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Morphological Differences in *Neoregelia* Trichomes and Uptake of Foliar-applied Copper

B.D. Fisher[1], E.R. Emino[2], and G.J. Gogue[3]

Department of Horticultural Sciences, Texas A&M University, College Station, TX 77843

Additional index words. bromeliad, leaf surface, foliar absorption, scanning electron microscopy

Abstract. **Four bromeliad species in the genus *Neoregelia* were studied by scanning electron microscopy (SEM) for morphological characteristics and density of trichomes that may influence absorptive capacities. Neoregelia trichomes were classically peltate, generally elliptic, exhibiting a web-like cap characteristic of the Bromelioideae. Minor variations were evident between species in morphology, density, and distribution. Copper from copper sulfate applied to leaf surfaces was absorbed and translocated as determined by atomic absorption spectrometry, but uptake was not related to trichome density as determined by SEM nondispersice technique.**

Considerable interest has been expressed in absorption by Bromeliaceae trichomes (3, 7, 9, 12). Studies (1, 2) have shown that trichomes of the subfamily Tillandsioideae play an active role in foliar absorption of water and solutes. Rapid absorption of acridine orange by trichomes has been shown with fluorescence microscopy (4). Further work (6, 10) suggested that penetration was greater through trichomes than through adjacent epidermal cells. Absorption by Tillandsioideae trichomes has been established; however, the role of trichomes in foliar absorption by the Bromelioideae, and more specifically the *Neoregalia* species has not been studied.

The objectives of this study were to characterize trichomes of 4 species of *Neoregelia* as to morphology, density, and distribution, and to investigate the relationship of these characteristics to absorption of foliar-applied copper.

Materials and Methods

Morphological and absorptive studies were conducted on 4 *Neoregelia* species: *N. marcon* L.B. Sm; *N. mcwilliamsii*, L.B. Sm.; *N. carolinea* 'Meyenforffii' (Vell) L.B. Sm.; and *N. compacta* (Mez) L.B. Sm. Mature specimens of each species were maintained at 21°C in a greenhouse under standard cultural conditions for several months prior to analysis.

Trichome morphology and distribution. Morphological characteristics and density of trichomes on the adaxial leaf surface of the 4 *Neoregelia* species were studied by scanning electron microscopy (SEM). Fully expanded mature leaf samples of each species were taken to obtain trichome density and distribution on the adaxial leaf surface. Additional micrographs provided detailed morphological characteristics of individual trichomes.

Five 1-cm^2 tissue samples from the distal and proximal regions of the adaxial leaf surface were used and prepared for the SEM by critical-point drying (8), then observed in a JEOL JMS-35 SEM at an accelerating voltage of 25kV.

The distal and proximal portions of the adaxial surface of excised leaves of *N. marcon*, which contain a high number of trichomes per unit area, and *N. compacta*, which contains a low number of trichomes per unit area, were treated with a 5 ml, pH 4.5 solution of 1000 mg/liter $CuSO_4$ in a randomized block design for 0, 1, 12, and 24 hr. The solution was placed in 2.5 cm diameter vials held in place on the leaf with lanolin. The central portion of each leaf was left unexposed, as the tissue was later analyzed for the presence of translocated copper.

After exposure to the $CuSO_4$ solution, five 1-cm^2 tissue samples were excised from distal and proximal regions of each leaf. Samples then were washed twice with distilled deionized water, prepared by acid digestion, and subsequently analyzed for copper by atomic absorption spectroscopy with a Perkin-Elmer 360 at 324.8 μm and a slit setting of 0.7 μm in an air-acetylene flame.

Further analyses of copper absorption were performed upon *N. marcon* and *N. carolinea* tissue with an SEM nondispersive X-ray attachment. Excised leaves were treated as before, and at 12 hr uniform samples were excised, washed as before, frozen in iospentane, freeze dried (8), and coated with 1Å gold/paladium. Photomicrographs with superimposed line scans were produced with an AMR 1200 SEM with a 5100 X-ray energy spectrometer.

Results and Discussion

Trichome morphology was similar to that previously reported (5, 6, 7, 12). Trichomes of all 4 *Neoregelia* species were peltate. Generally, they were elliptic and exhibited a web-like cap which has been proposed to be involved in solute absorption (12). Previous studies (12) suggested that the open "honeycomb" arrangement of the cap acts to absorb solutions prior to their absorption by the tricome stalk.

Despite this high degree of generic similarity, morphological variations, as determined by SEM analysis, were present in the 4 species (Fig. 1–8). The outer edges of the *N. marcon* trichomes (Fig. 1) were distinct, as were the inner walls, whose integrity was maintained throughout the trichome cap. The trichomes were numerous on the epidermal surface in both the distal and proximal regions (Fig. 5). There was, however, no apparent pattern in their distribution. All of the *N. marcon* trichomes were about the same size and configuration. No stomata were associated with trichomes in this species.

In contrast, *N. mcwilliamsii* trichomes appeared to be closely associated with the stomata (Fig. 6). The nature of the relationship between stomates and trichomes is unknown. Other differences between the trichomes of the 2 species were present. Trichome concentration on the *N. mcwilliamsii* leaf surface was less than that of the *N. marcon*, while trichome arrangement remained random. Individual *N. mcwilliamsii* trichomes had ex-

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[1]Former Graduate Research Assistant. Present address: 123 Cornwall, San Antonio, TX 78216.

[2]Present address: Dept. of Plant Sci., Univ. of Connecticut, Storrs, CT 06268.

[3]Present address: National Park Serv., Southeast Regional Office, 75 Spring Street, SW, Atlanta, GA 30303.

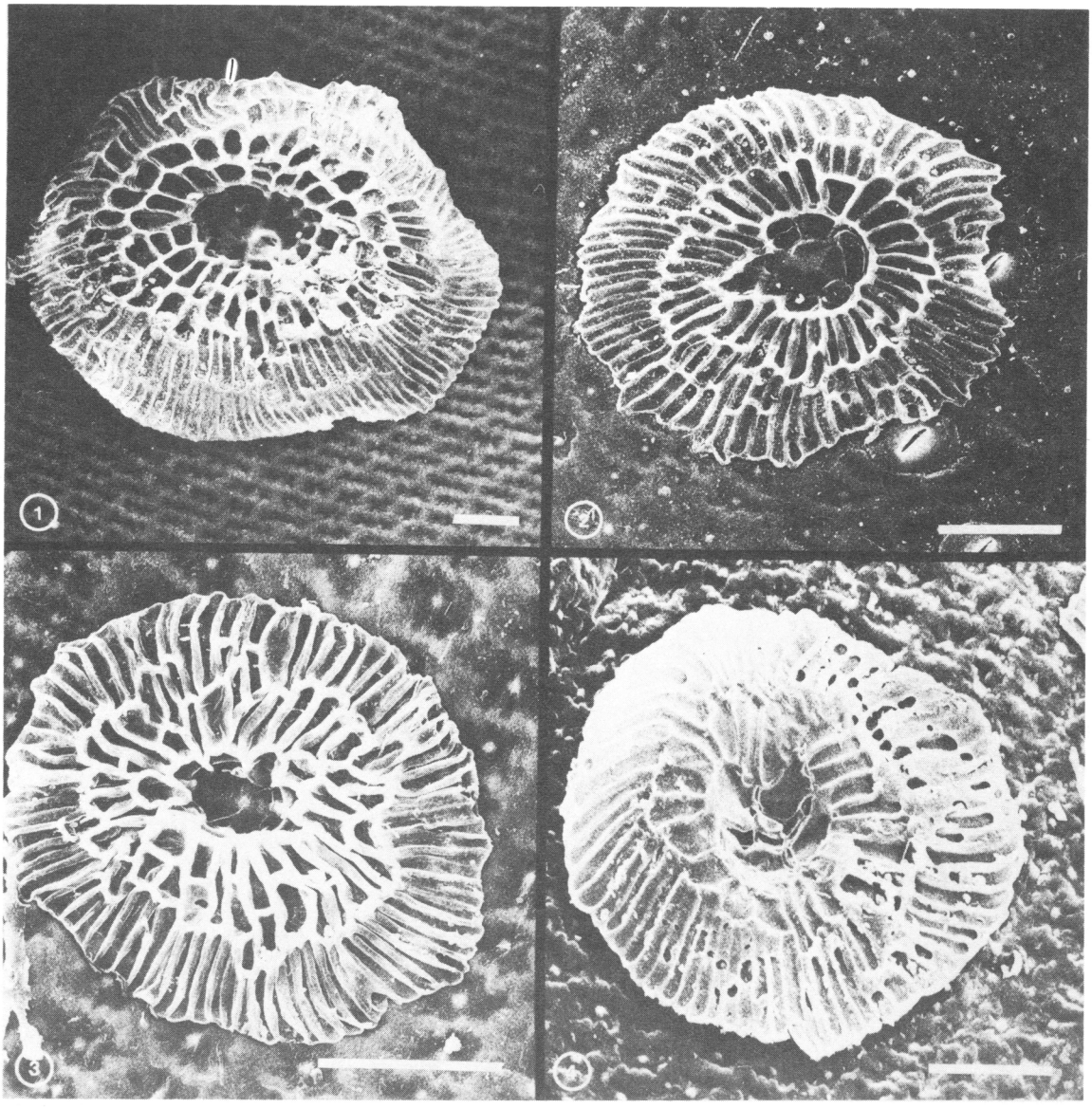

Fig. 1–4.Scanning electron micrographs of Neoregelia peltate trichomes on the adaxial leaf surface showing individual detail: 1. *N. marcon*, 2. *N. mcwilliamsii*, 3. *N. carolinea*, 4. *N. compacta*. White bar represents 50 μm.

tremely irregular margins and a complete, unfragmented inner web-like structure (Fig. 2).

The *N. carolinea* trichomes were characterized by distinct inner walls and slightly undulating margins (Fig. 3). Within the species, trichome distribution was similar in both the distal and proximal regions. This species along with *N. compacta* had fewer total trichomes per unit area than other species. The trichomes were widely spaced, with the stomates closely associated, similar to those of *N. mcwilliamsii* (Fig. 7).

The *N. compacta* trichomes were markedly different from the trichomes of the other 3 species in that the chambers of the web-like cap appeared less distinct (Fig. 4). This species also had fewer trichomes per unit area than *N. marcon* and *N. mcwilliamsii*. As in *N. marcon*, stomata were not associated with *N. compacta* trichomes. Trichomes of this species were arranged in orderly rows, coinciding with leaf veins. (Fig. 8).

Overall, the frequency and distribution of *Neoregelia* trichomes in the proximal and distal regions of the adaxial leaf surfaces showed individual species variation (Table 1). *N. marcon* had the greatest number of trichomes in distal samples whereas *N. mcwilliamsii* had the largest numbers near the base. *N. compacta* had fewest trichomes at both locations compared to the other species.

Both *N. marcon* and *N. carolinea* had significantly more trichomes at the tip than base whereas *N. mcwilliamsii* had significantly more in the base. There were no differences in *N. compacta* between tip and base.

Since trichomes have been implicated in foliar absorption (6, 11) it was speculated that *N. marcon*, with its larger size and number of trichomes, would absorb foliar applied compounds, such as $CuSO_4$, in greater amounts than *N. compacta*.

A SEM nondispersive line scan of each species indicated a higher concentration of copper in the center of trichomes than on foliar surfaces previously exposed to a 1000 mg/liter $CuSO_4$ solution. The same scan showed substantially reduced copper concentrations in those epidermal areas lacking trichomes. Untreated samples did not show evidence of copper in the trichome (Fig. 10).

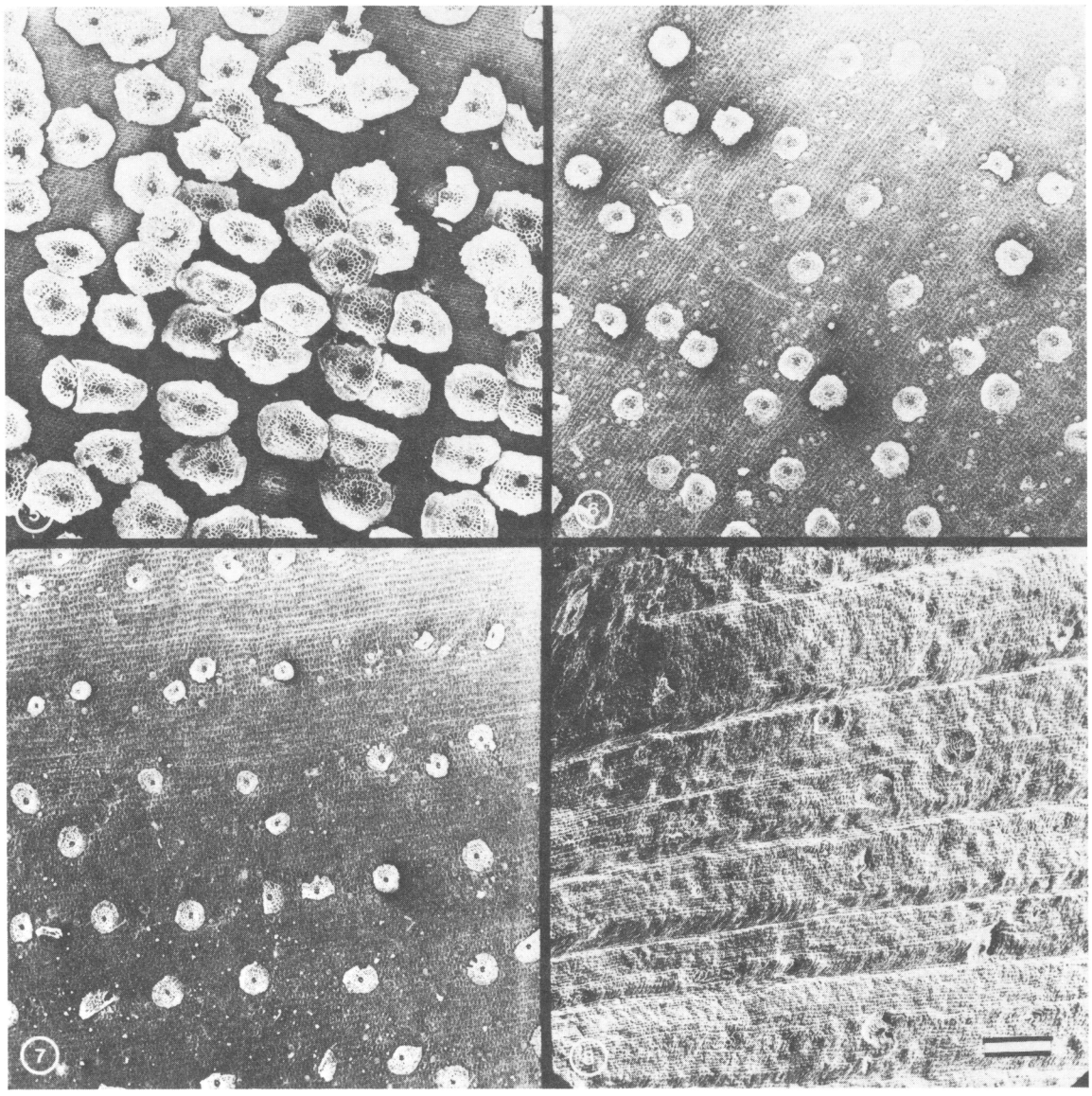

Fig. 5–8.Scanning electron micrographs of Neoregelia leaf adaxial surfaces illustrating relative peltate trichome size and distribution: 5. *N. marcon*, 6. *N. mcwilliamsii*, 7. *N. carolina*, 8. *N. compacata*. White bar represents 250 μm.

Table 1. Peltate trichome distribution in 4 species of *Neoregelia* leaf tips and bases.

Species	No. of trichomes per mm^2	
	Proximal	Distal
N. marcon	83.52 a[z]	54.48 b
N. mcwilliamsii	45.12 b	62.14 a
N. carolinea	44.28 b	40.88 c
N. compacta	22.64 c	22.30 d

[z]Mean separation in columns by Duncan's multiple range test, 5% level.

Both *N. marcon* and *N. compacta*, when exposed to copper and harvested over a range of times, had copper concentrations significantly higher than the control (Table 2). Maximum concentration occurred at 1 hr implicating translocation of copper in the excised leaf. There was a difference between species as well as between sampling locations.

To determine if applied copper was absorbed and then translocated, the experiment was repeated, and an adjacent untreated site was analyzed for copper (Table 3). These data show evidence for translocation of copper from distal and proximal absorption sites to sites in the central leaf region. At all translocation sites, a higher copper concentration was found than in corresponding control tissue. These results suggest that foliarly absorbed copper is readily translocated through the *Neoreglia* leaf tissue.

Incorporation of foliar applied material into epidermal cells via the trichomes and translocation of absorbed materials has been shown in previous studies (2, 7). Dybing and Currier (4) found rapid absorption of acridene orange by trichomes of some species, with subsequent radial spreading into the epidermal cells in the form of a multicolored, circular chromatogram around the base of each trichome.

This study indicates that there is considerable variation in trichome morphology and distribution with the *Neoregelia* spe-

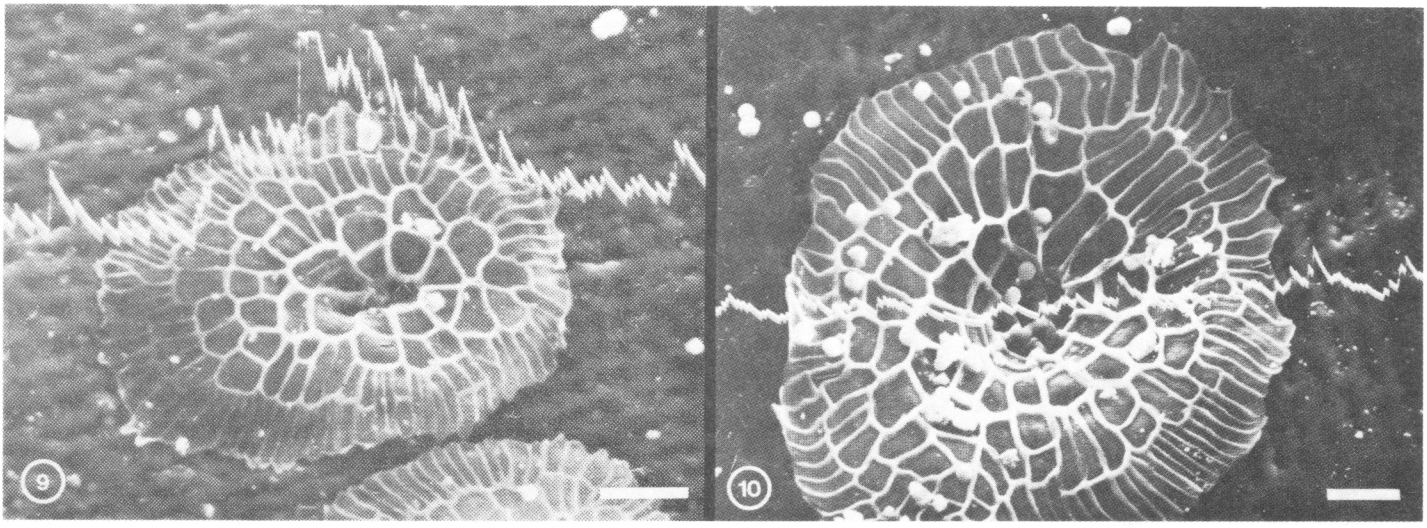

Fig. 9–10. Scanning electron micrograph of *N. marcon* trichomes with a superimposed line scan from a non-dispensive X-ray attachment detecting copper levels associated with treated (9) and nontreated (10) adaxial surfaces. White bar represents 50 μm.

Table 2. Copper concentration in *Neoregelia* species in washed treated areas over 4 exposure periods.

Exposure period (hr)	Copper (ppm)	
	N. marcon	*N. compacta*
0	25.5	44.0
1	175.2	225.4
12	160.0	202.4
24	115.2	168.0
$LSD_{(0.05)}$	52.6	79.1
$LSD_{(0.01)}$	71.7	107.9

cies studied. Also, there were differences in trichome distribution between proximal and distal ends. When treated with $CuSO_4$, trichomes could be identified with higher leaf Cu compared to untreated leaves. However, Cu uptake and translocation could not be related to trichome number and distribution since *N. compacta*, the species with fewest trichomes, had more Cu absorbed and translocated than *N. marcon*.

Although this study does not offer additional evidence that trichomes function in Bromelioideae in solute absorption, it does show leaf surface uptake and translocation as well as indirect evidence of increased copper concentration associated with the trichome. This apparent increased concentration also could result from measuring to a greater depth over a trichome. Benzing et al. (2) suggested that structural variations that distinguish peltate trichomes of Bromelioideae from those of Tillandsioideae may have functional significance in that trichomes described in this study are poorly disposed to provide entry points for moisture and solutes. If the trichomes are not the major point of entry, then other leaf surface features certainly are involved.

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Table 3. Copper concentration at treated and translocated sites of 2 *Neoregelia* species exposed to copper sulfate solution.

Exposure period (hr)	Species and site							
	N. marcon				*N. compacta*			
	Proximal		Distal		Proximal		Distal	
	Treated	Translocated	Treated	Translocated	Treated	Translocated	Treated	Translocated
	Copper conc (ppm)							
0	33.0 a[z]	22.0 a	25.6 a	22.0 a	58.6 a	40.3 a	47.6 a	29.3 a
1	303.3 a	92.0 c	217.6 b	88.0 c	335.0 a	203.3 b	221.6 b	121.1 c
12	251.6 a	151.3 b	181.0 b	58.6 c	270.0 a	170.0 ab	229.3 bc	140.3 c
24	177.6 a	77.0 b	150.6 a	47.6 b	240.3 a	144.0 bc	188.3 ab	99.3 c

[z]Mean separation in rows for each species by Duncan's multiple range test, 5% level. All translocated sites as determined by analysis of variance were significantly higher in Cu concentration than the control (0 exposure).

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Relationship between Rhizome Temperatures and Shoot Temperatures for Floral Initiation and Cut Flower Production of *Alstroemeria* 'Regina'

W.E. Healy[1] and H.F. Wilkins[2]
Department of Horticultural Science and Landscape Architecture, University of Minnesota, 1970 Folwell Avenue, St. Paul, Minnesota 55108

Additional index words. storage roots, photoperiod, number of nodes, soil cooling

Abstract. **When *Alstroemeria* 'Regina' shoots were grown in a continuous 13°C air temperature, and the underground structures (rhizomes and roots) were placed in a 5°, 10°, 15°, 20°, or 25° water bath, plants produced 22%, 33%, 13%, 14%, or 5% generative shoots, respectively (Expt. 1). When the underground structures were grown at 13°, there were no differences in percentages of generative shoots, regardless if shoots were in a 13° or 21° air temperature, and regardless if shoots were under short or long photoperiods. When soil temperature was 21° and air temperature was 13°, 12% generative shoots were produced only with a night interruption photoperiod (Expt. 2). Data from these 2 experiments led us to conclude that floral induction was controlled primarily by temperatures to which the underground structures were subjected, regardless of the air temperature or photoperiod. Storage root and rhizome dry weights were promoted by 13° air, 13° soil temperatures and night interruptions with incandescent light. Treatments which had a high percentage of generative shoots also had high root and rhizome dry weights.**

The exact control mechanisms for *Alstroemeria* 'Regina' flower induction, initiation, and development are not clearly understood (4, 5, 6, 7, 8). Several workers have shown the influence of air temperature on generative alstroemeria shoot induction and subsequent shoot and flower development (3, 5, 6, 7, 9, 10, 14). Work in Norway by Moum and Strömme (13) showed that air temperatures above 21°C reduced the flowering response of *Alstroemeria* 'Regina' and 'Orchid'. Work in Aalsmeer, The Netherlands, by Vonk Noordgraaf, (14) showed that with 'Walter Fleming', [syn. 'Orchid' (1)], an air temperature above 21° in conjunction with short days or with 25° and 16-hr days inhibited flowering. Vonk Noordegraaf further showed that a 25° soil temperature, in combination with a 9°, 17°, or 25° air temperature reduced the number of generative shoots (4).

Light treatments (photoperiodic and photosynthetic) have been reported to hasten generative (flowering) shoot development of alstroemeria (4, 8, 10, 12, 14). Long days, either as a night-interruption or as a day-continuation of 13- to 16-hr, depending on cultivar, accelerated flowering (4, 8, 10, 12). Generative shoot development ceased during high temperature periods and failed to respond to any light treatment (3, 4, 6, 7, 8, 9, 10).

If *Alstroemeria* cultivars are to continue to become an important cut flower crop in commercial floriculture, it is necessary to determine the control mechanism for flower control, the means to promote continuous flowering and ways to control dates of production. Cooper (2) and Wilkins et al. (16) have shown several growth responses to be affected by soil temperatures. Objectives of these experiments were to investigate the interactions of soil and air temperature and photoperiod on generative shoot development and production in *Alstroemeria* 'Regina' plants.

Materials and Methods

Expt. 1. Alstroemeria 'Regina' plants were divided on 1 Aug. into single rhizomes with attached storage roots and vegetative shoots. Each division was planted in a 15-cm plastic pot filled with 1 peat : 1 perlite : 1 soil (by volume) medium and grown in a glasshouse at a minimum day/night 13°C air temperature under natural-day conditions (45° north parallel) until 1 Jan., when plants were shifted into 28-cm plastic pots using the same medium. From 1 Jan. to 29 Dec., soil temperature treatments of 5°, 10°, 15°, 20°, or 25° (± 1°) were maintained by a specially designed waterbath (Fig. 1) by either cooling or heating the water circulating around the pots. Soil temperatures were monitored continuously. The 4 pots per treatment were covered with 2 sheets of 6 mil plastic before immersion to prevent waterlogging of the root system. The air temperature was a constant 13° until the day temperature could no longer be maintained, even with the use of fan and pad cooling. A 6-cm mulch of shredded polystyrene was used to cover the surface of all pots to reduce soil temperature fluctuations. Control plants were grown on an adjacent greenhouse bench at a constant 13° air temperature.

Expt. 2. Individual plants of *Alstroemeria* 'Regina' were divided on 10 Oct., using a similar medium and pot size as in Expt. 1 and were grown in a 21°C air temperature under natural days until 5 Jan., when they were shifted into 28-cm pots and placed into the temperature-controlled water baths as described

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[1]Presently Assistant Professor, Dept. of Hort., Univ. of Maryland, College Park, MD 20742.
[2]Professor.