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## Influence of Photoperiod and Daminozide Stock Plant Pretreatments on Ethylene and CO<sub>2</sub> Levels and Callus Formation from Dahlia Leaf Segment Cultures<sup>1</sup>

P. Gavinlertvatana, Paul E. Read, H. F. Wilkins, and R. Heins<sup>2</sup>

*Department of Horticultural Science and Landscape Architecture, University of Minnesota, St. Paul, MN 55108*

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**Abstract.** Ethylene (C<sub>2</sub>H<sub>4</sub>) and CO<sub>2</sub> levels and callus formation were influenced by photoperiods (8 hours-SD; 16 hours-LD) under which 'Nita' dahlia stock plants were grown and leaf segments incubated *in vitro*. Larger callus and higher levels of C<sub>2</sub>H<sub>4</sub> and CO<sub>2</sub> were detected when tissues from stock plants under SD were incubated under SD (SD-SD) than when incubated under LD (SD-LD). Tissue under LD-LD or LD-SD formed similar amounts of callus to SD-LD cultures, but C<sub>2</sub>H<sub>4</sub> and CO<sub>2</sub> levels were higher. Compared to water-sprayed controls, 2500 mg/liter butanedioic acid mono-(2,2-dimethylhydrazide) (daminozide) sprayed on SD stock plants one day before explant removal promoted callus formation, and C<sub>2</sub>H<sub>4</sub> and CO<sub>2</sub> production in tissue incubated under either SD or LD. Daminozide sprayed on LD stock plants had no influence on callus or C<sub>2</sub>H<sub>4</sub>, but CO<sub>2</sub> was increased under SD or LD incubation.

During incubation tissue growth and organogenesis in culture can be influenced by several factors, such as light, temperature, medium, and growth regulators (5, 7, 10, 13, 14, 19); but little is known of how stock plant conditions affect subsequent growth and development. It is known that conditions under which stock plants are grown influence rooting of cuttings (4, 15) and seed germination (6). Stock plant effects have been mentioned relative to tissue culture, e.g. ease of protoplast isolation (3), but without supporting data. In earlier work (17), we reported that C<sub>2</sub>H<sub>4</sub> was produced by tissues in culture and accumulated in the flask atmosphere at higher than recognized physiologically active levels. In addition, short photoperiods and daminozide stimulate tuberous root formation in dahlia cuttings (1, 15, 16); we, therefore, investigated the effect of these factors applied to stock plants on subsequent growth and C<sub>2</sub>H<sub>4</sub> and CO<sub>2</sub> levels resulting from *in vitro* culture of dahlia leaf segments.

### Materials and Methods

Six week old 'Nita' dahlia plants from rooted cuttings were grown under SD and LD at 64 μEm<sup>2</sup>sec<sup>-1</sup> of cool white fluorescent and incandescent light for 3 weeks before explants were taken. Daminozide at 2500 mg/liter or water was sprayed on stock plants to run-off 1 day before explants were harvested. New leaves which had just reached full expansion were harvested and surface disinfected in 0.5% (by volume) sodium hypochlorite and a few drops of Tween-20 for 15 min and rinsed 3 times with sterile deionized distilled water. Leaves were cut transversely through the mid-rib into small strips (1 × 0.5 cm) and 3 pieces were randomly transferred onto 50 ml modified Murashige and Skoog (MS) (10) medium in 125 ml Erlenmeyer flasks. In addition to MS high salt components and vitamins, 5 mg/liter NAA and kinetin were incorporated into the medium. The pH was adjusted to 5.8 ± 0.1 with 1N NaOH or 1N HCl and solidified with 0.8% Bacto agar prior to autoclaving at 121°C for 20 min. Explants were incubated in SD or LD growth chambers furnished with cool white fluorescent and incandescent light at 25°C. The 8 possible treatment combinations were replicated 4 times with 1 flask each. The experiment was repeated twice with similar results.

**Gas analysis.** Ethylene levels were determined by gas chromatography (GC) using a Varian model 1440 with a flame ionization detector. The column was 3.2 mm I. D. × 45 cm, packed with activated alumina 60/80 and maintained at 70°C. Air

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<sup>2</sup>Graduate Student, Professor, Professor and Graduate Student, respectively.

samples of 600  $\mu$ l were withdrawn from the culture flask for analysis with a 1 ml gas-tight syringe (Precision Sampling Corp.). The flask was immediately resealed with another layer of foil to prevent contamination. No contamination ever occurred.  $\text{CO}_2$  was determined using a Beckman G 2 gas chromatograph with a thermal conductivity detector at 130°C. Dual columns of molecular sieve 5A, 42/60 mesh, 6.4 mm I. D.  $\times$  180 cm and silica gel, grade 12, 4.7 mm I. D.  $\times$  168 cm preceded by a 244 cm by 6.4 mm empty column were used. At 2 to 3 day intervals air samples of 500  $\mu$ l were withdrawn from the culture flask for analysis.

**Callus formation.** Callus formation was scored at 25 days from 1 to 5 as follows: 1 = no callus, 2 = small amount of callus at cut edge of leaf, 3 = large amount at cut edge, 4 = large amount at cut edge and small amount on leaf surface, and 5 = large amount at cut edge and large amount on leaf surface.

### Results

When compared to water controls, daminozide stimulated more callus formation on explants taken from SD stock plants but did not affect callus formation on explants from LD stock plants (Fig. 1). Daminozide-sprayed SD-SD explants produced larger callus than daminozide-sprayed SD-LD tissues. Water-

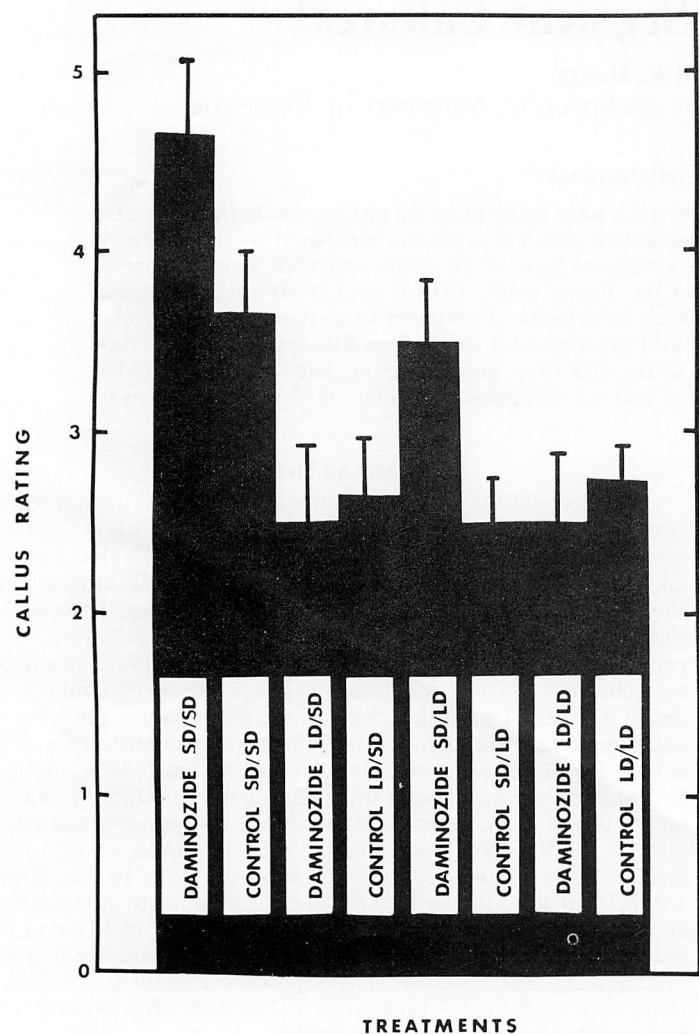


Fig. 1. Callus formation on dahlia leaf segments as affected by photoperiod and daminozide. Data taken at 25 days. Each histogram represents means of 4 replications.  $\phi$  represents a 95% confidence interval (CI). Nonoverlapping CI indicates significant difference at 5% level.

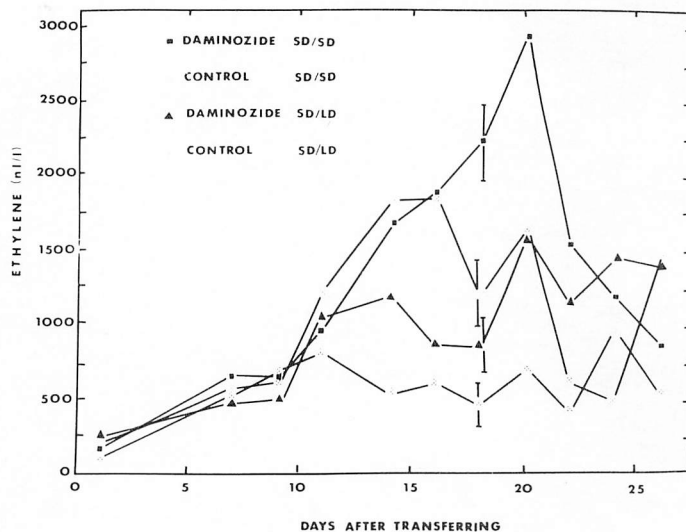


Fig. 2. Effect of daminozide, incubation and photoperiod on  $\text{C}_2\text{H}_4$  levels in flask atmospheres of dahlia leaf cultures from SD stock plants. Each point represents a mean of 4 replications,  $\phi$  represents a 95% confidence interval.

sprayed SD-SD explants formed a larger amount of callus than LD-SD, SD-LD, or LD-LD; but SD-LD explants formed a similar amount of callus to LD-LD and LD-SD tissues. Explants taken from LD stock plants formed the same amount of callus regardless of incubation conditions and daminozide or water treatments.

The highest  $\text{C}_2\text{H}_4$  levels were produced by the daminozide-sprayed SD-SD explants (Fig. 2 and 3). The  $\text{C}_2\text{H}_4$  produced by these explants reached a peak after 2 weeks incubation and then decreased during the 3rd and 4th weeks. There were no significant differences in  $\text{C}_2\text{H}_4$  levels between daminozide-sprayed LD-SD explants (Fig. 3). Significantly more  $\text{C}_2\text{H}_4$  was measured for water-sprayed SD-SD explants than for LD-SD or LD-LD explants. With SD-LD explants, significantly less  $\text{C}_2\text{H}_4$  was

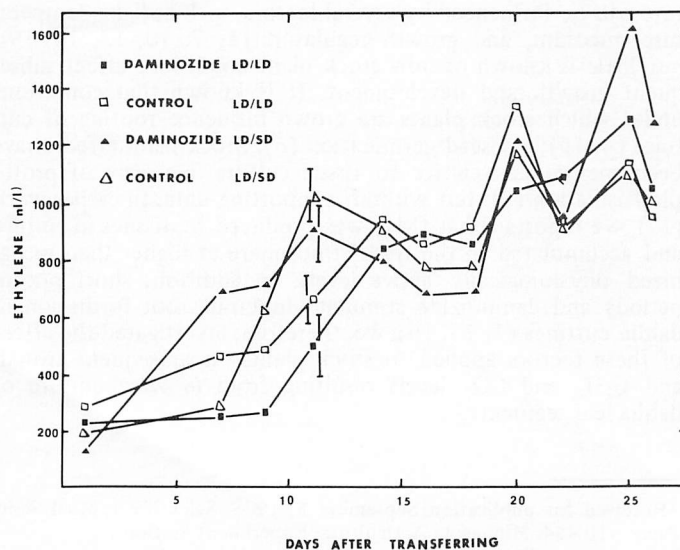


Fig. 3. Effect of incubation, daminozide and photoperiod on  $\text{C}_2\text{H}_4$  levels in flask atmospheres of dahlia leaf segments from LD plants. Each point represents a mean of 4 replications,  $\phi$  represents a 95% confidence interval.

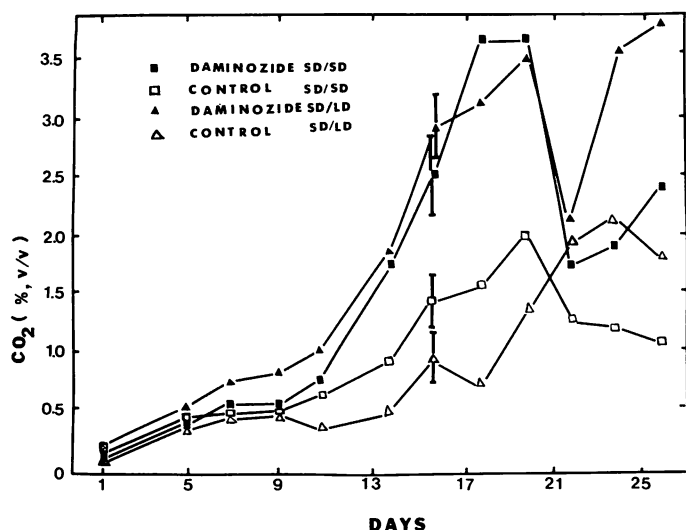


Fig. 4. Effect of incubation, daminozide and photoperiod on  $\text{CO}_2$  levels in flask atmospheres of dahlia leaf cultures from SD stock plants. Each point represents a mean of 4 replications,  $\phi$  represents a 95% confidence interval.

detected compared to SD-SD, LD-SD, and LD-LD tissues. In other treatments,  $\text{C}_2\text{H}_4$  levels slowly increased over time (Fig. 2 and 3).

Highest  $\text{CO}_2$  levels were detected in daminozide-sprayed SD-SD and SD-LD treatments but daminozide had no effect when applied to LD stock plants (Fig. 4 and 5).  $\text{CO}_2$  levels were similar for water-sprayed SD-SD or SD-LD explants to those of water-sprayed LD-SD and LD-LD tissues, although in later incubation stages, LD-LD explants produced more  $\text{CO}_2$  than SD-SD explants.

#### Discussion

From preliminary experiments, we determined that a known concentration of  $\text{C}_2\text{H}_4$  injected into flask atmospheres decreased at a constant rate of 50% in 2 hr. Therefore, measurable  $\text{C}_2\text{H}_4$  will represent a relative rate of production at a given measurement time.

$\text{C}_2\text{H}_4$  and  $\text{CO}_2$  levels gradually increased during the first 2 weeks of incubation and reached a peak during the 3rd and 4th weeks. This may be associated with anatomical transition of a normal leaf tissue to callus tissue as suggested in an earlier report (17).  $\text{C}_2\text{H}_4$  reached its peak at approximately the same time as  $\text{CO}_2$  did. The  $\text{C}_2\text{H}_4$  and  $\text{CO}_2$  levels and callus formation in control explants were observed to be correlated, ( $\text{CO}_2$  vs. callus  $r = 0.791$ ,  $\text{C}_2\text{H}_4$  vs. callus  $r = 0.782$ ). The interrelationship of  $\text{C}_2\text{H}_4$  and  $\text{CO}_2$  in climacteric fruits has been proposed as follows: a low level of  $\text{C}_2\text{H}_4$  stimulated respiration, thus providing energy (and  $\text{CO}_2$ ) for further  $\text{C}_2\text{H}_4$  biosynthesis and subsequent onset of climacteric (2). In our work, the higher the  $\text{C}_2\text{H}_4$  and  $\text{CO}_2$  levels, the greater the amount of callus observed. The high levels of  $\text{C}_2\text{H}_4$  and  $\text{CO}_2$  could be the result of anatomical and physiological changes during the transition stage of organized tissue to callus tissue similar to *Ruta* and *Rose* cell suspension cultures (9) or they could be the cause of callus formation as suggested in cotton ovule culture (7).

Relative respiration rate ( $\text{CO}_2$ ) and  $\text{C}_2\text{H}_4$  biosynthesis ( $\text{C}_2\text{H}_4$ ) appeared to be photoperiod responses, since explants taken from SD stock plants produced relatively high levels of  $\text{C}_2\text{H}_4$  and  $\text{CO}_2$ , while explants taken from LD stock plants produced relatively low levels. Photoperiod effects were probably a phytochrome response rather than photosynthetic, because

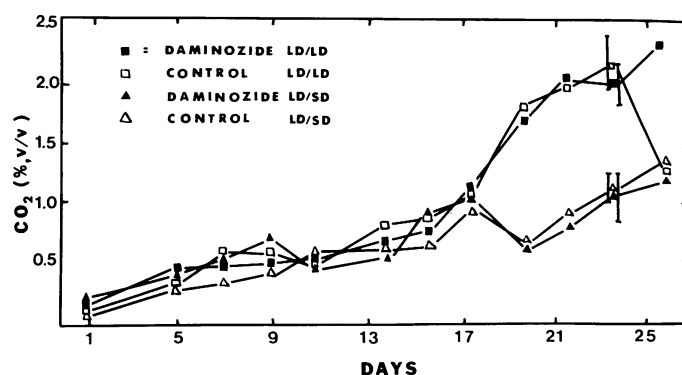


Fig. 5. Effect of incubation, daminozide and photoperiod on  $\text{CO}_2$  levels in flask atmospheres of dahlia leaf cultures from LD stock plants. Each point represents a mean of 4 replications,  $\phi$  represents a 95% confidence interval.

SD or LD stock plant conditions interacted with SD or LD incubation conditions in relative rate of  $\text{C}_2\text{H}_4$  and  $\text{CO}_2$  production from leaf explants, even though they were cultured on a medium containing adequate sucrose. Biran and Halevy (1) have reported that SD treatment of dahlia plants promoted  $\text{C}_2\text{H}_4$  evolution compared to LD treatment. A peak occurred after 2 weeks of SD treatment. In our experiments, leaves responded to SD by producing  $\text{C}_2\text{H}_4$  and it is known that leaves are the site of phytochrome activity.  $\text{C}_2\text{H}_4$  evolution from leaves may be associated with a hormone balance (1) which causes growth cessation (1) followed by a tuberous root formation (1, 9, 15, 16). To further evaluate the phytochrome control of  $\text{C}_2\text{H}_4$  and  $\text{CO}_2$  production hypothesis, experiments utilizing night interruption and red-far red light treatments should be explored.

The increased ethylene levels caused by 2500 mg/liter daminozide in these experiments is similar to results reported by Jindal et al. (8), who found that daminozide promoted ethylene production in apple shoots. Apple shoot growth was suppressed in the year of application and the year following, and was associated with ethylene production. Even though daminozide inhibits shoot growth, it has been proposed to interfere with auxin synthesis (18) or auxin transport (12); and it has been suggested that its effect may be exerted through increased ethylene production (8, 11).

It is clear that stock plant manipulation can modify relative rate of  $\text{C}_2\text{H}_4$  and  $\text{CO}_2$  emanation, thus resulting in profound physiological responses of tissues in cultures. We therefore suggest that culture failures may be overcome or partially overcome by stock plant treatments.

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