

# Heat-Induced Flower Abnormalities in Vero and the Marble Cultivars of *Dendranthema grandiflora*

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## ABSTRACT

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The Marble cultivars of the florists' chrysanthemum, *Dendranthema grandiflora*, often develop green bracts and displaced ligulate florets in the center of the inflorescence where normally only tubular florets are produced. These abnormal inflorescences were induced in plants of cultivars Pink Marble, Florida Marble, and the control Vero by exposing them to high (32 C) day temperatures, 6 and 12 days after they had been induced to flower by exposure to a 10-hr photoperiod at a cool (21 C) temperature. Plants remaining at 21 C, or exposed to 27 C day temperatures for 6 or 12 days after the cool-temperature induction, produced normal inflorescences. The normal floral receptacle contains outer ray (ligulate), pistillate florets and central disk (tubular), perfect florets. Abnormal structures produced on the floral receptacle of heat-treated plants included green bracts; displaced ligulate florets, and florets with characteristics of both tubular and ligulate florets. These included tubular florets with elongating corolla lobes and ligulate florets with sterile anthers. Scanning electron microscopy, light microscopy, and transmission electron microscopy revealed that green bracts in disks of abnormal inflorescences were indistinguishable from green bracts (phyllaries) composing inflorescence involucre. Pathogenic agents were not observed in the phloem or other cell types. Marble cultivars appear susceptible to "heat delay," a condition in which inflorescences revert to, or maintain, a more juvenile state when high temperatures occur during inflorescence development.

The Marble cultivars of florists' chrysanthemum, *Dendranthema grandiflora* (Tzveler), produce abnormal inflorescences that have been attributed to the presence of a mycoplasma-like organism (MLO) (3,7). Symptoms of this reported disease include formation of green structures among disk florets that resemble those observed in other chrysanthemum cultivars exposed to high temperatures during flower initiation (9,10).

High day and night temperatures in a greenhouse cause irregular budding and delayed flowering of chrysanthemums (10). Green disk bracts and undeveloped florets may form in the flower buds of plants exposed to high temperature (10). This observation, made many years ago, indicated that high temperatures can counteract the influence of short days on floral development, a condition called "heat delay." Although low temperatures may prevent bract development, temperatures that are too cool may cause "quilling," a condition in which the desired ratio of tubular to ligulate florets is disrupted (6). Among the symptoms associated with heat delay are green bract formation in the center of the inflorescence and increased

petalage. In recent studies, high temperatures appeared to delay the transition of the meristem to the reproductive state

(14). When buds formed, they were composed mostly of bracts with development of only the outer row of florets.

Symptoms associated with the MLO disease, chrysanthemum phloem necrosis (CPN), include reduced flower size, abnormal and (or) partial petal formation in flower heads and chlorophyllous disk florets with no stamens or pistils. Although symptoms of CPN were found to vary with temperature, the entire syndrome develops on plants grown at both 24 and 32 C (3).

In preliminary investigations, we found that the Marble cultivars are heat sensitive, developing green disk bracts when flowered at high temperatures (5). In this report, we compare anatomical features and cellular content of normal and abnormal inflorescences formed on plants grown at low and high temperatures.

## MATERIALS AND METHODS

**Composite inflorescence terminology.** Inflorescences in the Compositae typi-

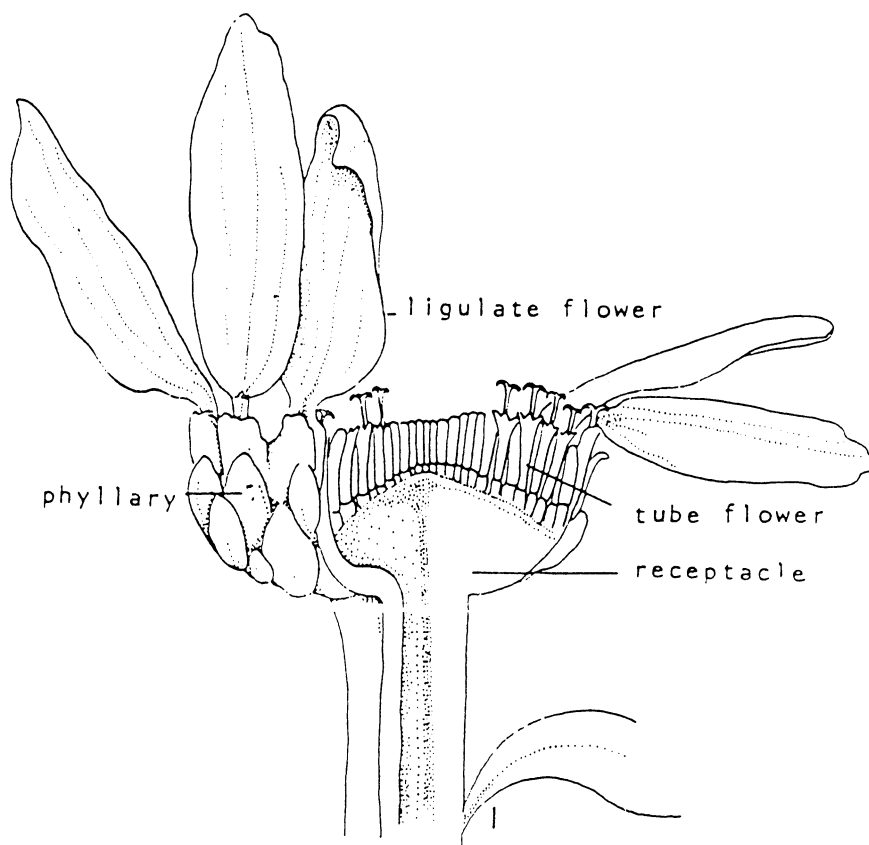


Fig. 1. Normal structure of daisy-type chrysanthemum inflorescence. Line drawing courtesy of James P. Smith (12). (Used by permission)

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cally form a head composed of many small florets on a conical or flattened receptacle (Fig. 1). Florets are of two types: tubular, perfect florets located in the center of the receptacle and ligulate, pistillate florets formed at the periphery. In daisy-type chrysanthemums, tubular florets (sometimes called disk florets) are yellow and are more numerous than ligulate florets. Ligulate florets (sometimes called ray florets) contain elongated strap-shaped corollas whose color varies with cultivar. Florets are subtended by an involucre of bracts called phyllaries. In this report, we use the terms tubular and ligulate florets and refer to that portion of the receptacle covered by tubular florets as the disk.

**Plant material.** Cuttings of *D. grandiflora* cvs. Florida Marble, Pink Marble, and Vero obtained from a commercial source were rooted in perlite and grown in Pro-Mix, a commercial potting mixture (Premier Brands, New Rochelle, NY). Plants were fertilized at weekly intervals with 20-20-20 and kept in a greenhouse with supplemental incandescent light on a 14-hr day at 21 C and night at 16 C. After 2 wk the plants were placed in growth chambers, and controlled treatments were initiated.

**Growth chamber environments.** At the beginning of the experiment, Pink Marble and Vero were exposed to a 10-hr photoperiod with fluorescent plus incandescent light with 300  $\mu$ E at a low inductive day/night (21/16 C) temperature for 7, 14, or 28 days. Treatments consisting of four plants in each combination were exposed to a moderate-temperature treatment (27/21 C) or a high-temperature treatment (32/27 C) for 6 or 12 days with a 10-hr photoperiod. Plants were then returned to the original low-temperature environment (21/16 C) in a chamber with the same photoperiod. Inflorescences were observed for abnormalities in developing terminal and axillary buds. When plants were in full bloom, inflorescences were rated for abnormal development of bracts and ligulate florets in the disk. The experiment was repeated twice.

Next, 10 Pink Marble plants per treatment in each of eight groups were grown in a greenhouse on a 14-hr day at the low temperature and transferred to a chamber at the same temperature on a 10-hr photoperiod at 300  $\mu$ E for 5 days. One group of plants was maintained for an additional 21 days at this temperature and photoperiod before being returned to a greenhouse. Three other groups were exposed to high temperature for 7, 14, and 21 days on the 10-hr photoperiod before they were returned to the low temperature and 10-hr photoperiod or to a greenhouse on a 14-hr day at the low temperature. Four other groups of 10 plants each were exposed first to low temperature for 5 days in a chamber. They were then trans-

ferred to high temperature for 7 days followed by low temperature for 7 days and either maintained at low temperature (control) or treated for 7, 14, or 21 days at high temperature. Plants were then exposed to low temperature on a 10-hr photoperiod for 14 days and finally returned to a greenhouse on a 14-hr photoperiod at the same low temperature. Disk bract formation was rated on a scale of 0-3, with 3 as the most severe

symptom. Disk bract abnormalities in flowers from terminal and axillary buds were recorded. The experiment was repeated twice with similar results. Data presented are from one experiment.

**Microscopy.** Inflorescences were dissected as immature, closed buds and as open flowers to determine the presence of specific floral organs and stages of inflorescence maturation. Normal and abnormal inflorescences from each culti-

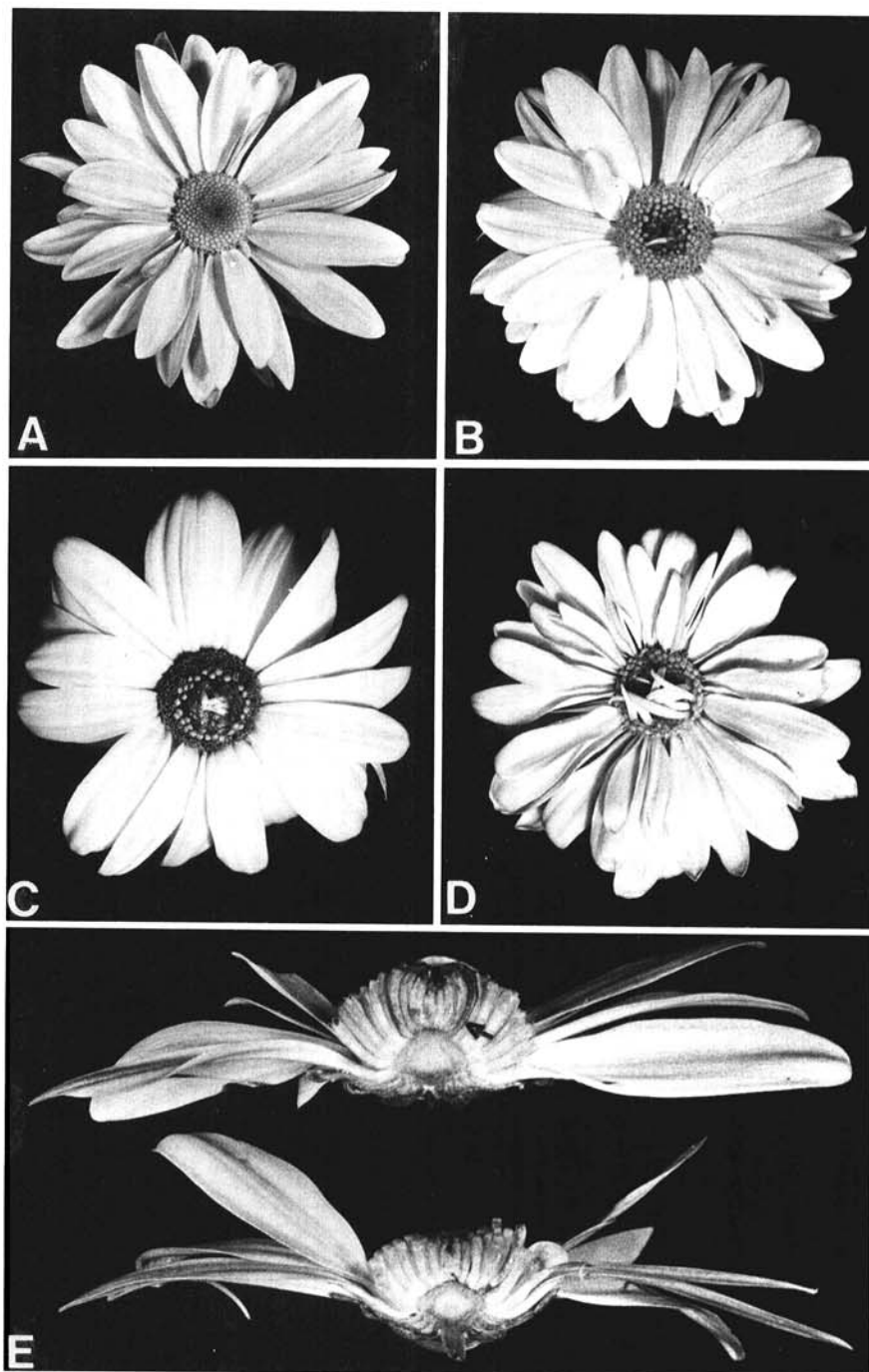


Fig. 2. Comparison between normal cv. Pink Marble inflorescences and those affected by heat delay. (A) Normal inflorescence grown at 21/16 C, day/night temperatures for 5 wk and returned to a greenhouse at the same temperature. (B) Abnormal inflorescence from axillary bud with bracts massed at the center of the disk with one ligulate floret protruding. Plant exposed to 7 days at 21/16 C followed by 12 days at 32/27 C. (C) Axillary flower with disk bracts surrounding ligulate florets. Plant exposed to 14 days at 21/16 C followed by 12 days at 32/27 C. (D) Similar to C, but ligulate florets are more mature. (E) Cross sections of inflorescence shown in A (bottom) and B (top). Disk bracts (arrow) originate from the receptacle.

var were examined by light microscopy scanning electron microscopy, and transmission electron microscopy to compare gross morphology, anatomy, and ultrastructure.

Samples for scanning microscopy included the entire receptacle or portions cut to expose the inner structure. Samples were fixed in 2% glutaraldehyde and 1.5% acrolein, or 5% glutaraldehyde, and dehydrated in a graded series of ethanol. Separate tubular florets with growth abnormalities were also sampled. After critical point drying, samples were sputter coated with gold-palladium and observed in a JSM 6100 scanning electron microscope. Similarities and differences in gross morphology and anatomy were examined.

For light and transmission electron microscopy, bracts from disks and involucre were laterally bisected and fixed in 2% glutaraldehyde and 1.5% acrolein, postfixated in osmium, dehydrated in alcohol, and embedded in LX112. Bracts were sectioned from apex to base to produce both thick and thin sections. Thick sections (5  $\mu$ m thick) were stained with 0.1 mg of methylene blue per milliliter of double distilled H<sub>2</sub>O, and examined with a Leitz Ortholux II microscope. Thin sections (500 nm thick) were stained in uranyl acetate and Reynolds lead citrate and observed with a JEOL 100 CX transmission electron microscope.

## RESULTS

**Normal flower structure.** Vero, Pink Marble, and Florida Marble plants exposed to low inductive temperatures produced daisylike flowers typical of the Compositae. In the transition from a

vegetative to a reproductive state, involucre phyllaries appeared first, enclosing the immature bud. Floral primordia matured from the edge of the receptacle progressively toward the center. Two or three rows of primordia adjacent to the involucre bracts differentiated to form pistillate ligulate florets, whereas the remainder formed perfect tubular florets.

As inflorescences matured, ligulate and tubular florets enlarged (Fig. 2A). After phyllaries began recurving, corollas on ligulate florets rapidly elongated and colored to yellow in Florida Marble and Vero and pink in Pink Marble. Tubular florets opened at maturity to reveal style and anthers, displaying a gradient in maturity from the periphery (mature) to the center (immature) of the disk (Fig. 2A). Occasionally a floret developed at the interface of tubular and ligulate florets that displayed characteristics of each. Corollas were small and lobed like those of tubular florets but with two lobes slightly enlarged and colored as ligulate florets. Such intermediate florets occasionally contained rudimentary anthers but typically were pistillate.

**Abnormal flower structure.** Inflorescences on plants exposed to high temperatures (32/27 C day/night) developed ligulate florets and green bracts in the disk (Fig. 2B–D). Some inflorescences formed bracts only or bracts with a single ligulate floret (Fig. 2B), whereas others contained numerous ligulate florets (Fig. 2D). Disk bracts subtended ligulate and tubular florets and often appeared in a circular formation overlapping developing florets (Fig. 2C) much as involucre phyllaries cover closed buds. Ligulate florets formed in the disk did not expand

to the size of those at the periphery of the receptacle (Fig. 2D) and sometimes contained rudimentary, sterile anthers. Florets were observed in the disk that displayed characteristics of both tubular and ligulate florets, similar to those normally located at the interface between tubular and ligulate florets. Cross sections of abnormal and normal inflorescences revealed that disk bracts developed directly from the receptacle (Fig. 2E).

**Effects of high temperature on floret development.** After an initial 7-day, 10-hr photoperiod, Vero and Pink Marble plants exposed for either 6 or 12 days produced normal inflorescences at 27/21 C. In contrast, a few abnormal inflorescences were present on plants of both cultivars after exposure for 6 days to a high temperature (32/27 C) regime (Table 1). More inflorescences, particularly axillary ones, developed abnormally on plants exposed to 32/27 C for 12 days (Table 1).

When the low temperature (21/16 C) inductive treatment was extended to 14 days, Vero and Pink Marble developed inflorescence abnormalities in the terminal but not in lateral buds after a 6-day exposure to a moderate (27/21 C) temperature, whereas three-fourths of those plants exposed for 12 days to 27/21 C had abnormalities (Table 1). On the other hand, Vero plants exposed to the 21/16 C regime for 12 days and Pink Marble plants exposed to 27/21 C for 6 or 12 days failed to develop abnormal terminal inflorescences, whereas two plants of Vero, one exposed for 6 and one for 12 days to the high temperature (32/27 C) developed abnormalities in axillary but not terminal inflorescences. No abnormal inflorescences were on plants maintained at 21/16 C from the initiation of short days until flowering. Similar results were obtained with Pink Marble in a repeat experiment, but only one plant of Vero showed a few ligulate florets in the terminal flower of a plant treated 12 days at 32/27 C following 14 days of short days at 21/16 C. Similar results were obtained in two separate experiments.

In the next experiment, plants exposed to 21/16 C on a 10-hr photoperiod for 12 days followed by 3 wk of exposure to 32/27 C showed more severe disk bract symptoms than did plants exposed to only 1 wk at 32/27 C (Table 2). Symptoms of green bract development were most prominent in the terminal flower. Symptoms were less pronounced in the first lateral flower with only slight green bract development in flowers at the second and third and fourth and fifth nodes from the apex. A similar pattern of ligulate floret development in the disk was also observed. The greatest number of ligulate florets formed in the disk of terminal flowers. Fewer ligulate florets were in the disk of flowers formed at the second and third nodes, and no

**Table 1.** Effect of sequential temperature on development of abnormal flowers of *Dendranthema grandiflora* cultivars Vero and Pink Marble<sup>a</sup>

Inductive treatment				No. plants with abnormal flowers <sup>c</sup>	
Days at 21/16 C	Days <sup>b</sup>	Temperature (C)	Cultivar	Terminal flower	Axillary flowers
7	6	32/27	Vero	3 <sup>d</sup>	1 <sup>d</sup>
7	6	32/27	Pink Marble	1 <sup>d</sup>	0
7	12	32/27	Vero	1 <sup>c</sup>	4 <sup>d,e</sup>
7	12	32/27	Pink Marble	0	4 <sup>c</sup>
14	6	27/21	Vero	4 <sup>c</sup>	0
14	6	27/21	Pink Marble	4 <sup>d</sup>	0
14	12	27/21	Vero	3 <sup>d</sup>	3 <sup>d</sup>
14	12	27/21	Pink Marble	3 <sup>c</sup>	3 <sup>d,e</sup>
14	6	32/27	Vero	4 <sup>d</sup>	0
14	6	32/27	Pink Marble	4 <sup>d</sup>	4 <sup>d</sup>
14	12	32/27	Vero	4 <sup>d</sup>	4 <sup>d</sup>
14	12	32/27	Pink Marble	3 <sup>d</sup>	4 <sup>d</sup>
28	6	32/27	Vero	0	1 <sup>d</sup>
28	6	32/27	Pink Marble	0	0
28	12	32/27	Vero	0	1 <sup>d</sup>
28	12	32/27	Pink Marble	0	0

<sup>a</sup>Plants were exposed to an inductive 10-hr photoperiod at low temperature and light intensity of fluorescent and incandescent illumination at the beginning of the experiment.

<sup>b</sup>Number of days plants exposed immediately following exposure to 21/16 C.

<sup>c</sup>Number of plants out of four treated plants. Results are from one of two experiments.

<sup>d</sup>Green bracts or phyllaries in the disk.

<sup>e</sup>Ligulate florets in the disk.

abnormalities were present in flowers formed at the fourth and fifth nodes.

When plants were subjected to the flower inductive 10-hr photoperiod treatment at 21/16 C for 5 days and then an alternating cycle of high temperature (32/27 C) followed by low temperature (21/16 C) and then high temperature for 7, 14, or 21 days, followed by low temperature (21/16 C), fewer green bract symptoms were in the terminal flower compared with more symptomatic flowers formed at the first, second, and third nodes. When plants were exposed to the high temperature treatment twice with an intervening low-temperature cycle, symptoms were present in flowers forming at the fourth and fifth nodes (Table 2). This is in contrast to the absence of symptoms in flowers formed at these node positions when plants were exposed to only one cycle of high temperature.

**Scanning electron microscopy. Normal inflorescences.** Disks were covered with closely packed tubular florets containing five-lobed corollas (Fig. 3A). Trichomes were present on phyllaries and florets, with T-trichomes prominent on phyllaries and glandular trichomes (12) prominent on ligulate and tubular florets (Fig. 3B). T-trichomes were more numerous on phyllaries towards the exterior of the inflorescence. Phyllaries were further distinguished from florets by their fanlike shape, a surface cellular pattern radiating outward from the center (Fig. 3B), and the presence of stomates. Ligulate florets occurred in two or three rows between tubular florets and phyllaries. Immature ligulate florets were smaller than tubular florets and contained distinctive scroll-like corollas (Fig. 3C) and, when immature, were smaller than neighboring tubular florets.

**Abnormal inflorescences.** Disk bracts often developed in circular rows among tubular florets (Fig. 3D). Their shape, trichomes, surface patterns, and stomates (Fig. 3E) were similar to those of involucre phyllaries (Fig. 3B). In some in-

florescences, rows of disk bracts curved over florets to delineate a zone of very small, immature tubular florets from the normal larger ones present on the rest of the disk (Fig. 3F). Bracts alternated with florets and appeared to originate from the receptacle, not from florets (Fig. 3G). Bracts also subtended individual ligulate florets present in the disk (Fig. 3H). Disk ligulate florets were generally similar in appearance to normal peripheral ligulate florets, although florets were observed that contained characteristics of both tubular and ligulate types. Such intermediate florets were five lobed, as in tubular florets, but with two lobes elongating and expanding like ligulate florets (Fig. 3I).

**Light microscopy of involucre phyllaries and disk bracts.** The anatomies of involucre phyllaries from Vero and disk bracts from Pink Marble were essentially identical. Both contained abaxial rows of subepidermal fibers, stomates, central vascular bundles, and an undifferentiated ground mesophyll with no palisade layer (Fig. 4A and B). Tissue on either side of the thick central region shown in Figure 4 tapered to a "wing" ultimately only two cell lengths wide, with fibers running throughout most of the length.

**Transmission electron microscopy of involucre phyllaries and disk bracts.** Ultrastructural observations had a two-fold purpose: First to find if fiber cells (revealed by light microscopy as a major anatomical feature) were identical in disk bracts and phyllaries and secondly, to examine phloem for the presence of MLOs. Disk bracts of Pink Marble could not be distinguished from involucre phyllaries of Vero on the basis of either of these criteria.

Subepidermal fibers contained living protoplasts and thick secondary walls with multiple pits in lateral walls (Fig. 4C). Content of central vacuoles included opaque nonmembrane-bounded spherical bodies with slightly dense cores (Fig. 4D) and vesicles forming beaded fila-

ments (Fig. 4E) or spheres. Vesicles appeared to be autophagic, i.e., associated with cellular breakdown, and many retained visible remnants of ER (Fig. 4E) and mitochondria. Others appeared as single membrane-bounded vesicles with variable cytoplasmic content. Autophagy was also a common feature in epidermal cells, and, as in fibers, electron density of autophagic vesicles matched that of nearby cytoplasm (Fig. 4F).

Phloem appeared similar to that previously described in Vero, Pink Marble, and Florida Marble leaves (1). In both Vero phyllaries (Fig. 4G) and Pink Marble disk bracts, sieve elements had thick nacreous cell walls and phloem parenchyma, and companion cells contained transfer cell wall ingrowths. Mature sieve element content included plastids filled with starch and electron dense granules, vesicles (Fig. 4G), p. protein, mitochondria, endoplasmic reticulum, and plasmalemmae.

## DISCUSSION

Photoperiod and temperature are important in synchronizing floral initiation and development in florists' chrysanthemum (11). Floral abnormalities may be induced by specific daylength and temperature treatments. Inflorescence buds, produced by vernalized chrysanthemum plants exposed to extended periods of long day include several different types of abnormalities in the receptacle (9). Delay in development of the inflorescence and formation of bractlike structures in the inflorescence have long been associated with high temperatures (7,8,11).

In our studies, floral abnormalities were more common in the terminal buds of Vero or Pink Marble plants that received 14 days of low temperatures before exposure to high temperatures than in plants that were exposed to low temperatures for 7 or 28 days. These results are consistent with interpretations that the chrysanthemum inflorescence is

**Table 2.** Sequence of temperature treatments and flower position on green bract development in floral disks of *Dendranthema grandiflora* cultivar Pink Marble

5 <sup>a</sup>	Days at day/night temperature									Flower position			
	7	7	7	7	7	7	7	7	7	T <sup>b</sup>	1 <sup>c</sup>	2-3 <sup>d</sup>	4-5 <sup>e</sup>
21/16 <sup>f</sup>	21/16	21/16	21/16	GH						0	0	0	0
21/16	21/16	32/27	21/16	21/16	GH					0.44 <sup>g</sup>	0.11	0	0
21/16	21/16	32/27	32/27	21/16	21/16	GH				0.5	0.25	0	0
21/16	21/16	32/27	32/27	32/27	21/16	21/16	GH			1.6	0.22	0.05	0
21/16	32/27	21/16	21/16	21/16	GH					0.11	0	0	0
21/16	32/27	21/16	32/27	21/16	21/16	GH				0	0.7	1.0	1.0
21/16	32/27	21/16	32/27	32/27	21/16	21/16	GH			0.2	1.4	1.7	1.4
21/16	32/27	21/16	32/27	32/27	32/27	21/16	21/16	GH		0.6	1.5	1.6	2.2

<sup>a</sup>Number of days plants were exposed to the day/night temperature designated before shifting to the next temperature.

<sup>b</sup>Terminal flower.

<sup>c</sup>First axillary bud.

<sup>d</sup>Second and third axillary buds.

<sup>e</sup>Fourth and fifth axillary buds.

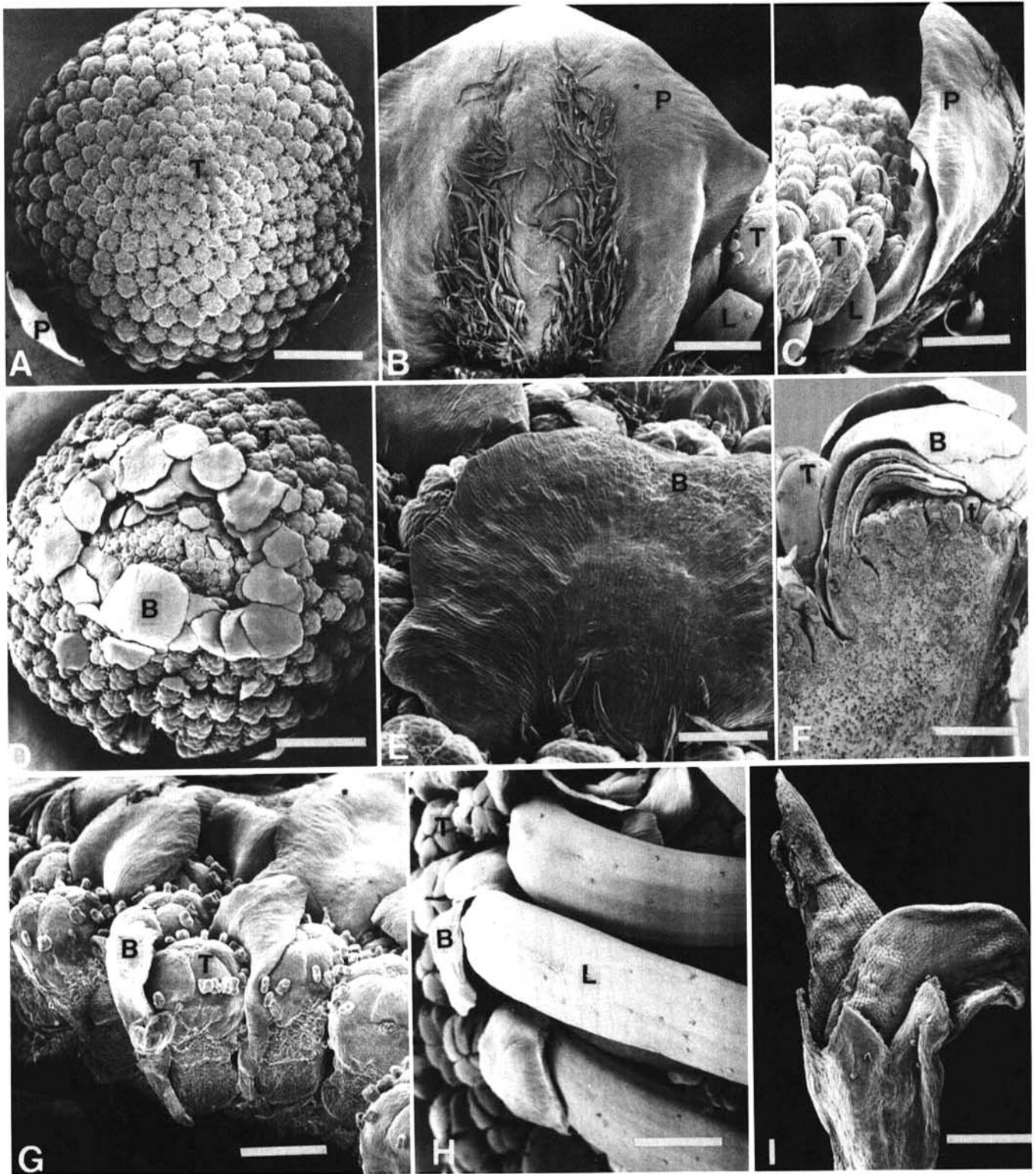
<sup>f</sup>Day/night temperature in C.

<sup>g</sup>Rating of 0-3 is based on the extent of green bract development in the disk. 0 = No green bracts. 1 = Disk is 25% covered. 2 = Disk is 50% covered. 3 = Disk is more than 50% covered with green bracts. Ratings are the percent average of four flower positions on each of the five plants.

susceptible to heat-induced abnormalities during a specific stage of development, probably during initiation and early differentiation of florets. Floret

initiation in daisy-type chrysanthemums is known to occur somewhere between 14 and 16 days. In the daisy-type cultivar Bittersweet, floret initiation first appeared

at the 14th day and in Orchid Queen, on the 16th day after beginning short-day treatment (6). By the 16th day, floret primordia covered about one-fourth of



**Fig. 3.** Scanning electron micrographs of normal (A-C) and abnormal (D-I) inflorescence structure in *Dendranthema grandiflora*. (A) Vero. Immature tubular florets form an unbroken array over the disk. Ligulate florets at periphery are not visible. Bar = 1.25 mm. (B) Pink Marble. Phyllaries are distinguished by epidermal cells radiating from the center and by the presence of T-trichomes. Bar = 750  $\mu$ m. (C) Pink Marble. Side view of receptacle showing normal placement of phyllary, ligulate florets and tubular florets. Bar = 833  $\mu$ m. (D) Pink Marble. Bracts form concentric rings among small tubular florets. Bar = 1.25 mm. (E) Disk bracts resemble phyllaries in shape, presence of trichomes, and epidermal cell pattern. Bar = 2.30 mm. (F) Pink Marble. Cross section of receptacle reveals significant size differential between tubular florets separated by clustered disk bracts. Bar = 750  $\mu$ m. (G) Disk bracts alternating with tubular florets. Bar = 375  $\mu$ m. (H) Disk bracts and ligulate florets protrude among tubular florets in the receptacle's center. Bar = 1.25 mm. (I) Two of five corolla lobes are elongated on a tubular floret located among displaced ray florets shown in H. Bar = 1.0 mm. B = disk bracts; L = ligulate florets; P = Phyllary; T = tubular florets; t = small tubular florets.

the receptacle in Bittersweet.

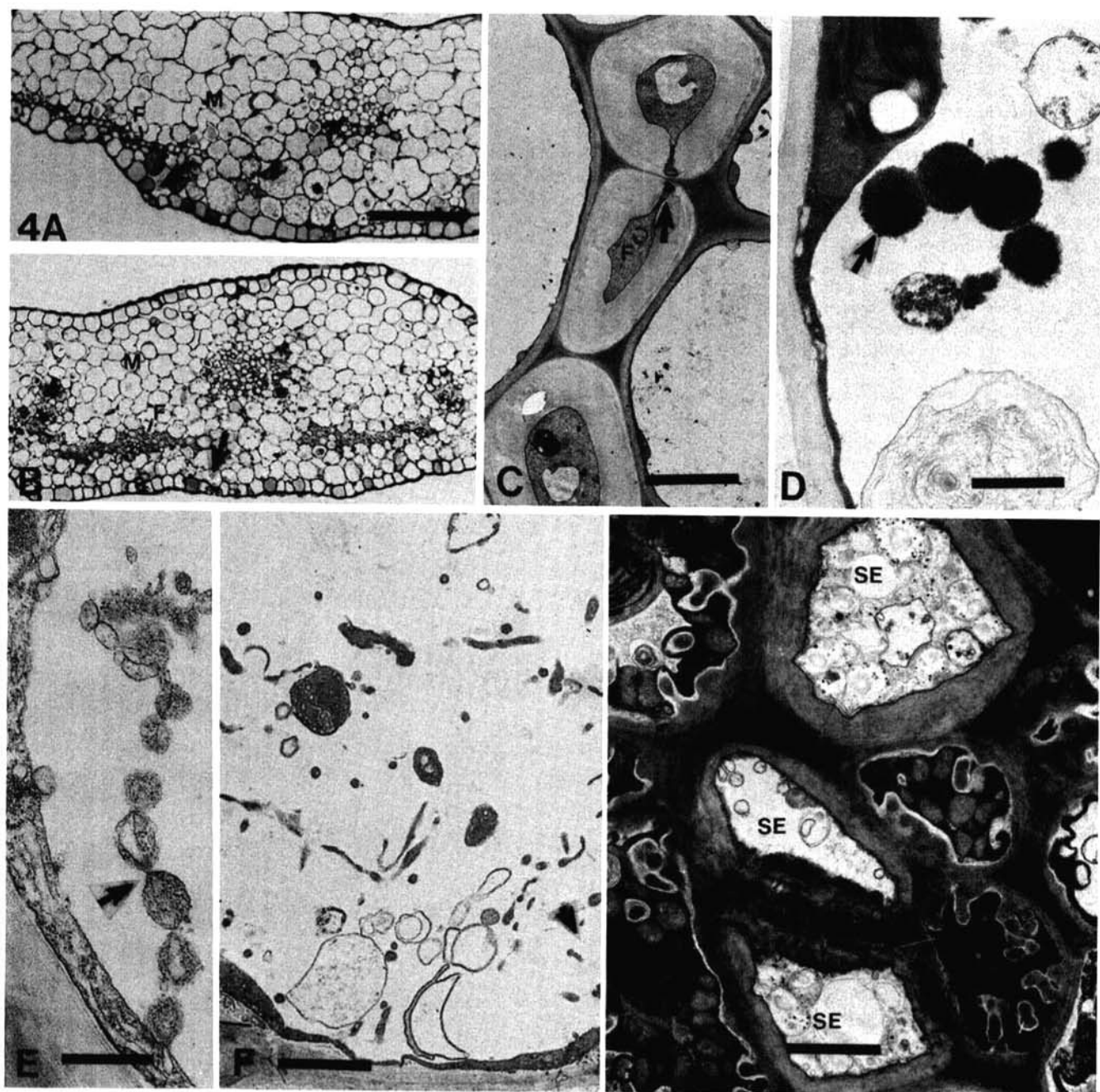
High temperatures appear to temporarily induce a more juvenile state that returns the receptacle to an earlier stage of development. When this condition prevails phyllaries and ray florets may be produced out of sequence. Because disk bracts originate directly from the receptacle and are not modified florets, their presence may serve as a "marker" indicating progress of floret initiation on the receptacle before high temperatures causes reversion to a more juvenile stage

of growth. High temperatures may also cause floral primordia that have already been initiated to revert to more juvenile forms. This may be the origin of florets in the disk that display both tubular and ligulate characteristics.

High temperature-induced abnormalities in the terminal and upper axillary flowers of Vero and Pink Marble exposed to 27/21 C or 32/27 C following an initial low-temperature inductive treatment for 2 wk is consistent with the conclusion that high temperature induces

floral abnormalities during a transition period from the vegetative to the flowering state after the onset of short days. Similar abnormalities did not occur in flowers of plants grown for 4 wk at 21/16 C before exposure to high temperature. Increasing the time of exposure to 32/27 C from 1 to 3 wk increased the number of flowers with green bracts in both the terminal and axillary flowers following a 12-day initial low temperature inductive treatment.

Axillary buds on plants exposed



**Fig. 4.** Light micrographs (A, B) comparing internal structure of a Vero involucre phyllary (A) and a Pink Marble disk bract (B). Transmission electron micrographs (C-G) of fibers (C-E), epidermal cell (F) and phloem (G). Involucre phyllary (A) and disk bract (B) have similar anatomy, including stomates (arrow), abaxial rows of subepidermal fibers (F) and undifferentiated ground mesophyll (M). A and B Bar = 715  $\mu$ m. C. Pink Marble. Fibers (F) have thick cell walls with pits (arrow) containing living protoplasts. Bar = 4.0  $\mu$ m. D. Vero. Vacuolar content includes dense core spherical structures (arrow). Bar = 625 nm. E. Vero. Autophagic vesicles (arrow) apparently released into the central vacuole. Bar = 1.7  $\mu$ m. F. Vero. Electron dense, spherical and beaded filamentous components (arrow) showing structures similar to beaded forms of mycoplasma-like organisms. Bar = 2.0  $\mu$ m. G. Vero. Normal sieve element (SE) content includes plastids, starch grains, and vesicles. Bar = 2.0  $\mu$ m.

initially to 12 days of 21/16 C are apparently past the stage of highest susceptibility to heat-induced abnormality, even when plants are subsequently exposed to up to 3 wk of 32/27 C. Pink Marble plants exposed only to 5 days of low-temperature induction before exposure to 32/27 C showed more plants with green bract abnormalities in flowers from the lower axillary buds and a more severe symptom. Apparently the lower axillary buds in this treatment are more susceptible to green bract development because they are less mature than on plants initially exposed to 12 days of the low-temperature inductive treatment.

Flowering in the cultivar Orange Bowl grown at 22/18 C during the first 4 wk of short days was not substantially delayed and flowers developed normally when plants were subsequently transferred to high temperature (13). High temperatures during the third and fourth week did, however, cause bract formation interior to the outer row of florets (13). Bracteate buds were produced when Orange Bowl was exposed to high temperature at the start of or at the third week of short days until flowering, and only the outer rows of florets developed. The receptacle was covered with noninvolucral bracts (13).

Development of heat-induced abnormal inflorescences may result from imbalances in endogenous plant growth substances (11). The effect is not limited to chrysanthemum; high temperature reportedly alters the endogenous auxin and gibberellin levels in tomato flowers and results in inhibition of fruit set in tomato flowers (2). An alternative explanation for inflorescence inhibition may be that reproductive and vegetative organs compete for a pool of assimilates reduced by rapid metabolic processes at higher temperatures (2).

MLOs have been invoked as the cause of chrysanthemum phloem necrosis, a disease associated with flower abnormalities similar to those reported here (7). Published illustrations of green structures in CPN-affected Marble inflorescences resemble those induced by high temperatures (7). We interpret these as displaced phyllaries rather than as chlorophyllous flowers described in CPN reports. Disk bracts are essentially identical to involucre phyllaries and show no remnants internally of floral structure.

Inflorescences of both the Marbles and Vero contained a range of vesicles and dense-core structures that resemble published descriptions and illustrations of the CPN MLO (3). We did not interpret these as MLOs, however. Vesicles appeared to originate from host cytoplasm in cells undergoing autophagy, whereas dense core structures lacked binding membranes and appeared to be metabolites.

In addition to causing floral abnormalities, CPN has also been associated with foliar necrosis in the Marble cultivar (7,3). Our work with the Marbles indicates that the floral and foliar syndromes appear to have distinct etiologies. Previous investigations into the foliar abnormality in the Marbles revealed plants were highly sensitive to manganese toxicity and did not contain an MLO (1,4).

The sensitivity expressed by the Marble cultivars to high temperatures and low concentrations of manganese limits the value of these cultivars to commercial growers. The cultivar Vero, with a similar flower but lower sensitivity to heat induced abnormalities may prove to be a practical substitute.

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