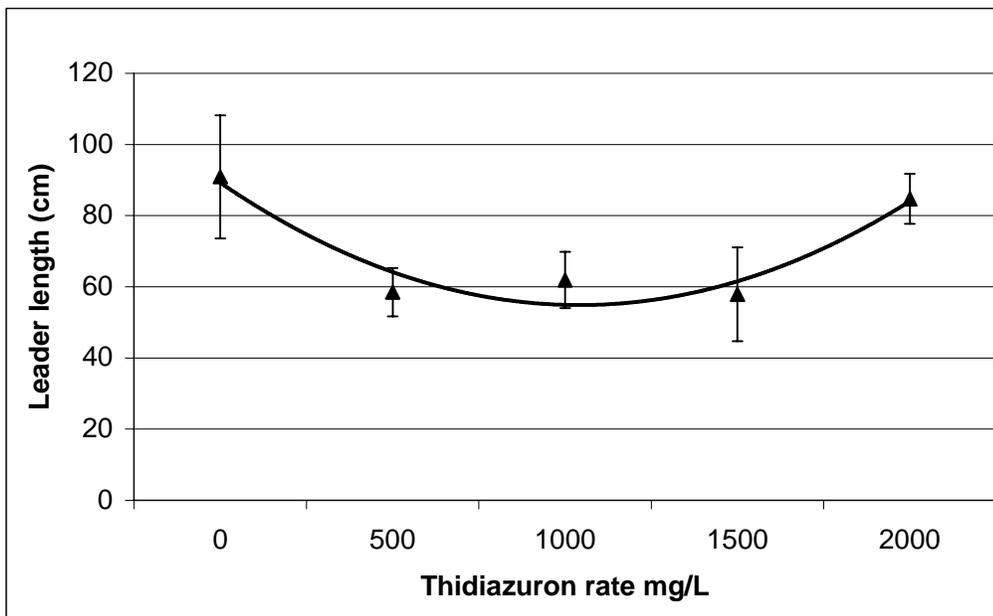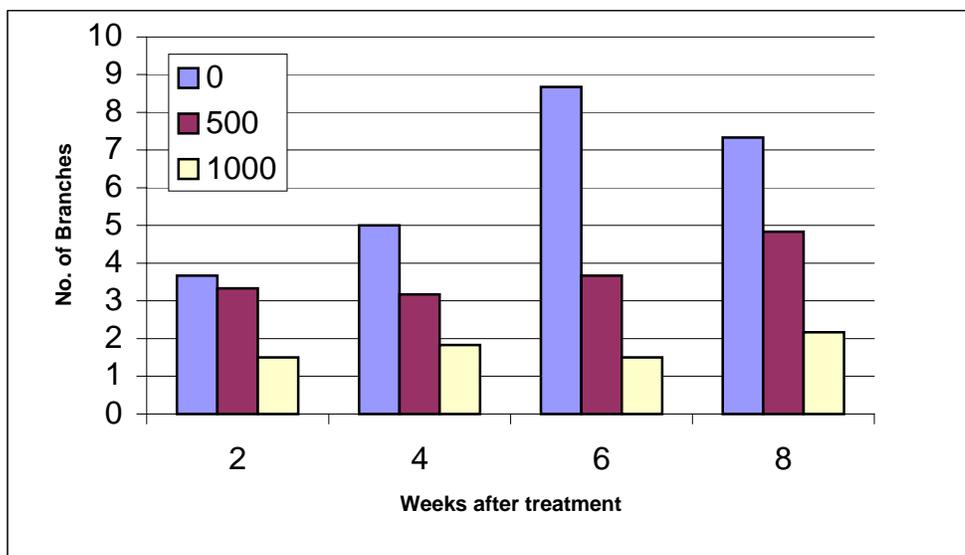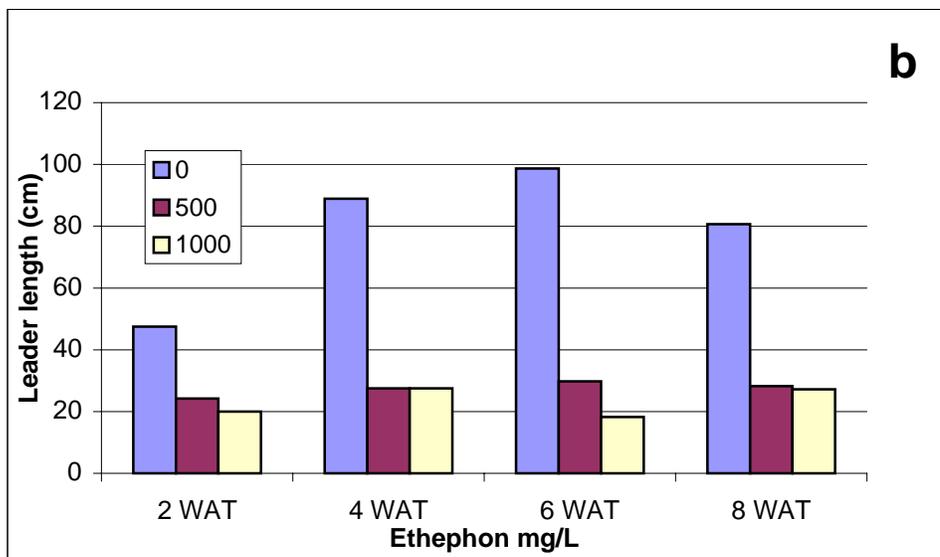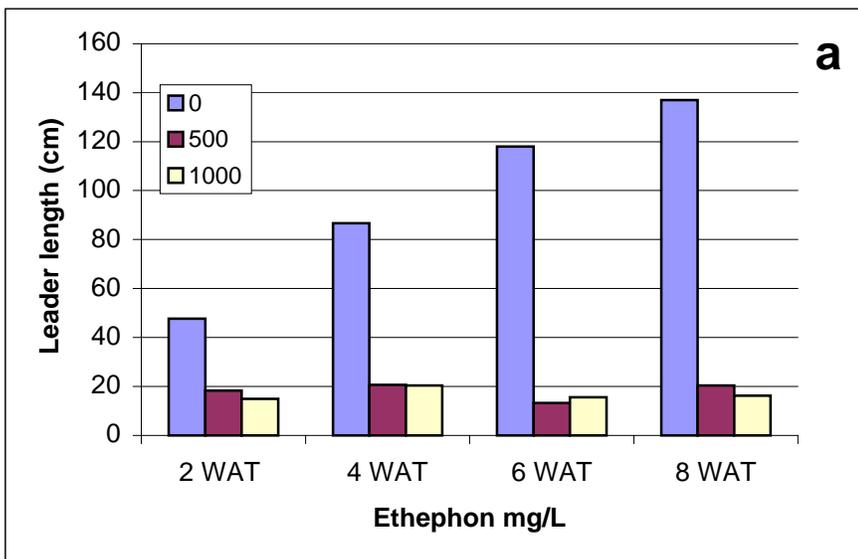
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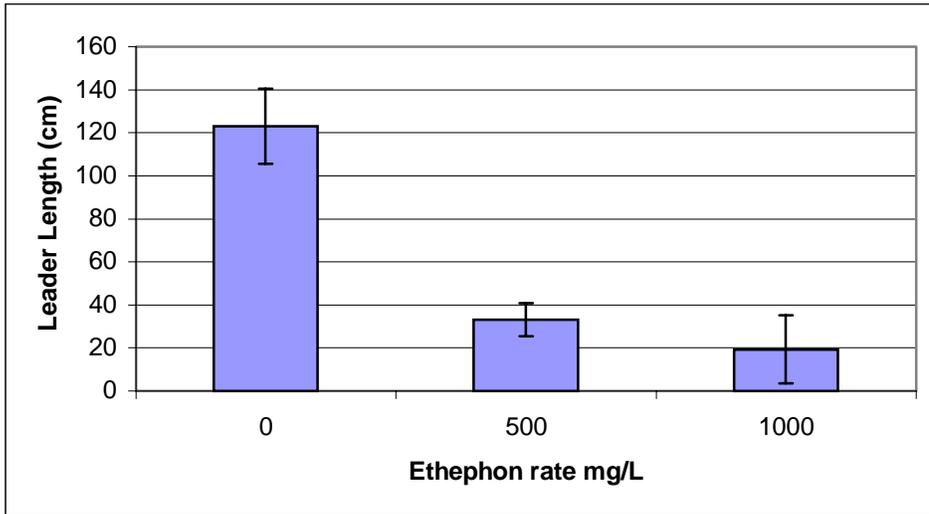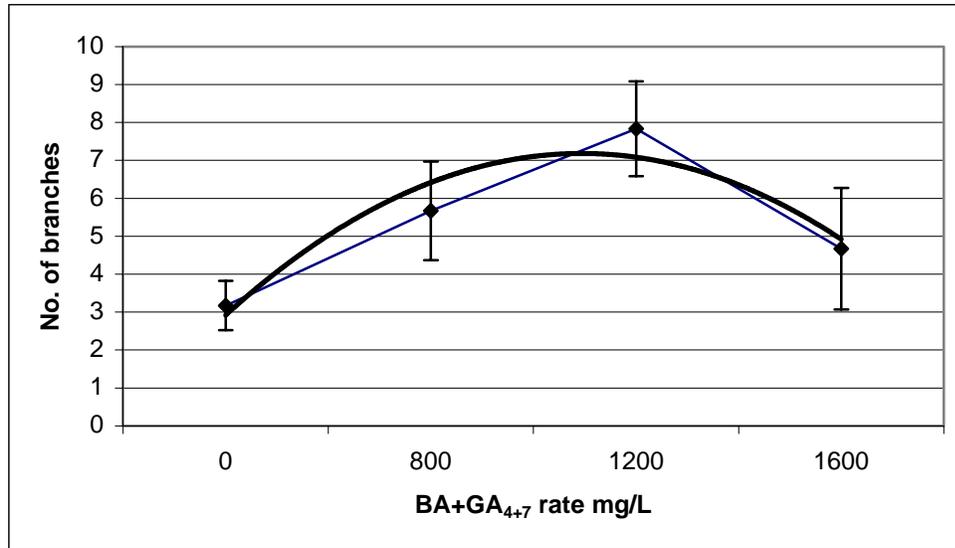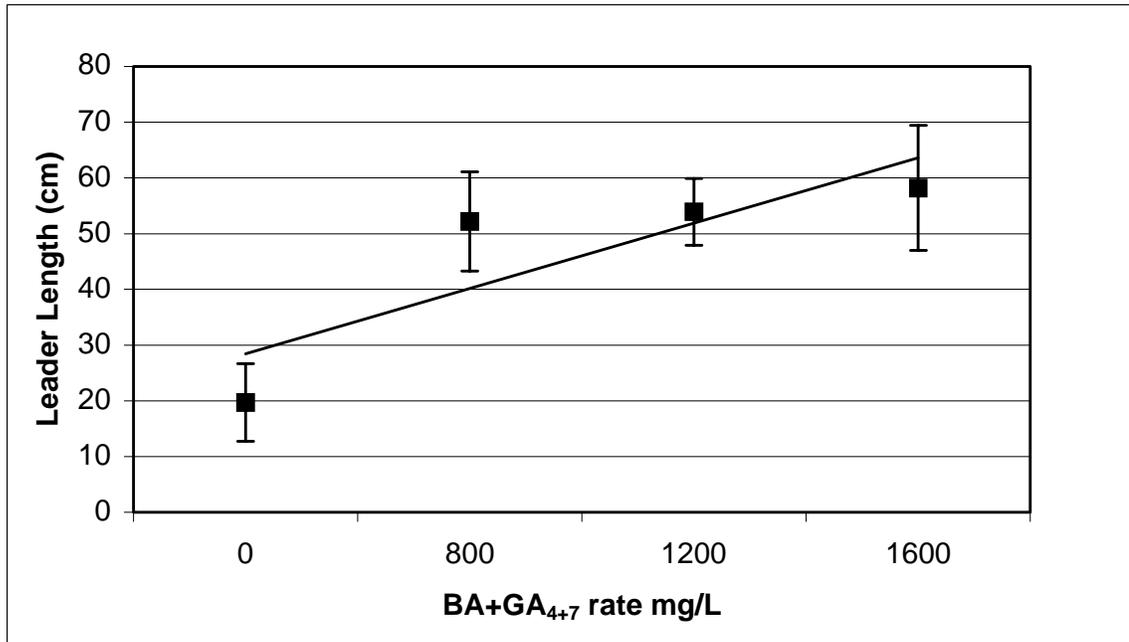
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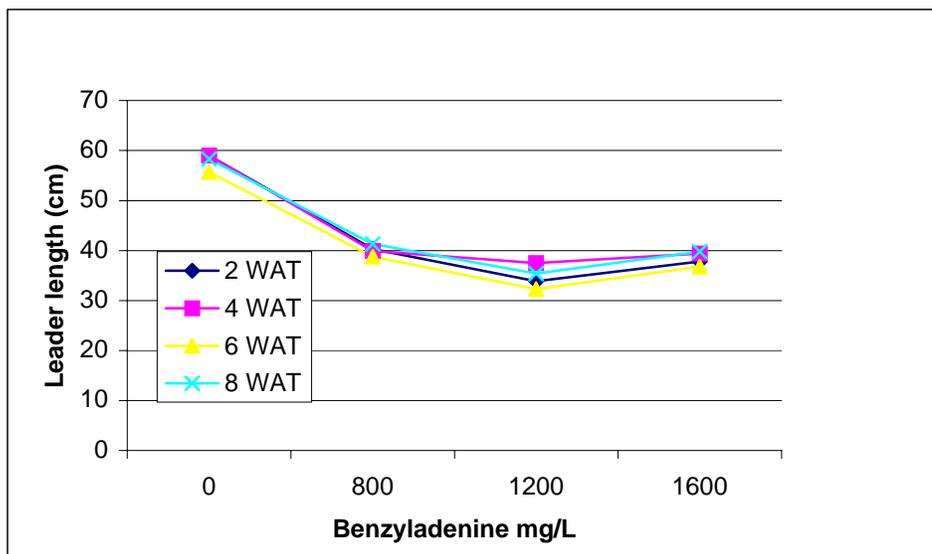
PGRs Replace Pinching Clematis spp. to Increase Branching

Sadie Puglisi[1]; Joyce Latimer[2] and Holly Scoggins[3]

Department of Horticulture, 301 Saunders Hall, Virginia Polytechnic Institute & State University, Blacksburg, VA 24061-0327

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[1]Research Assistant. This paper is based on a portion of a thesis submitted by the senior author in partial fulfillment of the requirements for the M.S. degree.

[2]Professor.

[3]Assistant Professor.

Developmental Physiology

PGRs Replace Pinching of *Clematis* spp. to Increase Branching

Key words: apical dominance, Fascination, BA, GA_{4+7}, Dikegulac, Atrimmec, pinch

Abstract

Foliar sprays of branch enhancing plant growth regulators (PGRs); dikegulac sodium

(Atrimmec) at 800 mg·L^{-1} or BA+GA_{4+7} (Fascination) at 800 or1200 mg·L^{-1}, were applied

to *Clematis* L. 'Comtesse de Bouchard,' 'Ernest Markham,' and 'Hagley Hybrid,' to test

for practical alternatives to hand pinching. Prior to spraying, vines were either pinched or

left intact. Branch number, leader lengths, and time to bloom were measured. Pinching

alone increased branch numbers of 'Comtesse de Bouchard' at 2 and 4 weeks after

treatment (WAT). Pinching suppressed the branch numbers of all cultivars at 6 WAT

and of 'Comtesse de Bouchard' and 'Hagley Hybrid' at 8 WAT. Pinching suppressed the

leader lengths of all cultivars through 4 WAT, and 'Hagley Hybrid' until 6 WAT.

Although some cultivars had a significant interaction effect between PGR treatment and

pinch treatment, PGRs alone produced the greatest number of branches, regardless of

pinch. Dikegulac produced the greatest number of branches for 'Comtesse de Bouchard'

and 'Ernest Markham', and greatest suppression of leader lengths for all cultivars with a

slight delay in bloom time. BA+GA_{4+7} effects did not differ between rates. 'Comtesse

de Bouchard' treated with BA+GA_{4+7} had fewer branches than control plants.

BA+GA$_{4+7}$ treated 'Ernest Markham' plants had an increase in branching at 2 and 4 WAT, which turned into suppression at 6 WAT and treated 'Hagley Hybrid' had a significant increase in branch numbers at 2 and 6 WAT. All cultivars treated with BA+GA$_{4+7}$ had an increase in leader lengths. 'Ernest Markham' experienced a delay in bloom. All plants treated with BA+GA$_{4+7}$ experienced phytotoxicity. Results from this project indicate that dikegulac could be used in clematis production to enhance branching, decrease labor needs associated with pinching and provide a blooming vine for retail sale. Chemical names used: dikegulac sodium, 2,3:4,6-bio-O- (1-methylethylidene) – O-(2)-hexulofuranosonic acid; 6-benzyladenine, N-(phenylmethyl)-1H-purine 6-amine; and gibberellins 4 and 7

Introduction

Clematis vines are grown for their climbing, twining habit and showy blooms that last for weeks. Hybridization of clematis has been a popular hobby of many since before 20th Century (Gooch, 1996). Plantsmen like Ernest Markham and William Jackman are credited with hybridizing varieties of clematis that have unique and brilliant flowers. However, many of these hybrids do not have the vegetative growth that consumer's desire. Growth of many clematis hybrids is from the main leader, producing a thin, unbranched plant with one cyme. Producers increase branch numbers by pinching the vines back to the first node above the soil line after transplanting of plugs (personal communication, Johnny Patterson, Lancaster Farms, Suffolk, VA). After new growth is seen, a second pinch is done to the second node above the soil line. From this point, vines can be pinched back as often as once a week until sale. Pinching also shortens leader lengths to prevent tangling and improve the ease of handling and shipping. Clematis take 6 to 8 weeks to bloom after the last pinch. The practice of pinching, or cutting back, is labor intensive and compromises flowers in favor of vegetative growth at the time of sale. An average of $887.00 is spent on cutting back 92.9 m^2 of *Vinca minor* 'Bowles,' a vine of similar growth habit (personal communication, Becky Moss, Riverbend Nursery, Riner VA). Under the same conditions, the labor cost to spray a similar area with PGR using a hand held CO_2 sprayer is $64. The cost of using a chemical PGR would vary depending on the cost of the product and the method of foliar

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application. However, the cost of PGR use would be less than that of manual pinching.

Although these PGRs are not currently labeled for clematis production, previous research

has demonstrated that one spray of the PGRs dikegulac or $BA+GA_{4+7}$ can increase

branch numbers to reduce the need for pinching and therefore the labor required (Puglisi

and Latimer, unpublished). In addition to branch enhancement, dikegulac suppresses

leader lengths to produce a more compact plant. The purpose of this experiment is to

incorporate previously studied PGRs, dikegulac and $BA+GA_{4+7}$, into the common

production practices for clematis to test if they are a viable alternative to mechanical

pinching.

Materials and Method

Clematis cultivars 'Comtesse de Bouchard,' 'Ernest Markham,' and 'Hagley Hybrid'

arrived well rooted in 4.7 cm pots on 4 Mar. 2002. Plugs were transplanted one week

later into trade gallon pots (3.7 L) using a medium of 6 bark fines : 2 Canadian sphagnum

peat moss : 1 perlite (by volume) (Scott's Sierra Perennial Mix, The Scott's Co.,

Marysville, Ohio). Greenhouse temperatures were set at 15 ℃ night/ 21 ℃ day; air

temperatures averaged 14 ℃ night/ 25 ℃ day throughout the 8 week experiment. Plants

were given a constant liquid feed of 150 ppm 20N-4.4P-16.6K (Peter's 20-10-20

Fertilizer, The Scott's Co.,). Media pH averaged 5.8 with a range of 5.1 to 6.0, and

electrical conductivity (EC) averaged 1.57 with a range from 1.00 to 3.00 dS/m^{-1}.

Plants were treated on 19 Mar. 2002. Treatments quantified included either a pinch or

non-pinch (intact), one foliar application of a PGR, and cultivar. Half of the plants from

each cultivar, 72 plants total, were pinched down to the first node above the soil line. The

remaining 72 were left intact. Plants were divided into six blocks based on foliage

surface area to ensure even uptake of PGRs at corresponding rates. Each block received

the same treatments to serve as six replications of each treatment combination. All

plants, pinched and intact, were given treatments of either 800 or 1200 mg·L^{-1} BA+GA$_{4+7}$

(Fascination, 6-Benzyladenine: Gibberellins A$_4$A$_7$ 1.8:1.8 w/w, Valent U.S.A Corp.,

Germantown, Tenn.); or 800 mg· L^{-1} of dikegulac (Atrimmec, PBI Gordon, Kansas City,

Mo.); or no PGR (control) for a total of six plants per chemical, per block. The

surfactant, Tween 20, at a rate of 0.1 ml·L^{-1} was added to all PGR solutions or applied as

a foliar spray to controls. PGRs were applied once as a foliar spray using a 2 L hand-held

CO$_2$ pressurized sprayer (R&D Sprayers Inc., Model AS, Opelousas, LA) with a single,

8002VS nozzle with a straight 22" boom. The spray was evenly applied over a square

meter area at 28 psi for 28 seconds for a total volume of 210 mL·m^2. At the time of the

PGR applications, environmental conditions were cloudy and cool, air temperature was

21 ℃ and relative humidity was 70%.

 The experiment was a randomized complete block design with six replications. Blocks

were set across four greenhouse benches (Appendix three). Each bench had six plants of

each block. One block contained eight plants of each cultivar; four pinched and four

intact, each plant was sprayed with one chemical treatment or left as a control.   Data

were taken once every 2 weeks from 2 weeks after treatment (WAT) until 8 WAT. Data

included a count of shoot number and branch number. Shoots were determined to be

leaders that began at the soil line; branches were determined to be any axillary branch

above the soil line greater than or equal to 2.5 cm in length. The longest leader was

measured in cm from the rim of the pot to the top of the growth point, flower bud, or

flower. Numbers reported are the mean number of shoots, branches or leader lengths of

each cultivar from the six replications of each combination. The number of plants in

bloom was noted every two weeks. A final count of blooms was taken at 8 WAT. After

data were taken at 8 WAT, plant shoots were harvested from the soil line and dried at

65ºC for 3 days. Dry weights were measured in grams. Statistical analysis was done

using SAS (Cary, NC). The Least Square Means of shoot, branches, leader lengths and

dry weights for each treatment were determined by an ANOVA. The interactions tested

in the ANOVA were between pinch and cultivar, cultivar and PGR, PGR and pinch, and

cultivar x PGR x pinch. The main interaction tested was pinch x cultivar. If this

interaction was significant, pinch treatment statistics were analyzed separately for each

cultivar. Secondly, if PGR and cultivar interactions occurred PGR treatment data were

analyzed separately by cultivar. Thirdly, if a PGR x pinch interaction occurred, PGR

data were analyzed separately for pinched and intact plants. If a three-way interaction

occurred, all data were analyzed separately for each cultivar. The main effect tested in

the ANOVA was the difference in pinch treatments, followed by differences in cultivar, then PGR. Differences between cultivars, chemicals, and pinch treatments were determined using the Least Square Difference (LSD). Data are presented by week to show trends in PGR effects throughout the production cycle.

Results

Shoot numbers were not significantly different for any cultivar in any treatment. Only branch number and leader length data will be presented.

Branching

Pinch

There was a significant cultivar x pinch interaction on numbers of branches so data are presented by cultivar. Pinching increased branch numbers of 'Comtesse de Bouchard' at 2 and 4 WAT (Table 9 and Table 10, respectively). By 6 WAT, intact plants surpassed pinch plants to show more branches through the end of the experiment (Table 11). Pinching did not have a significant effect on branching of 'Ernest Markham' except for 6 WAT where intact plants had more branches (Table 11). Intact plants of 'Hagley Hybrid' had significantly more branches at 4 and 6 WAT (Table 10 and 11, respectively).

<u>PGR</u>

Significant interaction effects occurred with cultivar x PGR, or cultivar x pinch x PGR

for all data weeks (Tables 9, 10, 11, and 12). Data presented are separated by cultivar.

Where there is an interaction between PGR treatment and pinch treatment, data are

separated by pinch treatment.

Comtesse de Bouchard: At 4 WAT, PGR treatments were significant (*P<0.0251*). Plants

treated with dikegulac had a greater number of branches compared to those treated with

BA+GA$_{4+7}$, regardless of rate, however, numbers were not significantly greater than

controls (Table 10).   There was a significant interaction between PGR and pinch

treatments at 6 WAT (P<0.0091, Table 11). For intact plants, those treated with

dikegulac had the greatest number of branches, followed by controls. BA+GA$_{4+7}$ treated

plants had significantly lower branch numbers compared to controls, regardless of rate.

For pinched plants, controls or dikegulac treated plants had the greatest number of

branches. Again, BA+GA$_{4+7}$ treated plants had significantly fewer branches, regardless

of rate. At the end of the experiment (8 WAT), plants treated with dikegulac tended to

have the greatest number of branches, regardless of pinch treatment, but branch numbers

were not significantly different from controls (Table 12).   BA+GA$_{4+7}$ both rates reduced

the number of branches.

Ernest Markham: At 2 WAT, plants treated with BA+GA$_{4+7}$ had the greatest number of

branches regardless of pinch or PGR (Table 9). At 4 WAT, pinch and PGRs had a

significant interaction (P<0.0232), but PGR treatments were not significant for pinched

plants and only slightly significant for intact plants where both PGR treatments gave

greater branch numbers than controls (Table 10). AT 6 WAT, plants treated with

dikegulac or controls had the greatest number of branches, regardless of pinch (Table 11).

Plants treated with BA+GA$_{4+7}$ had significantly fewer branches, regardless of pinch or

rate. Results were consistent until the end of the experiment (Table 12).

Hagley Hybrid: PGR treatments were only slightly significant at 2 WAT (P<0.0456).

No treatment differed from the control; BA+GA$_{4+7}$ at 1200 mg·L^{-1} had a greater number

of branches compared to dikegulac treatments (Table 9). PGR treatments were not

significant at 4 WAT (Table 10). At 6 WAT, the main effect of PGR was, again, only

slightly significant (P<0.0304) (Table 11).   An interaction occurred between PGR and

pinch treatments (P<0.0099). Controls of the pinched plants had the greatest number of

branches. Controls were not significantly different from plants treated with BA+GA$_{4+7}$ at

1200 mg·L^{-1}. The two BA+GA$_{4+7}$ treatments were not significantly different from each

other. The plants with the fewest branches were those treated with dikegulac, but this

number was not significantly different from plants treated with BA+GA$_{4+7}$ at 800 mg·L^{-1}.

Intact plants treated with BA+GA$_{4+7}$ at 800 mg·L^{-1} were the only plants with significantly

higher branch numbers than the control, but this was not significantly different from

BA+GA$_{4+7}$ at 1200 mg·L^{-1} (Table 11). At the end of the experiment, no treatment was

significant (Table 12).

Height

Pinch Treatment

Pinching suppressed the leader lengths of all cultivars at 2 and 4 WAT (Table 13 and Table 14). Due to significant cultivar x pinch interactions at 6 WAT (Table 15) data are presented by cultivar. Only leader lengths of 'Hagley Hybrid' were still suppressed at 6 WAT (Table 15). This trend continued through 8 WAT without statistical significance (Table 16).

PGR

There were no cultivar differences in plant height except data taken at 6 WAT (Table 15). There was a significant interaction between PGR and pinch treatment at 2, 4, and 6 WAT. Data presented are the sum of three cultivars organized by data week, then by pinch. Overall pinch results show leader lengths of all cultivars were suppressed by pinching throughout the experiment, and significantly so at 2 and 4 WAT ($P<0.0001, P<0.0001$ respectively) (Tables 13, 14, 15, and 16) and at 6 WAT for 'Hagley Hybrid' ($P<0.0053$) (Table 15).

At 2 WAT, plants treated with dikegulac were the shortest in both pinched and intact treatments (Table 13). BA+GA$_{4+7}$ treatments were both taller than the control, but not significantly so. At 4 WAT, plants treated with dikegulac were again the shortest of all treatments, in both pinched and intact treatments (Table 14). There was a significant

interaction between cultivar and PGR at 6 WAT (P<0.0467) (Table 15). 'Comtesse de Bouchard' did not have significant differences between pinch treatments, but an interaction between PGR and pinch treatments did occur (*P<0.025*). PGR treatments were not significant for pinched plants. Of those plants that were not pinched, those treated with dikegulac were the shortest, followed by both $BA+GA_{4+7}$ treatments. 'Ernest Markham' did not have significant differences between pinch treatments, or an interaction effect. PGR treatments were significant (P<0.0002). Dikegulac plants were the shortest, followed by the control and $BA+GA_{4+7}$ at 800 mg·L^{-1}. $BA+GA_{4+7}$ at 1200 mg·L^{-1} was significantly taller than all other treatments. Leader lengths of pinched plants of 'Hagley Hybrid' were not significantly different. Intact plants of 'Hagley Hybrid' treated with dikegulac were significantly shorter than all other treatments, controls followed, and the $BA+GA_{4+7}$ were the tallest, though not significantly taller than each other. At the end of the experiment pinch treatments and cultivar interaction were not statistically significant (Table 16). PGR treatments were significant (P<0.0001). Dikegulac had significantly shorter leader lengths than all other treatments. $BA+GA_{4+7}$ at 800 mg·L^{-1} and 1200 mg·L^{-1} both had the longest leader lengths but were not significantly different from each other. The control was significantly different from all treatments, shorter than plants treated with $BA+GA_{4+7}$, but taller than those treated with dikegulac.

Dry Weights

There was a significant interaction between cultivar and PGR for dry weights of clematis

(Table 17). Data presented are by cultivar. There was significant response of 'Comtesse

de Bouchard' to PGR treatments (*P<0.0007*). Control plants weighed the most, and were

the only plants that significantly differed from other treatments (data not presented).

Shoot dry weights of 'Ernest Markham' were significantly affected by PGR treatments.

Plants treated with dikegulac had the least mass but only differed significantly from

plants treated with BA+GA$_{4+7}$ at 800 mg·L^{-1} (4.1 g vs 7.7 g, respectively *P<0.0179*).

'Hagley Hybrid' was also significantly affected by PGR treatment. Plants treated with

dikegulac had the least mass and differed significantly from both the control and plants

treated with BA+GA$_{4+7}$ at 1200 mg·L^{-1} [5.2 g (dikegulac) vs. 7.7 g (control) vs. 8.4 g

(BA+GA$_{4+7}$ at 1200 mg·L^{-1}) *(P<0.0228)*].

Bloom time

By 8 WAT all intact of 'Comtesse de Bouchard' plants treated with PGRs were in bloom,

except two plants in each of the control and 800 mg·L^{-1} BA+GA$_{4+7}$ groups (Table 18).

Control plants had fewer plants in bloom than treated plants, showing chemical

treatments did not delay bloom time. Of the pinched plants, all the untreated controls

were in bloom. Plants treated with dikegulac were all in bloom save one plant. Plants

treated with BA+GA$_{4+7}$ had considerably fewer plants in bloom, leading to the

conclusion that pinch plus BA+GA$_{4+7}$ treatments delay bloom time of 'Comtesse de

Bouchard.' All 'Ernest Markham' intact controls and intact plants treated with dikegulac

were in bloom (Table 18). Intact plants treated with BA+GA$_{4+7}$ had a majority of plants

in bloom. Pinched plants of 'Ernest Markham' had less plants in bloom compared to

intact plants. BA+GA$_{4+7}$ treatments delayed blooming on pinched plants of 'Ernest Markham.' All intact plants of 'Hagley Hybrid' were in bloom at 8 WAT except two plants treated with dikegulac (Table 18). Pinched plants had less flowering, with only plants treated with 800 mg·L^{-1} BA+GA$_{4+7}$ having 100% flowering. Dikegulac delayed bloom of both intact and pinched plants.

Discussion

Data from 'Comtesse de Bouchard' illustrates that pinch treatments appear to increase branch number for 4 weeks, after which the effects diminish and plants need to be pinched again. Results of 'Comtesse de Bouchard' indicate that if pinching were to occur only once, on this particular cultivar, final branch numbers would be suppressed rather than increased. If pinching is done to suppress leader lengths, growers should be certain to continue pinching this cultivar until sale. Continuous pinching is common practice in clematis production. 'Ernest Markham' and 'Hagley Hybrid' had similar branch numbers between pinched and intact plants until 4 WAT. At 6 WAT, plants had a greater number of branches on intact plants but this enhancement was no longer significant at the end of the experiment.

Although frequent interaction effects between pinch and PGR treatments occurred, both pinched and intact plants responded to PGR treatments in much the same manner. When

interaction effects were significant, mostly intact plants had the greater number of branches, regardless of PGR treatments. A compound effect between pinch and PGR to increase branch numbers was not found. Previous studies indicate pinching does or does not improve the effects of PGRs. Hand pinching of greenhouse azaleas (*Rhododenron simsii* Planch.) followed by dikegulac treatments 2 days later yielded more branches than either treatment alone (deSilva et al., 1976). Malek et al. (1992) found contradicting results in azalea research. Their research indicates dikegulac applications on 'Flame' azaleas (*R. calendulaceum* Mixch.) increased branch number regardless of pinch. Results were similar to studies with 'Goldflame' honeysuckle (*Lonicera x heckrotti* Rehd.) (Bruner et al., 2000) where dikegulac linearly increased branch numbers without an interaction with pinch treatments. Dikegulac treatments produced more branches on Reiger begonia (*Begonia x hiemalis* Fotsch. 'Northern Sunset') than hand pinching or pinch plus dikegulac (Agnew and Campbell, 1983). Absorption of dikegulac is through the leaves. Absorption decreases as leaf age increases (Bocion and deSilva, 1977). Decreased effect of dikegulac with pinch treatments may be the result of decreased chemical absorption. Results from this research with clematis indicate that PGR effects are independent of pinch treatments and can be used alone to reduce labor inputs.

<u>Dikegulac</u>

Dikegulac increased branch numbers of 'Comtesse de Bouchard' and 'Ernest Markham' with a 4 and 6-week delay, respectively. This delay is common with dikegulac (Al-

Juboory and Williams, 1991; Foley and Keever, 1993) and growers should schedule accordingly. Effects of dikegulac lasted throughout the 8-week experiment for 'Comtesse de Bouchard' and 'Ernest Markham' ($P<0.0001$ for both cultivars 8 WAT).

New axillary growth appeared slightly chlorotic. Chlorosis from dikegulac has been documented on Rieger begonia (Agnew and Campbell, 1983) and azalea (deSilva et al., 1976). Light green buds on clematis plants turned dark green to match original foliage within 4 WAT, as was the case with begonias and azaleas. Adventitious buds were seen growing in axillary clusters from old and new growth. Axillary clusters were noticed on previous studies with clematis (Puglisi and Latimer, unpublished). Bud clusters grew long and developed into groups of branches recognizable from a distance. Clusters may be advantageous to consumer appeal as they are eye catching.

Dikegulac suppressed leader lengths on all cultivars throughout the experiment. At the end of the experiment plants treated with dikegulac were the shortest of all plants regardless of pinch. Suppression of leader length by dikegulac is also expressed by results of dry weights where plants treated with dikegulac were the smallest for two cultivars. Dikegulac suppressed hypocotyls elongation in cucumber cotyledons (Gressel et al., 1976). Length suppression may be due to the inhibition of GA to incorporate precursors into DNA (Gressel et al., 1976). Leader length effects did not have a treatment delay as branch effects did. Fast suppression of leader lengths is advantageous

to producers as shorter, more compact plants are easier to handle and ship. Suppression

of leader length could explain why dikegulac delayed flowering of 'Hagley Hybrid.' The

number of plants that had a delay in bloom was small compared to those that were

flowering by sale time.

BA+GA$_{4+7}$

There was no significant difference between rates of BA+GA$_{4+7}$ throughout most of the

experiment. The lesser rate should be used to avoid unnecessary chemical applications,

which increases potential runoff toxicity and lessens economical benefits of PGR use.

All three cultivars had different responses to BA+GA$_{4+7}$. As with all PGR

recommendations, producers are advised to test their plants before applying BA+GA$_{4+7}$ to

a large crop. 'Comtesse de Bouchard' treated with BA+GA$_{4+7}$ had fewer branches than

did control plants. 'Ernest Markham' had an increase in branching, which turned into

suppression at 6 WAT. Multiple applications after 2 weeks may extend increased branch

responses. Weekly applications of BA+GA$_{4+7}$ stimulated more bud outgrowth than

single applications on *Euphorbia lathyris* L. (Preece, 1990). 'Hagley Hybrid' had a

significant increase in branch numbers at 2 and 6 WAT, but with inadequate significance

for consumer recognition. BA+GA$_{4+7}$ was expected to have more of an impact on

clematis, as it had increased branch numbers in previous studies (Puglisi and Latimer,

unpublished). An explanation may be the phytotoxic symptoms seen on all plants at both

rates. Three days after treatment, leaves present pre-application began to curl and pucker.

Plants appeared to grow out of phytotoxic symptoms at 3 WAT. At 4 WAT, new buds

that had broken from dormancy post application began to turn brown and die (bud blast).

Bud blast occurred with BA+GA_{4+7} on previous experiments (Puglisi and Latimer,

unpublished), where phytotoxicity was thought to be due to the cytokinin component of

the chemical (6-BA).   Foliar sprays of BA caused phytotoxic symptoms on florist

azaleas (*Rhododendron* cultivars 'Gloria and Prize) (Bell et al., 1997), *Dieffenbachia*

'Welkeri' Lodd. (Wilson and Nell, 1983), and *Anthurium* Andre. (Imamura and Higaki,

1988). Bud blast may the reason for branch suppression of 'Comtesse de Bouchard' and

'Ernest Markham' as suppression did not begin until 4 and 6 WAT, respectively.

Phytotoxicity may explain the delay in bloom of 'Ernest Markham.' Plants treated with

BA+GA_{4+7} were taller than controls presumably due to the gibberellin component in the

chemical. Despite consumer appeal of large plants, height increase is undesirable to

producers. Tall plants often tangle in each other making shipping and handling difficult,

and frequent staking necessary. BA+GA_{4+7} may not be desirable to producers for its

increase in plant height and early phytotoxicity.

Foliar sprays of dikegulac at 800 mg·L^{-1} increased branch numbers of *Clematis*

'Comtesse de Bouchard,' 'Ernest Markham,' and 'Hagley Hybrid' more than foliar

sprays of BA+GA_{4+7} at 800 or 1200 mg·L^{-1}. Branch numbers increased as a result of

dikegulac sprays, regardless of pinch, eliminating the need to mechanically pinch these

cultivars to increase axillary branching during production. Suppression of the leader

lengths from dikegulac creates a compact plant for ease of handling and shipping by

producers. Time to bloom is delayed, but plants are blooming by time of sale. Dikegulac could be used as an alternative to hand pinching of clematis vines to reduce labor inputs and improve plant aesthetics.
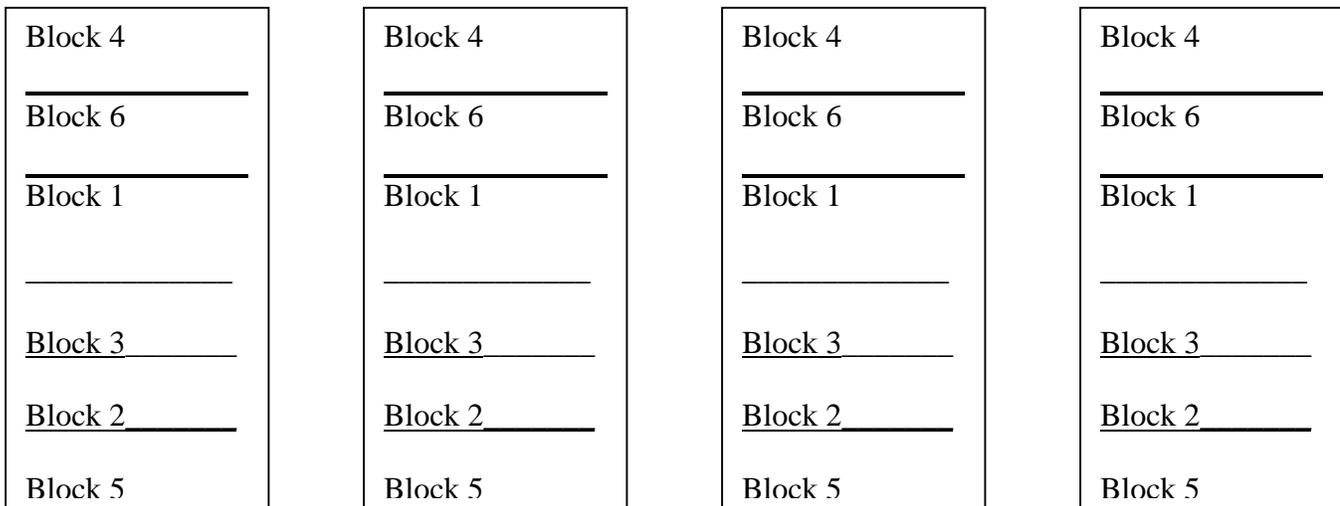
Literature Cited

Agnew, N.H., and R.W. Campbell. 1983. Growth of *Begonia x hiemalis* as influenced by hand-pinching, dikegulac, and chlormequat. HortScience 18(2):201-202.

Al-Juboory, K. H., and D.J. Williams. 1991. Effects of two growth regulators on lateral Branching and shoot growth of English ivy (*Hedera helix).* PGRSA Quarterly 19:35-40.

Bell, M.L., R.A. Larson, and D.A. Bailey. 1997. Vegetative growth responses of florist azaleas to dikegulac, GA_{4+7}, and benzylamino purine. HortScience 32(4):690-693.

Bocion, P.F., and W.H. de Silva. 1977. Some effects of dikegulac on the physiology of whole plants and tissues: Interactions with plant hormones. pp198-198. Proc. Intl. Conf. on Plant Growth Substances, Lausanne, Switzerland.

de Silva, W.H., P.R. Bocion, and H.R. Walther. 1976. Chemical pinching of azalea with dikegulac. HortScience 11(6):569-570.

Foley, J.T. and G.J. Keever. 1993. Chemically induced branching of *Vinca minor*. J Environ. Hort. 11(4):149-152.

Gooch, R. 1996. Clematis: The complete guide. Crowood, Ramsbury, Wiltshire.

Gressel, J., A Kadouri, D. Atsmon, and N. Cohen. 1976. Effects of dikegulac sodium on nucleic acid syntheses. Collected abstracts of the paper demonstrations:The 9th international conference on plant growth substances. pp.1-2.

Imamura, J.S. and T. Higaki. 1988. Effect of GA_3 and BA on lateral shoot production on *Anthurium*. HortScience 23(2):353-354.

Malek, A.A., F.A. Blazich, S.L. Warren, and J.E. Shelton. 1992. Growth response of seedlings of flame azalea to manual and chemical pinching. Proc. SNA Res. Conf. 37:251-252.

Preece, J.E. 1990. Growth stimulation of *Euphorbia lathyris* L. by GA_{4+7} and BA. J. Plant Growth Regulat. 9:85-88.

Wilson, M.R. and T.A. Nell. 1983. Foliar applications of BA increase branching of 'Welkeri' dieffenbachia. HortScience 18(4): 447-448.

Appendix

Cooling Pad

Each block contains 3 cultivars x 4 PGRs x 2 pinch treatments (pinched or intact) = 24 plants. Each table has 6 plants of each block.

Block 4 _____ Block 6 _____ Block 1 _____ Block 3_____ Block 2_____ Block 5	Block 4 _____ Block 6 _____ Block 1 _____ Block 3_____ Block 2_____ Block 5	Block 4 _____ Block 6 _____ Block 1 _____ Block 3_____ Block 2_____ Block 5	Block 4 _____ Block 6 _____ Block 1 _____ Block 3_____ Block 2_____ Block 5

Fan

Table 9: Effects of PGR and pinch treatments on branch numbers of three Clematis hybrids at 2 WAT.

		Cultivar		
Treatment		Comtesse de Bouchard	Ernest Markham	Hagley Hybrid
Pinch		2.5 a^z	2.5 a	3.6 a
Intact		0.6 b	2.0a	3.4 a
Main effect		***^{y}	NS	NS
PGR	Rate (mg·L^{-1})			
Control	0	1.4 a	1.3 b	2.9 ab
BA+GA$_{4+7}$	800	1.8 a	2.8 a	4.5 a
BA+GA$_{4+7}$	1200	1.4 a	3.8 a	3.3 ab
Dikegulac	800	1.5 a	1.2 b	2.6 b
Main effect		NS	***	*
PGR x Pinch		NS	NS	NS

		ANOVA
Source	df	Sums of squares
cultivar	2	65.4***
PGR	3	43.8***
pinch	1	30.1***
cv x PGR	6	33.3***
PGR x pinch	3	7.8^{NS}
cv x PGR x pinch	8	28.1^{NS}

^{z} Mean separation within main effect and columns by Tukey's $_{LSD}$ test at the P≤0.05 level
^{y} NS,*,**,*** Not significant at the P≤0.05 or significant at the P≤0.0001, P≤0.01, or P≤0.05 level, respectively.

Table 10: Effects of PGR and pinch treatments on branch numbers of three Clematis hybrids at 4 WAT.

		Cultivar			
Treatment		Comtesse de Bouchard	Ernest Markham		Hagley Hybrid
Pinch		3.3 a^z	1.9 a		3.9 b
Intact		2.0 b	2.9 a		5.7 a
Main effect		*^{y}	NS		**
PGR	Rate (mg·L^{-1})		Pinch	Intact	
Control	0	3.1 ab	1.5 a	0.7 b	5.1 a
BA+GA$_{4+7}$	800	1.8 b	2.7 a	1.3 ab	4.6 a
BA+GA$_{4+7}$	1200	1.8 b	1.5 a	5.0 ab	4.5 a
Dikegulac	800	4.1 a	1.8 a	4.5 a	5.0 a
Main effect		*	NS	*	NS
PGR x Pinch		NS	*		NS

	ANOVA	
Source	df	Sums of squares
cultivar	2	161***
PGR	3	33.7^{NS}
pinch	1	8.3^{NS}
cv x PGR	6	52.7^{NS}
PGR x pinch	3	68.1**
cv x PGR x pinch	8	89.9**

^{z} Mean separation within main effect and columns by Tukey's $_{LSD}$ test at the P≤0.05 level

^{y} NS,*,**,*** Not significant at the P≤0.05 or significant at the P≤0.0001, P≤0.01, or P≤0.05 level, respectively.

Table 11: Effects of PGR and pinch treatments on branch numbers of three Clematis hybrids at 6 WAT.

		Cultivar				
Treatment		Comtesse de Bouchard		Ernest Markham	Hagley Hybrid	
Pinch		4.9 b^z		3.4 b	9.1 b	
Intact		6.8 a		5.8 a	13.9 a	
Main effect		*^{y}		**	**	
PGR	Rate (mg·L^{-1})	pinch	intact		pinch	intact
Control	0	7.3 a	7.2 b	5.8 a	14.4 a	11.8 b
BA+GA$_{4+7}$	800	2.8 bc	2.2 c	1.9 b	7.2 bc	21.4 a
BA+GA$_{4+7}$	1200	2.3 c	3.8 c	3.2 b	12.6 ab	13.7 ab
Dikegulac	800	6.6 ab	14.2 a	7.4 a	5.3 c	9.8 b
Main effect		*	***	***	*	*
PGR x Pinch		**		NS	**	

	ANOVA	
Source	df	Sums of squares
cultivar	2	1347***
PGR	3	170**
pinch	1	301***
cv x PGR	6	848***
PGR x pinch	3	147*
cv x PGR x pinch	8	455**

^{z} Mean separation within branch numbers and columns by Tukey's $_{LSD}$ test at the P≤0.05 level.
^{y} NS,*,**,*** Not significant at the P≤0.05 or significant at the P≤0.0001, P≤0.01, or P≤0.05 level, respectively.

Table 12: Effects of PGR and pinch treatments on branch numbers of three Clematis hybrids at 8 WAT.

Treatment		Cultivar		
		Comtesse de Bouchard	Ernest Markham	Hagley Hybrid
Pinch		6.9 b^z	5.8 a	12.9 a
Intact		12.5 a	6.6 a	14.0 a
Main effect		**^{y}	NS	NS
PGR	Rate (mg·L^{-1})			
Control	0	12.0 ab	8.2 a	11.8 a
BA+GA$_{4+7}$	800	4.5 c	3.7 b	15.7 a
BA+GA$_{4+7}$	1200	7.1 bc	3.1 b	16.7 a
Dikegulac	800	15.3 a	9.6 a	11.6 a
Main effect		***	***	NS
PGR x Pinch		NS	NS	NS

		ANOVA
Source	df	Sums of squares
cultivar	2	1216***
PGR	3	349**
pinch	1	175**
cv x PGR	6	942***
PGR x pinch	3	89.7^{NS}
cv x PGR x pinch	8	374^{NS}

^{z} Mean separation within branch numbers and columns by Tukey's $_{LSD}$ test at the P≤0.05 level.
^{y} NS,*,**,*** Not significant at the P≤0.05 or significant at the P≤0.0001, P≤0.01, or P≤0.05 level, respectively.

Table 13: Effects of pinch and PGR on leader lengths of three Clematis hybrids at 2 WAT.

Treatment	Leader length (cm)	
Pinch	8.5 b^z	
Intact	51.3 a^y	
Main effect	***	
PGR	Rate (mg·L^{-1}) pinch	intact
Control	0 8.0 ab	57.4 b
BA+GA$_{4+7}$	800 10.8 a	66.7 a
BA+GA$_{4+7}$	1200 10.9 a	58.6 ab
Dikegulac	800 4.3 b	22.4 c
Main effect	***	
PGR x Pinch	**	

ANOVA

Source	df	Sums of squares
cultivar	2	1074**
PGR	3	13817***
pinch	1	65878
cv x PGR	6	492^{NS}
PGR x pinch	3	7623***
cv x PGR x pinch	8	1353^{NS}

^{z}Mean separation within main effect and columns by Tukey's $_{LSD}$ test at the P≤0.05 level
^{y} NS,*,**,*** Not significant at the P≤0.05 or significant at the P≤0.0001, P≤0.01, or P≤0.05 level, respectively.

Table 14: Effects of pinch and PGR effect on leader lengths of three Clematis hybrids at 4 WAT.

Treatment		Leader length (cm)	
Pinch		45.4 b[z]	
Intact		76.4 a	
Main effect		***[y]	
PGR	Rate (mg·L^{-1})	pinch	intact
Control	0	48.7 a	97.2 a
BA+GA$_{4+7}$	800	50.9 a	85.9 a
BA+GA$_{4+7}$	1200	52.2 a	98.2 a
Dikegulac	800	30.5 b	24.2 b
Main effect		**	***
PGR x Pinch		***	

	ANOVA	
Source	df	Sums of squares
cultivar	2	1085
PGR	3	55087***
pinch	1	33844***
cv x PGR	6	1654^{NS}
PGR x pinch	3	17463***
cv x PGR x pinch	8	2684^{NS}

[z] Mean separation within main effect and columns by Tukey's $_{LSD}$ test at the P≤0.05 level
[y] NS,*,**,*** Not significant at the P≤0.05 or significant at the P≤0.0001, P≤0.01, or P≤0.05 level, respectively.

Table 15: Effects of pinch and PGR effect on leader lengths of three Clematis hybrids at 6 WAT.

Treatment	Comtesse de Bouchard		Ernest Markham	Hagley Hybrid	
	Leader length (cm)				
Pinch	79.6 a[z]		82.1 a	77.9 b	
Intact	97.2 a		85.3 a	99.1 a	
Main effect	NS[y]		NS	**	

PGR	Rate $(mg·L^{-1})$	Pinch	Intact		Pinch	Intact
Control	0	88.7 a	142.2 a	86.8 b	82.9 a	89.2 b
BA+GA$_{4+7}$	800	74.1 a	111.2 b	86.8 b	80.7 a	134 a
BA+GA$_{4+7}$	1200	88.7 a	96.0 b	111.0 a	86.3 a	134 a
Dikegulac	800	67.0 a	39.3 c	52.1 c	69.5 a	44.0 c
Main effect		NS	***	***	NS	***
PGR x Pinch		***		NS	***	

ANOVA		
Source	df	Sums of squares
cultivar	2	583[NS]
PGR	3	53590***
pinch	1	6904**
cv x PGR	6	11417*
PGR x pinch	3	19158***
cv x PGR x pinch	8	9284[NS]

[z] Mean separation within main effect and columns by Tukey's $_{LSD}$ test at the P≤0.05 level
[y] NS,*,**,*** Not significant at the P≤0.05 or significant at the P≤0.0001, P≤0.01, or P≤0.05 level, respectively.

Table 16:Effects of pinch and PGR effect on leader lengths of three Clematis hybrids at 8 WAT.

Treatment		Leader length (cm)
Pinch		113 a[z]
Intact		124 a
Main effect		NS[y]
PGR	Rate (mg·L^{-1})	
Control	0	119 b
BA+GA$_{4+7}$	800	135 a
BA+GA$_{4+7}$	1200	140 a
Dikegulac	800	82.2 c
Main effect		***
PGR x Pinch		NS

		ANOVA	
Source			df
cultivar		2	Sums of squares
PGR		3	29090***
pinch		1	69299***
cv x PGR		6	3355[NS]
PGR x pinch		3	10674[NS]
cv x PGR x pinch		8	8037[NS]

[z] Mean separation within main effect and columns by Tukey's $_{LSD}$ test at the P≤0.05 level
[y] NS,*,**,*** Not significant at the P≤0.05 or significant at the P≤0.0001, P≤0.01, or P≤0.05 level, respectively.

Table 17: Mean comparisons as done by ANOVA on dry weights of three Clematis cultivars, 'Comtesse de Bouchard,' 'Ernest Markham,' and 'Hagley Hybrid' at 8 WAT.

	Dry weight ANOVA		
	df	SS	P value
Model	28	471.6	0.0001
Error	11	693.3	
Corrected total	139	1164.9	
R^2 0.4			
rep	5	99.8	0.0098
cultivar	2	23.2	0.1606
PGR	3	139.3	0.0001
pinch	1	2.11	0.5619
cv x PGR	6	114.4	0.0084
PGR x pinch	3	20.5	0.3547
cv x PGR x pinch	8	69	0.2122

Table 18: Percent of Clematis plants in bloom at 8 WAT.

Treatment	Rate (mg·L^{-1})	Comtesse de Bouchard	Ernest Markham	Hagley Hybrid
			Cultivar	
Intact				
Control	0	67	100	100
BA+GA$_{4+7}$	800	67	67	100
BA+GA$_{4+7}$	1200	100	67	100
Dikegulac	800	100	100	67
Pinch				
Control	0	100	83	83
BA+GA$_{4+7}$	800	17	50	100
BA+GA$_{4+7}$	1200	33	33	67
Dikegulac	800	83	100	83