Elevated Temperature Affects Auxillary Meristem Development in *Dendranthema ×grandiflorum* ‘Improved Mefo’

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Abstract. Effects of heat stress on viable and nonviable auxillary meristem development and subsequent lateral branching in ‘Improved Mefo’ chrysanthemum (*Dendranthema ×grandiflorum* Ramat. (Kitamura)) were studied. Plants grown at 33 °C day/27 °C night produced more nonviable buds than did plants grown at 23 °C day/18 °C night. A negative linear relationship \( y = 28.7 + [-0.66 (x \text{ days})], r^2 = 0.70 \) between timing of exposure to high temperatures and the number of nonviable buds was observed. Histological examination 28 days after exposure to 33 °C/27 °C revealed that plants showed both normal and abnormal bud development. Abnormal bud development occurred as a consequence of premature differentiation of auxillary meristematic tissue into nonmeristematic parenchyma tissue immediately after separation of auxillary from apical meristems.

Materials and Methods

In all experiments, rooted cuttings of ‘Improved Mefo’ chrysanthemum (Yoder Brothers Inc., Alva, Fla.) were planted in Vergro Klavix mix (Verlite Co., Tampa, Fla.); container size varied with the experiment. All plants were grown in air-conditioned glasshouses with temperature controlled at either 23 °C day/18 °C night (23/18 °C), or 33 °C day/27 °C night (33/27 °C). Relative humidity for all experiments was between 55% and 65% at 23/18 °C, and 70% to 80% at 33/27 °C. All temperature and RH readings were measured using an encapsulated thermocouple temperature and humidity sensor in an aspirated housing (Q-Com Corp., Irvine, Calif.). Long-day (14-h) photoperiod was maintained during all studies. Unless otherwise stated, plants were fertilized at each watering with a water-soluble fertilizer (20N–4.7P–16.6K) at a rate of 300 mg·L–1 N. For the July, August, September, and October experiments, average daily maximum PPF was 880, 830, 840, and 850 µmol·m –2 ·s –1, respectively. For the February, March, and May experiments, average daily maximum PPF was 915, 920, and 890 µmol·m –2 ·s –1, respectively.

Experiment 1. Effects of temperature on lateral bud viability. Rooted cuttings were potted in 20.7 × 18 cm (3.45-L) containers and were placed in the 23/18 °C greenhouse. Plants were soft-pinched at 14 d after planting, removing the apical meristem and all leaves < 1 cm². At that time, 40 plants either remained at 23/18 °C or were moved to 33/27 °C. Plants were spaced four per m², and temperatures were maintained for the duration of the experiment. For the next 8 weeks, all new growth was pinched weekly when emerging shoots had produced two nodes of growth above the preceding pinch. For a given shoot, this took ≈ 10 to 12 d. All pinches were done with razor blades when the third internode was sufficiently developed to allow for its removal without damage to the subtending bud. The area of leaf below the pinch was 3 to 5 cm², and was still expanding.

Number of viable and nonviable buds (auxillary meristems) per plant was determined at the end of the 8-week period. Viable buds were defined as those in which both growth and elongation occurred, and nonviable buds were defined as those that exhibited no visible signs of cell division, or if cell division did occur, it was in the form of hypertrophy, or disorganized production of parenchymatous cell masses. A completely randomized block design was used. Temperatures were randomly assigned among four temperature-controlled

Commercial growers of poinsettia [*Euphorbia pulcherrima* Willd. ex. Klotsch] and chrysanthemum from Canada to the southern regions of the United States have reported the problem of lateral bud loss (Barrett, personal communication). The loss of auxillary branching potential appears in the summer and fall production seasons and is generally attributed to high temperature and relative humidity. This combination of factors may reduce branching by limiting plant cooling and therefore biochemical processes. Considerable research has been devoted to the effects of temperature on other aspects of growth and development in chrysanthemum, such as stem and leaf development (Cockshull, 1988; Erwin and Heins, 1995). Karlsson et al. (1989) found a direct correlation between the rate of leaf unfolding in chrysanthemum and increases in day or night temperature between 10 and 30 °C. Additional research showed that increases in photosynthetic photon flux (PPF) augmented dry-matter accumulation when plants were grown between 10 and 30 °C (Karlsson and Heins, 1992).

Moe (1988) reported a direct relationship between temperature during stock plant production and subsequent growth and overall quality of rooted cuttings of poinsettia and chrysanthemum. Faust and Heins (1992) reported that night temperatures of 14 to 27 °C had no effect on the number of cuttings produced by stock plants of ‘Powerhouse’ chrysanthemum when day temperature was maintained at 35 °C.

Schoellhorn et al. (1996) reported that supraoptimal temperatures reduced lateral branching in heat-sensitive chrysanthemum cultivars, and that cut-flower cultivars generally produced fewer branches after pinch than did potted-plant cultivars. Most cultivars exhibited more branches in the spring than in the fall. The standard and spray-type cut-flower cultivars were more sensitive to stock plant environment and season of production than were the flowering pot cultivars studied. ‘Improved Mefo’ is a single-stem standard cultivar used in the production of cut-flower chrysanthemums. As such, branching is not a desirable characteristic in the production phase; the problem of reduced branching is more of a stock plant issue with this cultivar.

Reduced branching at elevated temperatures reduces not only rooting and branching potential of cuttings, but also cuttings per stock plant as well. The problem is prevalent in warmer climates, such as the southeastern United States, where high summer and fall temperatures, coupled with high relative humidity (RH), make it difficult for growers to keep production temperatures within optimal ranges. The problem of reduced lateral branching at supraoptimal temperatures has also been reported in poinsettia (Faust and Heins, 1996), peach (*Prunus persica* (L.) Batsch.) (Boonprakob and Byrne, 1996), impatiens (*Impatiens walleriana* Hook. F.), and fibrous begonia (*Begonia × semperflorens-cultorum* hybrids), and some hybrid cultivars of azalea (*Rhododendron L.*) (personal observation).

The physiological processes involved in this loss of bud viability are not clear, although high temperature seems to be a key environmental factor. This research was designed to explore high temperature responses during lateral bud development, and the timing of exposure to elevated temperatures required to affect bud development and viability in chrysanthemum.

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greenhouses, and were replicated three times, beginning on 14 Sept. 1994, 20 Oct. 1994, and 15 Mar. 1995. Data were analyzed using analysis of variance (ANOVA). Data for number of nonviable buds were analyzed using the total bud count of each stock plant as a covariant.

Experiment 2. Effect of timing of high temperature on lateral-bud viability. Rooted cuttings were planted in 10.5-cm (0.64-L) pots and placed in the greenhouse. Slow-release fertilizer (14N–6.2P–11.6K) 70-d formulation was incorporated at 603 g m\(^{-2}\) into the growing medium at planting, and plants received a liquid feeding of water-soluble fertilizer (20N–4.7P–16.6K) at 300 mg L\(^{-1}\) N once a week throughout the experiment.

After potting, plants were grown at 23/18 °C for 2 weeks and soft-pinched. The resulting lateral shoots were allowed to develop to the stage of two mature (area = 5 cm\(^{2}\)) leaves, which required ≈7 to 10 d, and then were soft-pinched. A control treatment remained at 23/18 °C for the duration of the experiment. Additional plants were moved from 23/18 °C to 33/27 °C at 0, 1, 2, 4, or 8 d after the second pinch. All but the uppermost emerging lateral shoots were removed 1 week later. This remaining lateral shoot was allowed to develop 10 leaves and then received a third, and final, soft pinch above the 10th node. Viable and nonviable bud numbers were determined 6 weeks after the final pinch.

The experiment was replicated three times, beginning on 8 Feb., 2 May, and 30 Aug. 1995. The experiment was a randomized block design with 50 plants per treatment. Data from the three experiments were pooled and analyzed using ANOVA.

Experiment 3. Anatomical studies. Plants were planted and fertilized as described in Expt. 2, and grown in an air-conditioned glasshouse at 23/18 °C. After 14 d, all plants were given a soft pinch. At that time 24 plants were left at 23/18 °C, and 24 were moved to a second glasshouse at 33/27 °C. Final spacing was four plants per m\(^{2}\). Developing laterals were removed and only the uppermost lateral remained on each plant.

Shoot apical meristems and 1.5 cm of the subtending stem were removed from the single stem of each plant 4 weeks later, fixed in formalin–acetic acid–alcohol (FAA), dehydrated in a tertiary-butyl alcohol series and embedded in paraffin. Embedded tissue was then sectioned at 10 μm, in serial transverse sections, with a rotary microtome (Spencer Lens, Buffalo, N.Y.). Sections were stained using either safranin-fast green or toluidine blue, viewed with a Nikon Optipol research microscope (Nikon, Tokyo), and photographed with an automatic Nikon UFX-II camera attachment.

Twenty shoot apices from plants in each temperature regime were sectioned and compared for lateral bud development and growth. In serial transverse sectioning of the shoot meristem, the number of leaf primordia was calculated by counting from the youngest leaf primordium emerging from the shoot meristem, in sequential order of development, to the oldest leaf primordium on a given section. Lateral bud development was referenced to leaf primordium number.

The experiment was conducted three times, beginning on 14 Sept. 1994, 2 May 1995, and 28 July 1995.

Results

Experiment 1. Effects of temperature on lateral bud viability. Branching potential in Improved Mefo was temperature dependent (data not shown). Plants grown continuously at 23/18 °C had an average of 20% nonviable buds, whereas those transferred to 33/27 °C had 56% (P ≤ 0.0001). Thus, transfer to 33/27 °C reduced production of viable buds.

Experiment 2. Effect of timing of high temperature on lateral-bud viability. Both temperature and timing of exposure to 33/27 °C had significant effects on the number of nonviable buds produced. In plants grown continuously at 23/18 °C, nonviable buds were 10% of the total buds per plant, whereas those moved from 23/18 °C to 33/27 °C after pinch developed 58%, 54%, 36%, and 20%, respectively (Fig. 1). The number of nonviable buds declined linearly as time of exposure to 33/27 °C after pinch increased (r\(^2\) = 0.70, P ≤ 0.0001). Timing of exposure to 33/27 °C had less effect on the number of already actively growing shoots per plant than did it on the number of quiescent and nonviable buds. Plants exposed to 33/27 °C after pinch produced 1–2 actively growing shoots, while plants exposed at a later date produced 3–4.

Experiment 3. Anatomical studies. Plants at 23/18 °C exhibited a normal sequence of bud development (Fig. 2A) as defined in the following manner. Residual meristems were visible by leaf primordium position three. The shell zone, which delineates the residual meristem from the apical meristem, became clearly defined at positions four to six (Fig. 2C), and had formed both tunica and corpus zones by positions seven to 10. The initial pair of unfolding leaf primordia was distinct from the surrounding tissue by positions 10 to 12. In plants grown at 23/18 °C this entire process occurred within 2 to 4 d. Plants grown at 23/18 °C formed viable buds at each node in each of the meristems sectioned, while those grown at 33/27 °C exhibited a variety of viable and nonviable bud formations. At 33/27 °C, buds occasionally were found that developed in the same manner as those at 23/18 °C, but the majority of residual meristems did not.

Time of separation of a residual meristem from the shoot meristem was more difficult to determine at the higher temperature because of the lack of a defined shell zone within the shoot meristem. Beginning at leaf primordium position three, the planes of cell division in plants grown at 33/27 °C were not as clear as those in plants grown at 23/18 °C, and by positions four to six (Fig. 2B), the shell zone was poorly defined and frequently absent (Fig. 2D), although a cluster of darker staining meristematic cells could still be seen in some sections.

By leaf primordium positions 10 to 12, when the axillary meristems of plants grown at 23/18 °C exhibited a well-developed tunica and corpus, the only indications of axillary meristems in plants grown at 33/27 °C were slight differences in cell arrangement within the cortex.

Lateral buds, which were visible (≥ 1 mm) at the time of exposure to 33/27 °C, appear to retain viability regardless of their proximity to the apical meristem (visual observations by author). Referencing of lateral bud development to leaf primordium number, or position in relation to the shoot meristem, showed that there was no viable bud present in chrysanthemum Improved Mefo exposed to high temperatures by the time the subtending leaf pri-

![Fig. 1. Effect of temperature on the percentage of nonviable buds produced on Improved Mefo chrysanthemum stems pinched at 10 nodes. Plants were grown continuously at 23/18 °C (control), or moved to 33/27 °C at 0, 1, 2, 4, and 8 d after pinching. Plants moved on day 0 were exposed to 33/27 °C 8 d earlier than were plants moved on day 8. Response to temperature duration was linear (y = 28.7 + [–0.66 (x days)], r\(^2\) = 0.70).](image_url)
mordium had reached position six from the shoot meristem. Anatomical study revealed disorganized development of residual meristem cells at and prior to leaf primordium position four, coupled with early differentiation of the meristematic cells; this resulted in a loss of meristematic potential.

**Discussion**

This research provides additional support for the concept of inhibition of bud development at supraoptimal temperatures. Plants produced more nonviable buds at 33/27 °C than at 23/18 °C. These results agree with those of Faust and Heins (1992), who reported ‘Powerhouse’ chrysanthemum formed progressively fewer shoots when grown at 35 °C day temperatures; however, Faust and Heins concluded that night temperature had no effect on the viability of lateral buds.

Faust and Heins (1996) reported heat-induced loss of viable buds in ‘Eckespoint Lilo’ and ‘Eckespoint Red Sails’ poinsettia, and reduced branching of rooted cuttings when stock plants were grown at temperatures above 27 °C. Stock plants were more variable in response to temperature than were the cuttings taken from them, perhaps because the number of axillary meristems on rooted cuttings is finite.

The anatomical measurement techniques we used were similar to those used by Popham and Chan (1950). The apical dome of ‘Improved Mefo’ meristems varied little in size or volume at 23/18 and 33/27 °C, which is in agreement with the work of Horridge and Cockshull (1979). Manual dissection of developing ‘Improved Mefo’ shoots showed that the meristem was surrounded by up to nine leaf primordia, in agreement with earlier work by Berg (1970) with ‘Improved Albatross’. Axillary meristems in ‘Improved Mefo’ develop originally as part of the apical meristem. As the subtending leaf moves away from the apical dome, an interceding layer of vacuolated cells separates the axillary meristematic tissue from the apical meristem, as reported by Esau (1960). The shell zone, which was used in this work as a marker for viable bud development, results from cell division around the separated axillary meristem. Without a shell zone there is no defined meristematic area within the leaf axil.

The only mechanism for bud loss seen in this study was differentiation of meristematic cells into parenchymatous tissue. Kundu and Rao (1954) studied a freely branching form and a nonbranching form of jute (*Corchorus capsularis* L.) and reported that the residual meristem in the latter apparently vacuolated before the tunica and corpus developed. Kundu and Rao’s use of the term vacuolate, or vacuolation, has no anatomical reference, but we assumed that they were referring to differentiation of the meristematic cells into parenchymatous cortical cells. Such premature differentiation would result in the loss of the meristematic potential of the bud as meristematic cells differentiate into parenchyma cells. The nonbranching characteristic was not dependent upon environmental stimuli, but was characteristic of a distinct genetic line.

In our study, time of exposure to high

![Fig. 2. Residual meristem development at leaf primordium position 6 showing development of shell zone in plants grown at (A) 23/18 °C and (B) 33/27 °C. (Magnification is 10×). Arrows indicate location of axillary meristematic cells. At 40× magnification, plants grown at (C) 23/18 °C exhibit a dense mass of meristematic cells, with prominent nuclei. The shell zone is distinguishable by the frequency and plane of cell division (indicated by arrow). In plants grown at (D) 33/27 °C, the axillary meristem has differentiated into parenchymatous cell masses with a smaller ratio of nuclei to total cell volume and the shell zone is no longer visible, with reduced cell divisions occurring and no distinguishable plane of cell division.](image)
temperatures after pinch was negatively correlated with the number of nonviable buds produced in chrysanthemum ‘Improved Mefo’. More nonviable buds occurred in plants exposed to 33/27 °C at pinch and immediately after than on plants moved to high temperatures at subsequently later dates. This work demonstrates that nonviable buds were produced as a result of changes in normal bud development beginning at separation from the apical meristem, rather than a later suppression of bud growth.

**Literature Cited**


