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NAA-induced Leaf Epinasty in Chrysanthemum

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Abstract. **Leaves of 'Mountain Snow' chrysanthemums (*Chrysanthemum morifolium* Ramat.), sprayed with 10 mM NAA or 10 mM NAAEE, exhibited severe epinasty after 24 hr, while leaves sprayed with 5 mM ethephon did not. Treatment with 100 μM AOA 24 hr before application reduced ethylene production rate of leaves, but not epinasty. Localized application of NAA to adaxial, abaxial, or both leaf surfaces resulted in similar amounts of leaf epinasty. Epinastic leaves had enlarged adaxial epidermal cells. Size of abaxial epidermal cells was unchanged. This study provides evidence that leaf epinasty of chrysanthemum following NAA application is not the result of auxin-induced ethylene production. Chemical names used: (aminooxy)acetic acid (AOA); 1-naphthaleneacetic acid (NAA); 1-naphthaleneacetic acid ethyl ester (NAAEE); and (2-chloroethyl)phosphonic acid (ethephon).**

NAA and NAAEE are effective in reducing the number and size of axillary flower buds of chrysanthemum by restoring apical dominance (3). NAA and NAAEE sprays, however, induced severe epinasty of leaves after 3 days (3). Ethylene involvement in auxin-induced epinasty was shown by Amrhein and Schneebeck (2), who reported that NAA-induced epinasty of tomato plants was accompanied by increased ethylene production and that application of AOA with NAA inhibited ethylene production and prevented epinasty. Reduction of epinasty and ethylene production also occurred in mechanically stressed poinsettia plants that were treated with AOA and [*S*-(E)]-2-amino-4-(2-aminoethoxy)-3-butanoic acid (AVG) (9). It has been suggested that epinasty of leaves is associated with the redistribution of auxin by ethylene or by the interference of auxin transport (5). In contrast, Reid et al. (7) suggested that ethylene was not responsible for the distribution of auxin during the reorientation of poinsettia petioles, and they concluded that the redistribution was simply in response to orientation itself.

The purpose of this study was to determine whether NAA- or NAAEE-induced leaf epinasty in chrysanthemums is mediated by ethylene.

'Mountain Snow' cuttings (four per pot) were planted in 20-cm-diameter standard plastic pots containing a 3 peat : 1 vermiculite : 1 perlite mix (by volume) and rooted under intermittent mist and continuous light for 7 days (10). Plants were then placed on benches in an unshaded fiberglass greenhouse and fertilized daily with 200N–50P–250K (ppm) through the irrigation system. Plants were pinched 10 days after planting.

Plants were treated 17 days after planting with foliar sprays of distilled water and 10 or 100 μM AOA. Twenty-four hours later, 10 mM NAA, 10 mM NAAEE, or 5 mM ethephon was applied to the distilled water-pretreated plants; NAA (10 mM) was also applied to the AOA-pretreated plants. NAA and NAAEE concentrations are expressed as acid equivalents of the parent acid. Spray volume was 4 ml/plant and was applied with a hand-operated chromatography sprayer (Sigma). Severity of leaf epinasty was determined 24 hr after NAA, NAAEE, or ethephon treatment by visual ratings. The control ratings were fixed at 1.0 (no epinasty) and the other treatments were rated against the control up to 5.0 (severe epinasty). Each treatment consisted of four replicated pots arranged in a randomized complete block design.

Three days after the NAA, NAAEE, and ethephon treatments, the most recently matured leaf was excised at the base of the petiole and enclosed in 25-ml (1.5 × 15.0 cm) glass tube. Each tube contained 3 g of potassium hydroxide (KOH) and a 1-cm layer of loosely packed spun woolfiber between the leaf blade and KOH. Tubes were stoppered with rubber septums and placed under 40-W cool-white fluorescent lights at 22°C. After 1 hr, 1.0-ml gas samples were removed from the tubes and analyzed for ethylene by gas chromotography (9). Each treatment consisted of 10 replicate tubes arranged in a completely randomized design.

NAA (10 mM) solution was applied with a cotton swab to the abaxial and/or adaxial surface(s) of a recently matured leaf. The leaf at the node above or below the NAA-treated leaf was swabbed with distilled water and used as the control. Leaf epinasty was measured after 48 hr with a protractor measuring the abaxial angle formed by the stem and leaf. The experiment was arranged in a randomized complete block design using eight pots per treatment. The experiment was con-

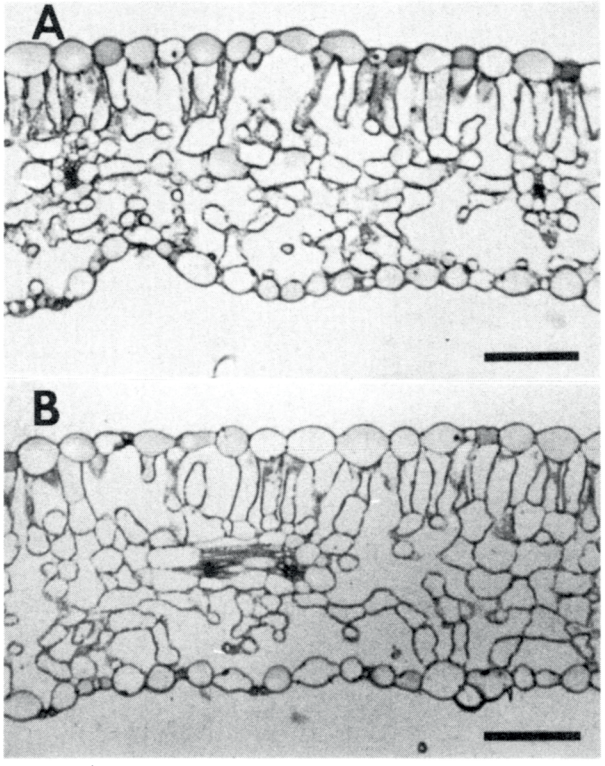

Fig. 1. Cross sections of untreated (**A**) and adaxial and abaxial treated (**B**) chrysanthemum leaves with 10 mM NAA. Bar = 120 μM.

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Table 1. The influence of various chemical treatments on leaf epinasty and ethylene production from leaves of 'Mountain Snow' chrysanthemums.

Treatments	Concn	Rating of leaf epinasty[x]	Ethylene production ($nl \cdot g^{-1} \cdot hr^{-1}$)
		Time after spray treatment	
		24 hr	25–26 hr
No pretreatment with AOA			
Ethephon	5 mM	1.2 c[y]	65.1 a[z]
NAAEE	10 mM	2.5 b	7.9 c
NAA	10 mM	3.5 ab	16.0 b
Control		1.0	2.8 c
Pretreatment with AOA 24 hr before 10 mM NAA application			
AOA	10 μM	4.0 a	30.3 b
	100 μM	3.9 a	3.9 c

[x]Rating value for controls were fixed at 1.0 and the other treatments were rated against the control with the highest value being 5.0, severe epinasty.
[y]Mean separation in columns by Duncan's multiple range test, 5% level.
[z]Statistical analysis conducted with square root transformation. Control was not included in the analysis since epinasty ratings for control were fixed to 1.0.

Table 2. Effect of localized application of NAA on leaf epinasty of potted 'Mountain Snow' chrysanthemums 48 hr after treatment.

Treatment	Leaf epinasty[x] (degree)
Adaxial surface	
Control	133.0 a[y]
NAA (10 mM)	49.5 b
Abaxial surface	
Control	118.5 a
NAA (10 mM)	51.0 b
Both surfaces	
Control	125.0 a
NAA (10 mM)	57.5 b

[x]Values were obtained by protractor measurement of the abaxial angle formed by the stem and the leaf.
[y]Mean separation in columns by Duncan's multiple range test, 5% level.

ducted twice. Since the results from both experiments were similiar, the data were pooled before statistical analysis.

Leaf tissues, 1 cm distal from the base of the leaf blade, were fixed in 4% glutaraldehyde for 2 days, dehydrated using the normal acetone series, and imbedded in resin. One micrometer cross-section 2 to 3 mm to the side of the midvein were made with an ultra-microtome, stained with Toluidine Blue O (8), and photographed using light microscopy.

Effect of spray treatments on leaf epinasty. Leaf epinasty was not evident at 6 hr after treatment with NAA or NAAEE. After 24 hr, epinasty ratings were greatest (3.5) in the NAA-treated plants, followed by NAAEE (2.5) and by ethephon (1.2), which did not display epinasty in comparison to the control (Table 1). Pretreatment with 10 and 100 μM AOA did not reduce epinasty of plants subsequently treated with 10 mM NAA. Epinasty ratings of plants pretreated with 10 and 100 μM AOA did not differ and were similiar to NAA-treated plants that received no AOA.

Ethylene production by leaves. Highest ethylene production rate occurred following ethephon treatment (Table 1). Ethylene production rate (16.0 $nl \cdot g^{-1} \cdot hr^{-1}$) from NAA-treated leaves was significantly greater than NAAEE (7.9 $nl \cdot g^{-1} \cdot hr^{-1}$) and control treatments (2.8 $nl \cdot g^{-1} \cdot hr^{-1}$). There was no difference in ethylene production rate between NAAEE and control. NAA-treated leaves that were pretreated with 10 μM AOA had significantly more ethylene production than the controls. However, pretreatment with 100 μM AOA reduced ethylene production to control levels.

Effect of adaxial and abaxial application of NAA on leaf epinasty and anatomy. NAA application to abaxial, adaxial, and both leaf surfaces resulted in similiar leaf angles and significantly smaller leaf angles compared to control plants (Table 2). NAA-treated leaves had enlarged adaxial cells (Fig. 1). The size of the abaxial epidermal cells was unchanged.

Palmer (6) showed that an increase in mean elongation rate of the upper half of *Helianthus* petioles occurred during epinasty and speculated that the cellular basis of epinasty was increased cell expansion in this region. Our results agree with this conclusion, since epinasty of chrysanthemum leaves was associated with enlargement of epidermal cells on the adaxial surface (Fig. 1).

A redistribution of auxin into the convex halves of epinastic leaves has been shown (5) and may have accounted for the increased growth along the upper surface of chrysanthemum leaves. Changes in auxin distribution have been attributed to increased ethylene concentration (4, 5). Our results show that the epinasty response to NAA can occur independent of ethylene production, since AOA, an inhibitor of ethylene synthesis (1), reduced ethylene production but did not reduce epinasty. Also, leaf epinasty of chrysanthemum was not evident after ethephon treatment, although substantial levels of ethylene were evolved (Table 1). The nonresponse was unexpected, since epinasty of leaves after exposure to ethylene has been reported by several workers (2, 5–7). Apparently, chrysanthemums are more responsive to auxin than to ethylene. Thus, it appears that ethylene production may not be necessary to achieve a different response in the upper and lower surface of the leaf after treatment.

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