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Mr. Charles Hall
Executive Secretary
PGRSA
P.O.Box 2945
LaGrange, GA 30241
Telephone (706) 845-9085

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**Ethephon and Trimming of *Scaevola aemula* Stock Plants Influence Vegetative Cutting Quantity and Quality**[1]

**James L. Gibson**[2] **and Brian E. Whipker**[3]

**Abstract:** This study determined the effects of ethephon foliar sprays at 250, 500, 750, or 1000 $mg{\cdot}L^{-1}$ and trimming on cutting quantity and quality of scaevola stock plants. Trimming stock plants to 15.2 cm of vegetative growth resulted in a 151% increase in cutting number, 126% greater total cutting weight, and 10% greater shoot length, than trimming more extensively to 7.6 cm. Cutting stem diameter, used by the propagation industry as an indicator of cutting quality, was similar for both trimming lengths. In another experiment, cutting length was larger with ethephon foliar sprays of 250 to 1000 $mg{\cdot}L^{-1}$ than the untreated control. The number of cuttings from trimmed (to 12.7 cm) and untrimmed plants were similar during both harvests 1 and 2 on a per plant basis, however, more cuttings per $m^2$ of bench area were generated from stock plants that were trimmed to 12.7 to 15.2 cm, an important economic consideration, than from untrimmed or extensively trimmed (7.6 cm) stock plants.
Nomenclature: Ethephon [(2-chloroethyl) phosphonic acid (Florel)]. Scaevola (*Scaevola aemula* R. Br.)
***Additional index words:*** Ethylene, fan flower, Florel, propagation, and vegetative cuttings

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## INTRODUCTION

Vegetative annuals are propagated asexually by tip cuttings from stock plants in order to achieve large quantities of genetically identical plants. The Australian fan flower (*Scaevola aemula* R. Br.) has increased in popularity within the U.S. floriculture industry over the past 13 years as a vegetatively-propagated annual because of its durability in the landscape (Rader, 1998). Scaevola stock plants are typically grown for 6 to 12 weeks to establish a large canopy so that during peak cutting demand, a large number of cuttings are produced (Jack Williams, Paul Ecke Ranch, personal communication). Manual pinching and trimming during stock plant establishment encourages the development of multiple shoots from the main stem (Healy, 1994).

To increase production of cuttings, ethephon (Florel, Rhone-Poulenc Ag. Co., Research Triangle Park, NC) is also applied to stock plants. Ethephon has been shown to stimulate lateral branching, inhibit flower formation, and decrease leaf area on stock plants of fuchsia (*Fuchsia* x *hybrida*), geranium (*Pelargonium* x *hortorum*), and lantana (*Lantana hybrida*) (Peter Konjoian, Floriculture Education Services, personal communication). Tsujita and Harney (1978) reported that ethephon at 500 $mg{\cdot}L^{-1}$ resulted in a smaller stem length and stem diameter of geranium cuttings. Applying ethephon at pinch or 2 weeks before pinching increased the number of geranium cuttings (Carpenter and Carlson, 1972). Therefore, the objective of this study was to determine the effects of ethephon foliar sprays and trimming on cutting quantity and quality of scaevola stock plants.

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[1]Received for publication Jan. 15, 2004 and in revised form Dec. 31, 2004.
[2]Department of Horticulture and Crop Science, The Ohio State University, Columbus, OH 43210
[3]Department of Horticulture, North Carolina State University, Raleigh, NC 27695

## MATERIALS AND METHODS

*Experiment 1*

Stem cuttings of 'Purple Fan' scaevola, rooted in 3.5 x 3.8 cm cells, were transplanted (2 per pot) into 1.2-L (16.5 cm diameter) round plastic containers on 9 Jan. 1999. The root substrate was Fafard® 4-P (Fafard, Anderson, SC), which contained: 4 sphagnum peat : 2 pine bark : 2 vermiculite: 1 perlite (by volume). Plants were fertilized at each watering with N at 150 $mg{\cdot}L^{-1}$ from Peters® 20-10-20 (Scotts, Marysville, Ohio) (20N-4.4P-16.6K). Plants were grown under natural daylength and irradiance for 68 days with greenhouse day/night setpoint temperatures of 19.7/18.3°C. Scaevola stock plants were pinched on 13 Jan. by removing 2 cm of growth from the terminal tip.

On 14 Feb., 25 stock plants were trimmed to 15.2 cm of vegetative growth from the pot rim and 25 were trimmed to 7.6 cm. Following trimming, four ethephon foliar sprays ($mg{\cdot}L^{-1}$) at 250, 500, 750, or 1000 were applied using a volume of 204 $mL{\cdot}m^{-2}$. An untreated control was included in both trimming treatments.

The experiment was a completely randomized design with five single-plant replications for each of the ten treatments. On 2 and 18 Mar. the total number of flowering and vegetative cuttings ($3 cm from the cutting base to the longest fully expanded leaf) were harvested and recorded. Cuttings were excised at the second node from the terminal tip. On 2 Mar. a subsample of 5 cuttings per replication from each treatment was selected randomly to measure basal stem diameter and shoot length (measured from the cutting base to the terminal tip). All cuttings collected per stock plant were dried to determine total and average cutting dry weight.

*Experiment 2*

Stem cuttings of 'Purple Fan' scaevola, rooted in 3.5 x 3.8 cm cells, were transplanted (2 per pot) into 2.54-L (19.0 cm diameter) round plastic containers on 5 Dec. 1999. Plants were fertilized at each watering with 150 $mg{\cdot}L^{-1}$ N from Peters® 20-10-20 (Scotts, Marysville, Ohio) (20N-4.4P-16.6K). Plants were then grown under natural daylength and irradiance for 94 days with greenhouse day/night setpoint temperatures of 19.7/18.3°C. Scaevola stock plants were pinched on 10 Dec. by removing 2 cm of growth from the terminal tip.

On 2 Jan. 2000, 25 stock plants were trimmed to leave 12.7 cm of vegetative growth from the pot rim, while the remaining 25 were left untrimmed. Four ethephon foliar sprays (in $mg{\cdot}L^{-1}$) at 250, 500, 750, or 1000 were applied on 10 Jan. and again on 15 Feb. using a volume of 204 $mL{\cdot}m^{-2}$. An untreated control was included in both trimming treatments. The experiment was a completely randomized design with five single-plant replications for each of the ten treatments.

On 1 Feb. and 7 Mar., total number of flowering and vegetative cuttings ($3 cm from the cutting base to the longest fully expanded leaf) were harvested and recorded. Stem cuttings were excised at the second node from the terminal tip. A subsample of 5 cuttings per replicate from each treatment were selected randomly to measure basal stem caliper and shoot length (measured from the cutting base to the longest fully expanded leaf). All cuttings collected per stock plant were dried to determine average cutting dry weight.

Data for number of cuttings, cutting basal stem diameter and shoot length, and total and average cutting dry weight were tested by analysis of variance by general linear model (SAS Inst., Cary, NC). Means were separated by least significant differences (LSD) at $P \leq 0.05$.

## RESULTS AND DISCUSSION

*Experiment 1*

*Trimming Effects*. During the first harvest, smaller sized 7.6 cm-trimmed stock plants produced 1 flowering cutting while larger 15.2 cm-trimmed stock plants produced 7 flowering cuttings. Flowering is a negative attribute on cutting quality, as floral tissue can serve as a host for *Botrytis* under intermittent mist systems during propagation (Gibson and Williams, 2002). However, during the first harvest, larger stock plants generated 130% more vegetative (non-flowering) cuttings (49.2) than smaller stock plants (21.4).

The total number of cuttings generated from larger stock plants was greater (119.8) than from smaller stock plants (62.3) for both harvests. Cutting number increased 151% (22.2 to 55.8) and 60% (40.1 to 64.0) when more biomass remained on the stock plant for harvests 1 and 2, respectively. Total dry weight (2.67 versus 1.18 g) and length of the cuttings (2.47 versus 2.25 cm) was greater with stock plants trimmed to 15.2 cm compared to 7.6 cm trimmed stock plants, respectively. The stem diameter (0.24 cm) and average dry weight (0.10 g) of the cuttings were similar between the two stock plant sizes. Ethephon foliar sprays of 0 to 1000 $mg{\cdot}L^{-1}$ produced a similar number of cuttings per stock plant (40), which may have been due to trimming and spraying on the same day. Ethephon applications are recommended for actively growing plant material and should be avoided during periods of plant stress (Konjoian, 2002).

*Experiment 2*

In Expt. 2 stock plants were treated with ethephon 8 and 14 days after trimming for harvest 1 and harvest 2, respectively. Ethephon was not sprayed earlier because half of the stock plants were trimmed to 12.7 cm and ethephon does not translocate to other plant parts (Gaston et al., 2002).

*Concentration Effects.* Ethephon was not effective in reducing the number of flowering cuttings because all ethephon treatments produced similar or a greater number of flower-

**Table 1. Effect of ethephon on scaevola cutting quantity and quality (Expt. 2).**

| Etephon ($mg{\cdot}L^{-1}$) | Flowering shoots | Vegetative shoots | Stem diameter (cm) | Shoot length (cm) |
|---|---|---|---|---|
| 0 | 19.4 | 45.8 | 0.23 | 5.73 |
| 250 | 24.0 | 39.1 | 0.24 | 6.16 |
| 500 | 21.8 | 39.3 | 0.26 | 6.43 |
| 750 | 19.4 | 46.7 | 0.26 | 6.52 |
| 1,000 | 26.1 | 36.8 | 0.26 | 6.28 |
| LSD | 3.5 | 5.6 | 0.02 | 0.33 |
| Significance[z] | *** | *** | *** | *** |

[z] ***; Significant by column $P = 0.001$, n = 20.

**Table 2. Scaevola cutting quality values for harvest 1 and harvest 2 (Expt. 2).**

| Harvest | Flowering shoots | Vegetative shoots | Total shoots | Stem caliper (cm) | Shoot length (cm) | Average dry weight (g) |
|---|---|---|---|---|---|---|
| 1 | 8.3 | 32.3 | 40.6 | 0.28 | 6.75 | 0.10 |
| 2 | 35.9 | 50.7 | 86.6 | 0.22 | 5.70 | 0.08 |
| Significance[z] | *** | *** | *** | *** | *** | *** |

[z] ***; Significant by column $P = 0.001$, n = 50.

ing shoots as the untreated control (Table 1). Ethephon at all concentrations had no effect on the total number of cuttings harvested or individual cutting dry weights (data not shown). Cutting stem diameter increased with the use of ethephon (500 to 1000 $mg{\cdot}L^{-1}$) and shoot length increased with ethephon concentrations $\geq 250\ mg{\cdot}L^{-1}$, compared to the untreated plants. The increase in cutting stem diameter with $\geq 500\ mg{\cdot}L^{-1}$ ethephon contradicts Tsujita and Harney's (1978) research with geraniums where cutting stem diameter decreased with $\geq 500\ mg{\cdot}L^{-1}$ ethephon.

*Trimming Effects.* The trimming x harvest interaction was not significant, therefore only trimming and harvest main effects are presented. Trimming the stock plants to 12.7 cm resulted in a larger shoot length (0.3 cm longer) and three fewer flowering cuttings per stock plant compared to the untrimmed plants (data not shown).

*Harvest Effects.* The number of flowering, vegetative, and total cuttings increased by 333%, 57%, and 113%, respectively, from harvest 1 to harvest 2 (Table 2). Stems were wider, cutting length was longer, and average cutting dry weight was greater during the first harvest, when compared to cuttings from the second harvest.

Although the actual number of cuttings from trimmed and untrimmed plants were similar during both harvests 1 and 2 on a per plant basis, commercially the comparison should be based on the density of stock plants per $m^2$ of bench area. In Expt. 2, 10.8 stock plants trimmed to 12.7 cm or 5.4 untrimmed stock plants in a 19 cm container would occupy 1 $m^2$ of bench area. Therefore, calculating the cutting yield on a $m^2$ of bench area basis, the stock plants trimmed to 12.7 cm would produce 439 cuttings, while 219 cuttings would be produced from the untrimmed stock plants. For the second harvest, 935 cuttings could be produced per $m^2$ of bench area from stock plants trimmed to 12.7 cm and 468 cuttings could be produced from the untrimmed stock plants. Therefore, trimming stock plants to 12.7 cm (1374 cuttings) would result in 100% more cuttings generated per $m^2$ for harvests combined compared to untrimmed plants (687 cuttings).

However, there is a limit to how extensively stock plants can be trimmed. Based on Expt. 1, 14.3 stock plants trimmed to 7.6 cm or 10.8 stock plants trimmed to 15.2 cm in a 16.5 cm container would occupy 1 $m^2$ of bench area. For both harvests, 1294 cuttings could be produced per $m^2$ of bench area from stock plants trimmed to 15.2 cm and 891 cuttings could be produced from the 7.6-cm trimmed stock plants. In conclusion 45% more cuttings were generated from stock plants that were not extensively trimmed.

Commercial propagators should trim scaevola stock plants from 12.7 to 15.2 cm to increase plant density per $m^2$ of bench area. This higher density could result in up to 100% more cuttings being produced per $m^2$ than untrimmed or extensively trimmed stock plants. Ethephon foliar sprays had no effect on cutting quantity, but improved cutting quality. A 250 to 750 $mg \cdot L^{-1}$ foliar spray would increase production costs only $0.002 to $0.007, respectively, per pot (based on the cost of $23.44/L), but its use would improve cutting quality by increasing cutting length and stem diameter.

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# A One-Step *in vitro* Procedure for Induction of Callus and Shoots in Cassava (*Manihot esculenta* Crantz)

**Kanyand Matand[1*], George Acquaah[2], and Marvin Burns[3]**

**Abstract**: Portions of young leaf lobes, stem, and root tissues were excised from cassava plantlets and cultured on Murashige and Skoog nutrient medium, in which 10 or 20 mM kinetin, thidiazuron (TDZ), or 6-benzylaminopurine (BA) and 2,4-dichlorophenoxyacetic acid (2, 4-D) were used alone or in combination for callus and shoot formation. After three months of culture, some organogenic calli were transferred to shoot-enhancing media for an additional three months. Other calli were subcultured on their initial treatment media throughout the six-month-culture period. The results showed that a single growth regulator treatment could be used to elicit callus and shoot formation and shoot development in cassava. Also, decreasing the concentration of 2, 4-D in the secondary medium by half of that of cytokinins consistently enhanced shoot formation, irrespective of the cytokinin source. The highest average shoots per callus were 9.4, 8, and 6.7 for kinetin, BA, and TDZ with 2, 4-D, respectively. Any one of these three combination treatments may be recommended for shoot production. The transfer of organogenic calli to a medium containing 2, 4-D alone resulted in increased root formation and little or no further shoot formation.

***Keywords****:* Cassava *in vitro* regeneration, cassava tissue culture, *in vitro* regeneration, *Manihot esculenta*.

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## INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is the most important root crop grown in the tropics, supplying more than 80 percent of the caloric intake for Sub-Saharan Africans (McMahon and Sayre, 1998) and for over 500 million people worldwide (Cock 1982). Cassava leaves are eaten as a vegetable in Central Africa, while the tuber peels make an excellent livestock feed. It is also widely grown in Asia and Latin America. Although cassava improvement using classical breeding methods has been undertaken for many years, modern biotechnological methods have the potential for more effective genetic manipulation of this crop (Acquaah, 2002).

For cassava as for other crops, the use of specific growth regulators and adjustment of their concentrations are the keys to successful plant tissue culture (Danso *et al*., 1999; Joseph *et al*., 1999; Trigiano and Gray, 1996). Individual growth regulators can be used alone or in combination to induce callus, shoot or somatic embryo (SE) or roots, or to develop shoots or SE into whole plants (Danso *et al*., 1999; Ihemere, 2003; Joseph *et al.,* 1999; Trigiano and Gray, 1996). Cassava researchers have generally used specific treatments to obtain each of the four outcomes - callus, shoot or SE and plant development, and root formation (Guohua, 1998; Joseph *et al.,* 1999; Peng *et al.,* 2001). This is because a single treatment could not result in callus, SE, or shoot and root formation, and plant development. Studies on other

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Center for Biotechnology Research and Education[1,2]; Department of Research and Extension[1]; Department of Agriculture and Natural Resources[2]; School of Agriculture and Applied Sciences[1,2,3], Langston University, Langston OK 73050; *Corresponding Author: kmatand@luresext.edu, Fax: (405) 466 3138.

crops have reported success in controlling those morphogenic developmental stages using a single treatment (Trigiano and Gray, 1996; Matand *et al.,* 1994). The study reported herein was undertaken to develop a single growth regulator treatment to induce callus and regenerate plants, and to assess the effect of three cytokinin sources and their ratios to auxin for cassava organogenesis.

## MATERIALS AND METHODS

### *Plant materials*

Cassava plantlets of cv MCol 1505 used in this study were provided by CIAT (Centro Internacional de Agricultura Tropical, Cali, Colombia). All plant materials received were sterile, placed in test tubes, and were maintained on revised Murashige and Skoog (MS) (1962) culture medium supplemented with 2% sucrose, 4 g/l phytagel, and 500 mg/l magnesium chloride (Sigma-Aldrich Co.) for a month.

### *Procedure*

a) *Callus and shoot formation* - Under sterile conditions of a laminar flow hood (Baker Company, Inc.), sterile portions of young leaf lobes (1-5 $mm^2$), stem (1-3 mm), and root (1-4 mm) tissue were excised from the stock material received from CIAT and cultured onto MS nutrient medium. The medium was supplemented with 10 or 20 mM kinetin, thidiazuron (TDZ), or 6-benzyl-aminopurine (BA) and 2, 4-dichlorophenoxyacetic acid (2, 4-D) used alone or in combination for callus and shoot formation. MS nutrient medium was also supplemented with 3% sucrose, 4 g/l phytagel, and 500 mg/l magnesium chloride. The pH of the different treatment media was adjusted to 5.7 with KOH prior to autoclaving. The basal MS nutrient medium was used as control. Some of the organogenic calli resulting from three-month culture were transferred to shoot-enhancing secondary media for an additional three-month culturing. Other calli were main-

Table 1: Organogenic response of cassava explants cultured on primary media treatments

| Treatment | Leaf callus | Shoots/leaf callus | Stem callus | Root callus |
|---|---|---|---|---|
| TDZ(20µM) + 2,4D (20µM) | +++ | 3.8 $^{b}$ | +++ | +++ |
| TDZ(10µM) + 2,4D (10µM) | +++ | 4.1 $^{b}$ | +++ | +++ |
| BA (20µM) + 2,4D (20µM) | +++ | 5.0 $^{a}$ | +++ | +++ |
| BA (10µM) + 2,4D (10µM) | +++ | 5.3 $^{a}$ | +++ | +++ |
| Kinetin(20µM)+2,4D(20µM) | +++ | 5.6 $^{a}$ | +++ | +++ |
| Kinetin(10µM)+2,4D(10µM) | +++ | 5.0 $^{a}$ | +++ | +++ |
| TDZ (20µM) | – | 0.0 $^{c}$ | – | – |
| TDZ (10µM) | – | 0.0 $^{c}$ | – | + |
| BA (20µM) | + | 0.0 $^{c}$ | + | + |
| BA (10µM) | – | 0.0 $^{c}$ | + | + |
| Kinetin (20µM) | + | 0.0 $^{c}$ | + | + |
| Kinetin (10µM) | + | 0.0 $^{c}$ | + | + |
| 2, 4 D (20µM) | ++ | 0.0 $^{c}$ | +++ | +++ |
| 2, 4 D (10µM) | ++ | 0.0 $^{c}$ | +++ | +++ |

Coefficient of variation: 54%; +: little callus formation; ++: moderate callus formation; +++: massive callus formation; -: no callus formation; Means within the same column and followed by the same letter are not significantly different by Duncan's Multiple Range Test (P= 0.05).

tained on their respective primary treatment media throughout the six-month culture period.
b) *Shoot formation enhancement* - Calli were allowed to develop shoot initials of up to about 1mm long and possess meristematic regions prior to transfer to media for further shoot formation. Calli were transferred to MS medium supplemented with 10mM kinetin, BA, or TDZ and 2, 5, 8, 10, 15, or 20mM 2, 4-D for an additional three months of culturing for shoot formation enhancement. Treatments of 10mM kinetin, BA, or TDZ and 10mM 2, 4-D were used as control on secondary media.
c) *Statistical design and analysis* - A completely randomized design with factorial arrangement was used for treatment allocation in callus and shoot formation, and shoot enhancement studies. In each study, five plates with 5 explants or organogenic calli each were randomly assigned to each treatment. SAS statistical package was used for statistical analysis (SAS Institute, 1990). Differences among shoot mean values were evaluated using the Duncan Multiple Range Test, 0.05.
d) *Cultural conditions* - All cultures were maintained in a growth chamber at a temperature of 28 ± 2 °C under a 16 h photoperiod and a photosynthetic photon flux of 100 µmol $m^{-2}$ $s^{-1}$ provided by cool white fluorescent lamps. Explants were subcultured onto fresh media

Table 2a: Effect of TDZ and varying 2, 4-D concentrations in secondary media for shoot production

| Treatment | Callus with Shoots (%) | Shoots/ callus |
|---|---|---|
| TDZ(10µM) + 2,4D (2µM) | $100.0^{a}$ | $6.7^{a}$ |
| 2,4D (5µM) | $100.0^{a}$ | $6.3^{a}$ |
| 2,4D (8µM) | $100.0^{a}$ | $5.2^{b}$ |
| 2,4D(10µM) (control) | $100.0^{a}$ | $3.6^{c}$ |
| 2,4D(15µM) | $90.0^{b}$ | $1.5^{d}$ |
| 2,4D(20µM) | $68.0^{c}$ | $1.4^{d}$ |
| TDZ (10µM) | $42.0^{d}$ | $0.0^{e}$ |
| 2, 4 D (10µM) | $3.0^{e}$ | $0.0^{e}$ |

Coefficient of variation: 35%; Means within the same column and followed by the same letter are not significantly different by Duncan's Multiple Range Test (P= 0.05).

Table 2b: Effect of BA and varying 2, 4-D concentrations in secondary media on shoot production

| Treatment | Callus with Shoots (%) | Shoots/ callus |
|---|---|---|
| BA (10µM) + 2,4D (2µM) | $100.0^{a}$ | $8.0^{a}$ |
| 2,4D (5µM) | $100.0^{a}$ | $6.0^{b}$ |
| 2,4D (8µM) | $100.9^{a}$ | $4.0^{c}$ |
| 2,4D(10µM) (Control) | $100.0^{a}$ | $4.1^{c}$ |
| 2,4D(15µM) | $85.0^{b}$ | $2.1^{cd}$ |
| 2,4D(20µM) | $78.0^{c}$ | $2.0^{e}$ |
| BA (10µM) | $40.0^{d}$ | $3.0^{d}$ |
| 2, 4 D (10µM) | $3.0^{e}$ | $0.4^{f}$ |

Coefficient of variation: 43%; Means within the same column and followed by the same letter are not significantly different by Duncan's Multiple Range Test (P= 0.05).

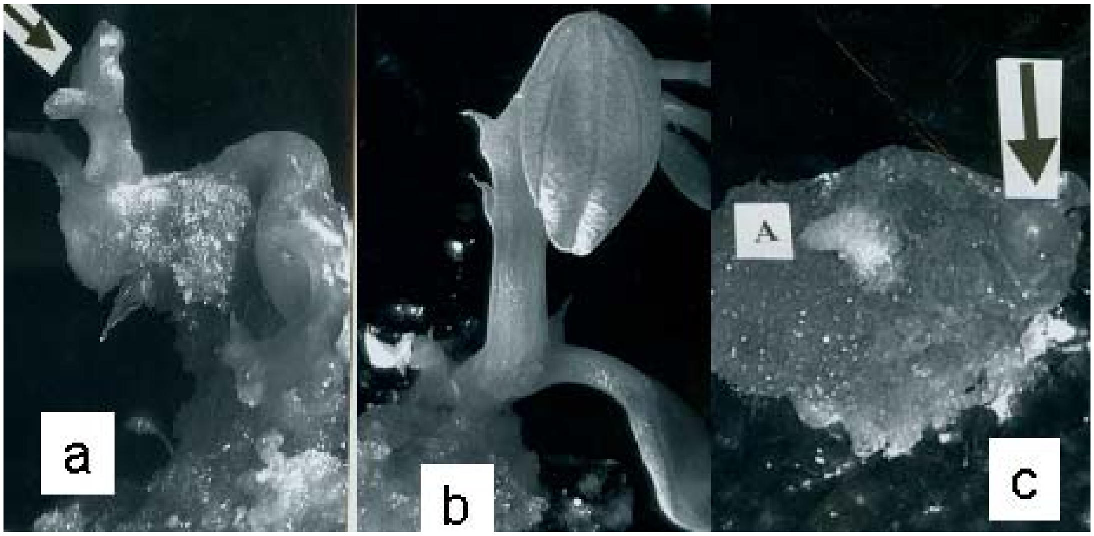

**Figure 1**

every 25 days, and data were collected over a six-month period.

## RESULTS

*Callus formation on primary media*

Callus formation was initiated and proliferated from cassava leaf, stem, and root tissues that were cultured on primary media that contained 2, 4-D used alone or in combination with TDZ, BA, or kinetin (Table 1). No callus was formed on control basal MS medium. Generally, treatments consisting of BA, kinetin, or TDZ in combination with 2, 4-D showed the best callus proliferation for leaf, stem, and root explants (Table 1). However, treatments of BA, kinetin, or TDZ alone either produced no callus, or produced non-vigorous and browning calli.

*Adventitious shoot formation:*
*Shoot formation on primary media*

A single, two-component growth regulator treatment produced both callus and shoots in leaf explants (Table 1). Both somatic embryos (Figure 1a) and adventitious shoots (Figure 1b) were observed. Some calli developed localized green regions (meristems) that subsequently developed into adventitious shoots (Figure 1c). Shoots also formed on leaf calli, but only when the primary media consisted of BA, kinetin, or TDZ and 2, 4-D (Table 1). These media are the same as those that produced the greatest callus growth on leaf, stem, and root explants (Table 1). However, it should be noted that no shoots were observed on stem or root calli. There were no differences in average number of shoots formed on primary media in which kinetin or BA was used in combination with 2, 4-D (Table 1). When TDZ, BA, or 2, 4-D were used alone in the primary media, no shoot formation was observed.

*Enhancement of shoot formation on secondary media*

Generally, transferring of organogenic calli with early shoots and meristems from primary shoot-forming media (10 μM BA, kinetin, or TDZ and 2, 4-D) to similar secondary media with low concentrations of 2, 4-D enhanced shoot production (Tables 2a, b, c). By decreas-

Table 2c: Effect of kinetin and varying 2, 4-D concentrations in secondary media on shoot production

| Treatment | Callus with Shoots (%) | Shoots/ callus |
|---|---|---|
| Kinetin (10µM) + 2,4D (2µM) | 100.0$^{a}$ | 9.4$^{a}$ |
| 2,4D (5µM) | 100.0$^{a}$ | 5.8$^{b}$ |
| 2,4D (8µM) | 100.0$^{a}$ | 4.0$^{c}$ |
| 2,4D(10µM) (Control) | 100.0$^{a}$ | 3.5$^{cd}$ |
| 2,4D(15µM) | 75.0$^{b}$ | 3.5$^{cd}$ |
| 2,4D(20µM) | 68.0$^{c}$ | 3.0$^{d}$ |
| Kinetin (10µM) | 35.0$^{d}$ | 3.0$^{d}$ |
| 2, 4 D (10µM) | 2.0$^{e}$ | 0.1$^{e}$ |

Coefficient of variation: 33%; Means within the same column and followed by the same letter are not significantly different by Duncan's Multiple Range Test (P= 0.05).

ing 2, 4-D concentration by at least half of that of cytokinins, shoot production was significantly enhanced, compared with the control by average increases of about 6, 4, and 3 for kinetin, BA, and TDZ respectively. Treatment combinations with the lowest concentration of 2, 4-D (2µM) formed more shoots than any other treatments, irrespective of the cytokinin source (Tables 2a, b, c). The greatest average shoots per callus were 9.4, 8, and 6.7 for kinetin, BA, and TDZ with 2µM 2, 4-D, respectively. Results also showed that, irrespective of the cytokinin source, all two-component treatments whose 2, 4-D concentration was lower than that of the control by at least 50% produced shoot averages that were significantly greater than that of the control (Tables 2a, b, c). Further, there were no significant differences for percent calli with shoots (100%) among treatment combinations in which the concentration of 2, 4-D was lower or equal to that of the control, irrespective of the cytokinin source (Tables 2a, b, c). All related combination treatments in which the concentration of 2, 4-D was higher than that of the control or the cytokinins (10 µM) had lower percent calli with shoots, and formed fewer shoots per callus than the control (Tables 2a, b, c).

## DISCUSSION

This study provides a one-step method alternative for rapid propagation of cassava plants. A single two-component growth regulator treatment was used to induce callus and SE and to develop plants. The best combination treatments of this study were 10µM kinetin, BA, or TDZ and 2µM 2,4D. All induced shoots in 100 percent of the cultured calli with average shoots of 9.4, 8, and 6.7 per callus, respectively. Although these average numbers of shoots were higher than those reported by Groll *et al.*, 2002 (8.6 plants/gram of callus), they were lower than those reported by Li *et al.*, 1998 (24.1 shoots/explant) and Joseph *et al.*, 2001 (24.3 somatic embryos/explant). However, the overall plant regeneration frequency from this study (100%) was higher than those reported by Groll *et al.*, 2002 (98.5%), Li *et al.*, 1998 (84%) and Joseph *et al.*, 2001 (86.7%).

Although the manipulation of growth regulator concentrations in cassava studies has permitted *in vitro* plant regeneration (Danso *et al.* 1999; Joseph *et al.* 1999; Peng *et al.* 2001), callus and somatic embryos (SE) or adventitious shoot formation as well as plant development, could not be achieved when using a single growth regulator treatment (Groll *et al.*, 2002; Joseph *et al.*, 2001). Generally, cassava *in vitro* plant regeneration has been achieved with media treatments specific to each of the developmental stages that were previously indicated (Danso *et al*. 1999; Joseph et al. 1999; Peng *et al.* 2001).

With the exception of the multiplication of plants from specialized shoot meristematic tissues derived from apical or axillary buds (Escobar, 1997; Konan *et al*., 1997), cassava plant regeneration methods generally have involved four major steps as follows: (1) direct or indirect SE induction that used a single auxin (Joseph *et al.*, 1999; Joseph et al., 2001; Raemakers *et al*., 1993), (2) somatic embryo maturation that used a single cytokinin (Joseph *et al.*,1999; Raemakers *et al*., 1993; Zhang *et al.*, 2000) or auxin (Danso *et al.*, 1999), (3) shoot development that used a two component growth regulator treatment (Joseph *et al.*, 2001; Li *et al.*, 1998; Zhang *et al.*, 2000), and (4) rooting of regenerated shoots on a nutrient medium without growth regulators (Joseph *et al.*, 1999; Joseph *et al*., 2001; Sofia and Raemakers, 1998). Although induction of SE (step 1) is commonly achieved with the use of a single growth regulator treatment, most explants from which SE are induced are generally excised from cassava plants that have been pretreated with growth regulators, which had been included in their maintenance media (Raemakers *et al.*, 1993; Sofia and Raemakers, 1998; Zhang *et al.*, 2000). Further, the residual effect of thidiazuron, for instance, that had been included in the pre-treatment medium was observed to have caused the induction and regeneration of peanut plants from explants grown on basal MS nutrient medium (Matand *et al.*, 1994). Accordingly, it might be inferred that the residual effect of cytokinins that had been included in the maintenance media of most reported cassava regeneration methods might have contributed also to the induction of SE in step 1.

This report also describes a procedure that enhanced shoot production on the secondary medium treatments. By keeping constant the concentration of cytokinin while decreasing that of 2, 4-D, average shoots were increased by up to about 2.7, 1.9, and 2 folds for the treatment combinations involving kinetin, TDZ, and BA, respectively, compared with the control. Most importantly, all treatments in which the concentration of 2, 4-D in the secondary media was decreased by at least half of that of the cytokinin, irrespective of the cytokinin source, significantly enhanced shoot formation. However, when the concentration of 2, 4-D in related treatments was increased, the number of shoots per callus decreased; further growth of shoots and shoot meristems, which formed prior to their transfer to the secondary media, was inhibited in favor of root formation. The increase of 2, 4-D may have resulted in the enhancement of the potential effect of the endogenous auxin in causing shoot apical dominance (George, 1993; Trigiano and Gray, 1996). In a related study conducted by Li *et al.* (1998) in which 0.5µM IBA was combined with increasing concentrations of BA (0.1 to 2 µM) and vice versa, shoot frequency and the average transplantable shoots increased with BA concentrations up to the concentration twice that of IBA (0.5 mg/l) before it declined. The highest shoot induction rate and the greatest average number of transplantable shoots per explant were 59% and 8.7 respectively. This study and the current one may support the popular concept that a cytokinins to auxin ratio that is less than 1 would influence shoot formation (George 1993).

In conclusion, this study reports a simple protocol in which a single two-component growth regulator treatment may be used to induce the callus were increased compared with the control, irrespective of the cytokinin source. The transfer of organogenic calli to a medium containing 2, 4-D alone resulted in more root formation and little or no further shoot formation.

## ACKNOWLEDGMENT

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## Leaves Required for Floral Induction of Lychee[1]

**Zhentu Ying and Thomas L. Davenport**[2]

**Abstract**: Two cultivars of lychee (*Litchi chinensis* Sonn.), Mauritius and Brewster, were evaluated to determine if leaves are required for flowering. After the onset of cool season temperatures, three experimental branches were girdled approximately 1 meter back from terminals to isolate the treated stems from the rest of the tree, and each was treated in one of three ways. Stems on each branch were either tip pruned and defoliated, tip pruned with leaves left intact, or received no pruning treatment. Comparison of results showed that tip pruning stimulated more total and reproductive shoot production in stems and that defoliation prevented induction of reproductive shoots in favor of only vegetative shoots in both cultivars. This latter result infers that lychee flowering requires leaves as the source of the putative florigenic promoter. Tip-pruned 'Brewster' branches with intact leaves in which the girdles failed to close during fruit development had significantly greater fruit set over those in which the girdles closed or those of non-pruned branches regardless of girdle closure.

**Nomenclature**, lychee, litchi, *Litchi chinensis* Sonn.
***Additional index words*:** litchi, flowering, florigenic promoter

### INTRODUCTION

The vegetative or flowering response of some subtropical species such as citrus, mango, and lychee have been proposed to be the result of interaction between a putative vegetative promoter that decreases in activity with stem age and a putative florigenic promoter that is up-regulated by cool temperatures (Davenport, 2000; Davenport, 2003b). The putative florigenic promoter has been demonstrated to be produced in stems of citrus (Davenport, 2003a) and in leaves of mango (Davenport and Nuñez-Elisea, 1997; Nunez-Elisea et al., 1996; Reece et al., 1949). It is graft transmissible (Kulkarni, 1986; 1988) and readily translocated over long distances from leafy stems to leafless stems in mango branches (Davenport and Ying, 2003; Nunez-Elisea et al., 1996).

Two cultivars of lychee (*Litchi chinensis* Sonn.), Mauritius and Brewster, are commonly grown in south Florida. Both, especially 'Brewster', are irregular in their flowering and fruit setting behaviors from year to year, causing unreliable cropping (Davenport et al., 1999; Zheng et al., 2001). As in citrus and mango, it has long been recognized that cool winter temperatures promote flowering of lychee (Batten and McConchie, 1995; Davenport, 2003b; Groff, 1921; Menzel, 1983). Davenport and Stern (2004) proposed that cool temperatures up-regulate production of a putative florigenic promoter in lychee. The purpose of this study was to determine whether leaves or stems are the source of the putative florigenic promoter in this crop.

### MATERIALS AND METHODS

**Plant Materials** - Experiments were conducted on ten-year-old, 4-meter-high

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[2] University of Florida, IFAS, Tropical Research and Education Center, 18905 SW 280 St., Homestead, FL 33031. This research was supported by the Florida Agricultural Experiment Station and a grant from USDA-CSRS, TSAR Special Grant no. 01-72792-6912 and approved for publication as Journal Series No. _R-10212_.

'Brewster' and 'Mauritius' lychee trees during winter floral inductive conditions in an orchard located in Homestead, Florida (25ºN latitude, 3 meters above sea level). Three similar branches with basal diameters of 2 to 3 cm at a point 1.0 to 1.5 m inside the edge of the canopy were randomly selected, tagged, and treated as described below in each of five replicate trees of each cultivar in late December of 2002, soon after passage of the first winter cold front producing floral inductive night temperatures of 10° to 15°C. The number of resting terminal stems, from which growing shoots emerged upon shoot initiation (Davenport, 2000; Davenport, 2003b), in the experimental branches of the three replicated treatments ranged from 33 to 149 in 'Brewster' and 24 to 86 in 'Mauritius. Although variation in the number of stems among the selected experimental branches within and among trees was unavoidably great, individual stems served as the experimental unit responding to branch treatments by either initiating lateral or terminal shoots on tip-pruned or on non-pruned stems, respectively. Shoots displaying only leaves were classified as vegetative. Mixed shoots displayed both leaves and inflorescences inserted at the base of each leaf, and generative shoots displayed only inflorescences forming leafless, determinate panicles (Davenport and Stern, 2004).

**Treatments** - *Girdling* prevents phloem transport of the putative florigenic promoter from other branches into experimental branches as was demonstrated in mango (Davenport and Ying, 2003; Nuñez-Elisea et al., 1996). A girdling ring of branch bark and cambium about 1 cm in width was removed at a location 1.0 to 1.5 m inside the edge of the canopy on all three experimental branches selected for treatment on each of the five replicate trees per cultivar. *Tip pruning* stems stimulates synchronous initiation of lateral shoots on all of the stems on an experimental branch at the same time. Without tip pruning, individual stems normally initiate vegetative or reproductive shoots sometimes many weeks apart. In such cases, reproductive shoots are timed by the passage of arctic cold fronts providing the cool temperature conditions necessary for floral-induction. Two of the three experimental branches from each replicate tree were tip-pruned below the third node from the apex on every stem to stimulate synchronous lateral bud break during the cool, floral inductive conditions that was present at the time.

**Table 1.** Effects of tip pruning and defoliation of girdled branches on the number of shoots per stem (Sht/stm) and proportion (%) of each shoot type in two lychee cultivars, Brewster and Mauritius. All results are means of five replicate branches among five trees.

| Cultivar<br>Branch treatment | Total # stems | Vegetative | | Mixed | | Generative | | Total | |
|---|---|---|---|---|---|---|---|---|---|
| | | Sht/stm* | % | Sht/stm | % | Sht/stm | % | Sht/stm | % |
| Brewster | | | | | | | | | |
| Tip pruned & defoliated | 417 | 1.33a | 100 | 0.00c | 0 | 0.00a | 0 | 1.33ab | 100 |
| Tip pruned | 208 | 0.01b | 1 | 0.01a | 1 | 1.59a | 98 | 1.61a | 100 |
| Non pruned | 223 | 0.00b | 0 | 0.00a | 0 | 1.00b | 100 | 1.00b | 100 |
| Mauritius | | | | | | | | | |
| Tip pruned & defoliated | 325 | 1.13a | 100 | 0.00b | 0 | 0.00c | 0 | 1.13a | 100 |
| Tip pruned | 169 | 0.29b | 23 | 0.33a | 26 | 0.63b | 51 | 1.25a | 100 |
| Non pruned | 227 | 0.00c | 0 | 0.08b | 8 | 0.89a | 92 | 0.97a | 100 |

*Means within a column for each cultivar followed by different letters are significantly different using Duncan's new multiple range test at P=0.05.

*Defoliation* removes a potential source of the putative florigenic promoter. One of the above two tip-pruned branches was completely defoliated by hand, and the other was left with leaves intact.

The three treatments randomly assigned to branches on each of five trees of the two cultivars thus compared were:

*Tip pruned & defoliated* - providing synchronously growing lateral shoots initiating from leafless stems on isolated branches during, cool, floral-inductive conditions.

*Tip pruned* - providing synchronously growing lateral shoots initiating from leafy stems on isolated branches during, cool, floral-inductive conditions.

*Non pruned* - providing asynchronously growing shoots initiating from leafy stems on isolated branches during, cool, floral-inductive conditions.

**Data Recording and Analysis** - The total number of resting stems on each experimental branch on the five replicate trees was recorded at the time of treatment. The total number of new lateral or terminal shoots and their type (vegetative, mixed, or generative) was recorded for each branch in early March of 2003 when the above three shoot types were evident. The total number of fruit on each test branch was counted in early May of 2003 to determine the number of fruit per stem in 'Brewster'. Stem number for each branch was summed among the replicate treatments for each cultivar. Shoot and fruit observations were averaged over the five replicate branches within each cultivar, analyzed for significance by ANOVA, and subjected to Duncan's new multiple range test (P< 0.05) to determine differences in means.

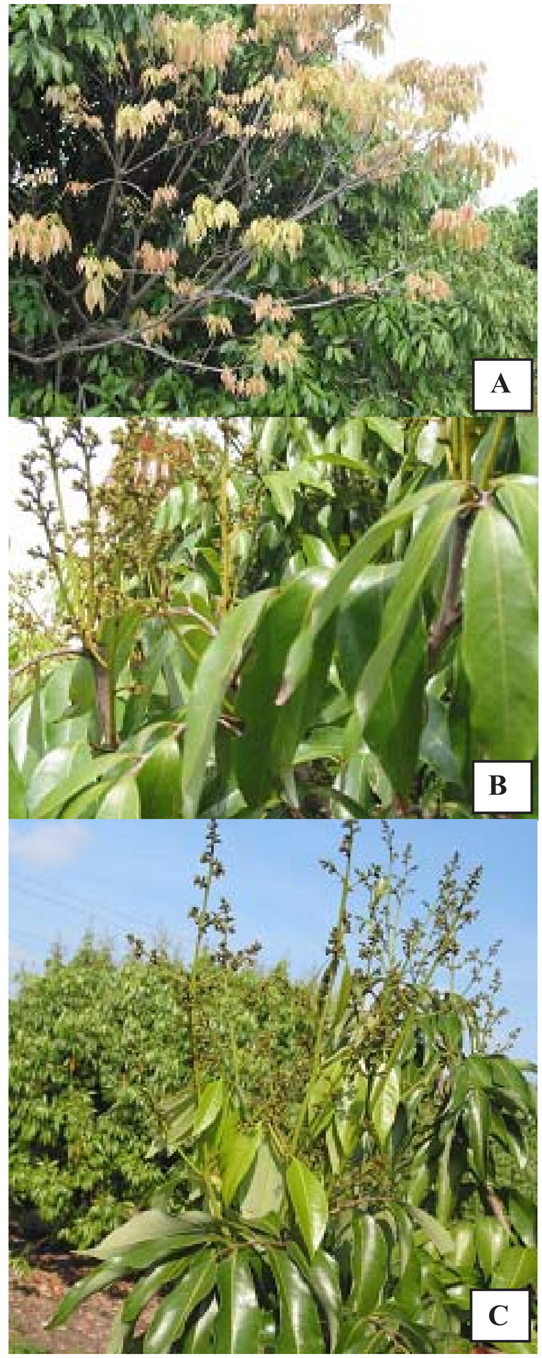


**Figure 1 -** A. Lateral vegetative shoots on girdled branches that were tip pruned and defoliated; B. lateral generative shoots on girdled branches that were tip pruned; C. terminal generative shoots on non-pruned girdled branches, similar to shoots on non-experimental branches in the rest of the tree canopy.

**Table 2.** Effect of girdle closure and tip pruning on fruit set in girdled branches of 'Brewster' lychee trees.

| Treatment | No. of stems | No. of fruit* | Fruit/stem |
|---|---|---|---|
| Tip pruned | | | |
| Open girdle | 175 | 808 | 4.62a** |
| Closed girdle | 33 | 16 | 0.48b |
| Non pruned | | | |
| Open girdle | 165 | 84 | 0.51b |
| Closed girdle | 58 | 25 | 0.43b |

* Data were collected on May 2, 2003

** Means followed different letters are significantly different at P=0.05 based on Duncan's new multiple range test.

## RESULTS AND DISCUSSION

As is typical of other tropical fruit crops we have studied in south Florida (Nunez-Elisea et al., 1996; Southwick and Davenport, 1986), tip pruning of stems stimulated synchronous initiation of new shoots from lateral buds soon after treatment in both lychee cultivars. If shoot initiation occurs in these crops during cool temperature conditions, as occurred in the present experiment, the shoots are typically induced to flower (Davenport, 2000; Davenport and Stern, 2004; Menzel and Simpson, 1988; 1995). Girdling the experimental branches did not alter the flowering behavior of the experimental branches compared to those of the rest of the tree canopy in any replicate tree of either cultivar. For example, flowering in the non-pruned treatment of the girdled branches was the same as those in the non-treated portions of the canopy (data not shown, Fig. 1C). Shoot initiation in the non-pruned experimental branches of 'Mauritius' and 'Brewster' trees was similar but less synchronous than that of the tip-pruned branches with bud break occurring over a longer period of time. Shoot initiation in the non-pruned branches and in the canopy of 'Brewster' occurred later than those of 'Mauritius', which is typical of the cultivar. Despite differences in shoot initiation times among the treatments, the two branch treatments that were tip pruned tended to form more vegetative (Fig. 1A) and reproductive (Fig. 1B) shoots than the non-pruned treatment (Fig. 1C) due to the fact that the latter still had apical buds that resulted in initiation of one terminal shoot per stem, whereas the tip pruned stems forced more shoots from lateral buds (Table 1). A small number of 'Mauritius' stems did not initiate an apical shoot in the non-pruned branches resulting in the 0.97 shoots per stem.

The absence of leaves on the stems of tip pruned and defoliated branches at the time of shoot initiation during floral inductive conditions completely inhibited reproductive shoot production and stimulated induction of only vegetative lateral shoots in both 'Brewster' and 'Mauritius' (Table 1, Fig. 1A). These results imply that the putative lychee florigenic promoter proposed by Davenport (2003b) and Davenport and Stern (2004) is most likely synthesized in leaves and transported into buds. Controlled condition experiments have demonstrated that the flowering or vegetative response is not governed by differences in available carbohydrates or nitrogen in leaves of either lychee or mango (Batten and McConchie, 1995; Davenport and Ying, 2003; Davenport et al., 2001). The same response to defoliation in floral inductive conditions has also been demonstrated in mango (Davenport and Nuñez-Elisea, 1997; Nuñez-Elisea et al., 1996).

Nearly all of the shoots that formed in the tip-pruned-treated branches of the 'Brewster' trees were generative (Table 1, Fig. 1B). There was, however, a significant proportion of vegetative and mixed shoots that formed in the same treatment of 'Mauritius' branches. We have no clear explanation for this difference in performance of the two cultivars, but it could be linked to the temperatures at the time that shoot initiation occurred.

We noticed an unusually high amount of fruit set in the tip-pruned branches compared to the non-pruned branches in most of the 'Brewster' trees (Table 2). No such correlation was seen in the 'Mauritius' trees. Closer examination revealed that this difference was related to whether the girdles had partially closed, i.e. if the phloem and bark of the branch girdle had reestablished connections (closed) across the girdle or the girdle had remained open during fruit development. A nearly ten-fold increase in fruit set occurred in tip-pruned branches in which the girdles had remained open (Table 2). Girdles that closed on the branches with tip-pruned stems had the same amount of fruit set as those of both closed and open girdles on the non-pruned branches.

There is no clear explanation for this phenomenon. The only thing unique about the branches with greater fruit set from the other treatments is the fact that all inflorescences developed from lateral buds as a result of tip pruning (Fig.1B) combined with lack of girdle closure. Further investigation may provide an answer that could lead to increased lychee productivity.

Flowering of mango, citrus, and lychee appears to be governed by similar mechanisms (Davenport, 2003b). Flowering or vegetative induction in these crops appears to be regulated by interaction of a putative vegetative and a florigenic promoter at the time of shoot initiation. The florigenic promoter has been demonstrated in a number of studies to be synthesized in leaves and can be transported over long distances in branches to developing shoots via phloem (Davenport and Ying, 2003; Nuñez-Elisea et al., 1996). This is the first report indicating the source of the putative florigenic promoter of lychee also resides in leaves.

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**32nd Annual Conference**
**Plant Growth Regulation Society of America**
**July 24-27, 2005**
**Radisson Newport Beach Hotel**
**Newport Beach, California**

The 32nd Annual Conference of the Plant Growth Regulation Society of America will be held in Newport Beach, California July 24-27, 2005. Newport Beach is located in Orange County, California which is centrally located to several major tourist attractions including Disneyland, Knott's Berry Farm, and the jewel of the California missions—Mission San Juan Capistrano. In addition to symposia, a workshop, an industry update session, and contributed papers and posters, the conference will feature a pre-conference tour to Catalina Island, one of the top ten Southern California experiences. This idyllic isle, often compared to Mediterranean jewels such as Capri and Malta, sits just 22 miles from the mainland. Swimming, snorkeling and kayaking are just a few popular water activities. You can also stroll the cobblestone streets of the tiny town of Avalon, visit the Art Deco Casino pavilion, ride a glass-bottom boat, or tour the conservation-protected interior of this channel island.

The conference will be held in the Radisson Newport Beach Hotel and features benefits such as Travel and Best Student Paper/Poster Awards and a Young Scientist Award. Meeting updates may be found on the PGRSA website at http://www.griffin.peachnet.edu/pgrsa. The final Wednesday evening reception will be held in the Botanical Gardens at the Huntington Library, one of the world's greatest cultural, research, and educational centers. For more information about the meeting, topics, or location, contact:

Dr. Sonja Maki
Biology Department
Carleton College
One North College Street
Northfield, MN 55057
Tel: (507)646-4544
Fax: (507)646-5757
E-mail: smaki@carleton.edu

## PGRSA CURRENT NEWS ITEMS

### PGRSA Officers Elected

Dr. Jeffery P. Norrie was elected 2nd Vice President. He is joined on the 2004-2005 Steering Committee by two other newly elected officers, Dr. Steven J. McArtney, Secretary and Dr. C. Thomas Chao, Member-at-Large. Jeff, Steve, and Thomas assumed their responsibilities following the annual meeting in 2004..

### PGRSA Officers Appointed

Business Manager, Executive Officer, and *PGRSA Quarterly* Editor positions are or are in the process of being vacated/filled. The position of Executive Officer is in transition as Dr. Wayne A. Mackay is in the process of replacing Dr. Thomas J. Tworkoski by the summer of 2005. The position of *PGRSA Quarterly* Editor will be filled by the end of 2005. The position of Business Manager will be filled by the end of 2006. If you are interested in or know of someone who may be interested in either the Business Manager or Editor position, contact Dr. Louis Ferguson, PGRSA President.

Mr. Charles Hall of Associated Services Group was reappointed Executive Secretary of the PGRSA for 2004 – 2005. He has served in that position since 1998. Associated Services Group handles daily financial activities, maintains a current membership and mailing list, archives society publications, and distributes all society mailings. The contact at Associated Services Group is Kaye Lindsey.

### Nominations for the Steering Committee

The Nominations Committee will be seeking nominees for two positions on the 2005-2006 Plant Growth Regulation Society of America Steering Committee. The positions are 2nd Vice President and Member-at-Large. If you are interested in being nominated, contact Dr. Ronald F. Smith. The election for these positions will be held in the spring of 2005.

### PGRSA Conferences

Dr. Sonja Maki, 1st Vice President, will be the program coordinator for the 2005 annual meeting to be held in Newport Beach, California, and Dr. Jeffery Norrie, 2nd Vice President, will be the program coordinator for the annual meeting to be held in Quebec, Canada in 2006. Information about the meetings will be mailed and posted on the Web site.

### Membership

Beginning in 2005, registrants attending the annual meeting will automatically be members for the following year. Registration fees will increase slightly to accommodate the change.

# 2004 PGRSA

**Charles T. Hall, Jr. - Executive Secretary**
**PGRSA**
**P.O. Box 2945**
**LaGrange, GA 30241**
**Phone: 706-845-9085**

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**Dr. Eric A. Curry (Past President)**
USDA/ARS Tree Fruit Research Laboratory
1104 Western Ave., Wenatchee, WA 98801
509-664-2280 ext. 209 (phone) 509-664-2287 (fax)
curry@tfrl.ars.usda.gov

**Dr. Louise Ferguson (Vice President)**
University of California – Davis
Pomology Dept. - Kearney Agricultural Center
9240 South Riverbend Avenue, Parlier, CA 93648
559-646-6541 (phone) 559-646-6593 (fax)
louise@uckac.edu

**Dr. Sonja L. Maki (1st Vice President)**
Carleton College
Biology Dept., One North College St.
Northfield, MN 55057
507-646-4544 (phone) 507-646-5757 (fax)
smaki@carleton.edu

**Dr. Jeffrey P. Norrie (2nd Vice President)**
Acadian Seaplants Ltd.
R&D Division
30 Brown Ave., Dartmouth, Nova Scotia
CANADA B3B 1 X8
902-468-2840 (phone) 902-468-3474 (fax)
jnorrie@acadian.ca

**Dr. Steven J. McArtney (Secretary)**
North Carolina State University
Dept. of Horticultural Sciences, 455 Research Drive
Fletcher, NC 28732
828-684-3562 (phone) 828-684-8715 (fax)
steve_mcartney@ncsu.edu

**Dr. Ricardo A. Menendez (Member at Large – 3)**
Valent BioSciences Corporation
870 Technology Way, Suite 100
Libertyville, IL 60048
847-968-4715 (phone) 847-968-4801 (fax)
ricardo.menendez@valent.com

**Dr. Ronald F. Smith (Member at Large-2)**
Canadian Forest Srvc. – Atlantic Forestry Center
P.O. Box 4000, Regent Street
Fredericton, New Brunswick, CANADA E3B 5P7
506-452-3533 (phone) 506-452-3525 (fax)
rosmith@nrcan.gc.ca

**C. Thomas Chao (Member at Large – 3)**
University of California-Riverside
Department of Botany and Plant Sciences
4106 Batchelor Hall Ext., Riverside, CA 92521
909-787-3441 (phone) 909-787-4437 (fax)
ctchao@citrus.ucr.edu

**Dr. Richard T. Dunand (Business Manager)**
LSU Agricultural Center Rice Research Station
1373 Caffey Road, Rayne, LA 70578
337-788-7531 (phone) 337-788-7553 (fax)
rdunand@agcenter.lsu.edu

**Dr. Caula A. Beyl (Editor, PGRSA Quarterly)**
Alabama A&M University
Institutional Planning, Research and Evaluation
P.O. Box 272, Normal, AL 35762
256-372-8093 (phone) 256-372-8162 (fax)
cbeyl@asnaam.aamu.edu

**Dr. Thomas J. Tworkoski (Executive Officer)**
USDA/ARS Appalachian Fruit Research Station
2217 Wiltshire Road, Kearneysville, WV 25431
304-725-3451 ext.390 304-728-2340 (fax)
ttworkos@afrs.ars.usda.gov

**Dr. Wayne Mackay (Associate Executive Officer)**
Texas A&M Research & Extension Center
17360 Coit Road, Dallas, TX 75252-6599
972-231-5362 972-952-9216 (fax)
w-mackay@tamu.edu

**Mr. Charles T. Hall, Jr. (Executive Secretary)**
ASG/PGRSA
P. O. Box 2945, LaGrange, GA 30241
Shipping address:
301 Broome St., Suite 203, LaGrange, GA 30240
706-845-9085 (phone) 706-883-8215 (fax)
chall@asginfo.net
PGRSA accounts – Kaye Lindsey
klindsey@asginfo.net

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## Till We Meet Again

**June 5-8, 2005**
2005 In Vitro Biology Meeting
Hyatt Regency, Baltimore, MD
www.sivb.org/meetings.asp

**June 26 - 30, 2005**
10th International Symposium on Plant Bioregulators in Fruit Production
Saltillo, Mexico
www.saltillo2005.org

**July 10 -15, 2005**
Xth International Turfgrass Research Conference
Llandudno, North Wales, UK
www.aber.ac.uk/itrc2005

**July 18 -21, 2005**
ASHS Annual Conference
The Riviera Hotel, Las Vegas, NV
ashs@ashs.org: ashs.org

**July 24-27, 2005**
32nd Annual Conference
Plant Growth Regulation Society of America
Radisson Newport Beach, Newport Beach, California
www.griffin.peachnet.edu/pgrsa

## Down the Road

**July 27-30, 2006**
ASHS Annual Conference
Sheraton New Orleans Hotel, New Orleans, Louisiana
www.saltillo2005.org

**August 13-19, 2005**
XXVIIth International Horticultural Congress
COEX Convention Center, Seoul, South Korea
www.ishs.org/calendar/index.htm