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The Effect of Temperature, Photoperiod, and Freeze Treatment on the Morphology of *Chrysanthemum* X *morifolium* Ramat. 'Astrid'[1]

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Abstract. **More rhizomes were initiated by plants of 'Astrid' chrysanthemum grown in short day and cool air temperature than in long day and warm air. Rhizome development was greatest, shoot growth was enhanced, and root length and dry weight increased with warm compared to cool soil temperature. Rhizomes grown at a cool soil temperature either in long or short days had the least cellular injury after exposure to −8°C.**

Rhizome development in lowbush blueberry and *Agropyron repens* L. has been correlated with vigorous aerial growth under optimum environments (11, 16). Long photoperiod and high temperature were more effective in promoting rhizome development in lowbush blueberry and *Bromus inermis* Leyss. than short photoperiod and cool temperature (11, 20). Temperature and photoperiod influenced plant morphology; soil temperature repeatedly had greater effect on vegetative growth than did air temperature (2, 5, 10, 12, 13, 14, 15).

This investigation was undertaken to determine the effect of temperature and photoperiod on vegetative growth including root length and dry weight, shoot height and dry weight, and rhizome number and dry weight of 'Astrid' chrysanthemum plants, and to examine the anatomy of the rhizomes under different environmental regimes before and after freezing.

Materials and Methods

'Astrid' chrysanthemum rooted cuttings were grown in an arcillite medium (referred to as "soil") in polyethylene-lined 0.9 liter (1 quart) cardboard containers for 65 days in the greenhouse at: 1) a warm air and soil temperature of 24° ± 2°C (W/W); 2) a warm air 24° ± 2° and a cool soil 8° ± 2° (W/C); 3) a cool air 4° ± 1° night and 12° ± 2° day with a warm soil 24° ± 2° (C/W); and 4) a cool air 4° ± 1° night and 12° ± 2° day and a cool soil 8° ± 2° (C/C). There were 14 plants in each of these 4 regimes; 7 were grown under short days (SD) and 7 under long days (LD).

Plants in SD received 8 hr of natural light; plants grown in LD received natural daylength plus an interruption of the night by 4 hr of incandescent light (medium intensity > 110 lux) from 2200 until 0200 HR.

Plants were watered with 1/2-strength modified Hoagland's (9) nutrient solution every other day. Two greenhouse corrugated transite benches with flat 20-cm-high transite sides were enclosed with Styrofoam sheets with 30 openings into which plant containers were inserted. Soil temperatures were maintained by thermostatically controlled electric heating cables and constant fan circulation within the enclosure. The 8° ± 2°C soil temperature was maintained by constant cold-water circulation in coiled plastic tubes encircling the containers which were embedded in perlite in Styrofoam chests.

Rhizomes were severed 65 days after planting and cut into 1-cm segments. Five to 6 segments of rhizomes were wrapped in aluminum foil, placed in glass jars, and surrounded with dry arcillite. Controls were kept at 5°C. All samples were replicated 9 times for each treatment. A thermocouple was inserted into the pith of 1 rhizome segment of each treatment. To prevent supercooling, the jars were placed in Styrofoam covered with perlite, and placed in a freezer with a temperature drop of 3° per hour. Two hours after the segments cooled to −8°C the jars were removed and held at 5° for a minimum of 15 hr after which they were processed for anatomical examination.

Shoot height, root length, rhizome number, and root, shoot, and rhizome dry weight were obtained. To determine the effect of freeze stress on rhizome segments, 5-mm sections from the middle portion of the rhizomes were taken from the 8 treatments before and after stressing to −8°C and fixed in formalin-aceto-alcohol (FAA), aspirated, dehydrated with tertiary butyl alcohol, and infiltrated and embedded in Paraplast (7). Transverse sections were cut 15 μm on a rotary microtome and stained with safranin O, crystal violet and light green SF yellowish (6).

Results and Discussion

In warm and cool air environments root length and dry weight were significantly higher in LD and warm soil compared to SD and cool soil (Table 1). With warm air, shoot height and dry weight were significantly greater in LD and warm soil than in SD and cool soil. In cool air, shoot length was significantly greater in LD and warm soil than in SD and cool soil. However, shoot dry weight was significantly higher under LD with C/W compared to other treatments. At warm air temperature, there were more rhizomes initiated in SD than LD. The number of rhizomes was similar regardless of soil temperature when the air was cool except in SD and LD C/C. In both warm- and cool-air environments rhizome dry weight was higher with a warm soil and greatest in SD C/W.

The cellular structure of rhizomes grown under W/W conditions in either SD or LD without subsequent cold stress are shown in Fig. 1 and 3. Rhizomes grown in the same environments but exposed to −8°C showed separation of cells in the

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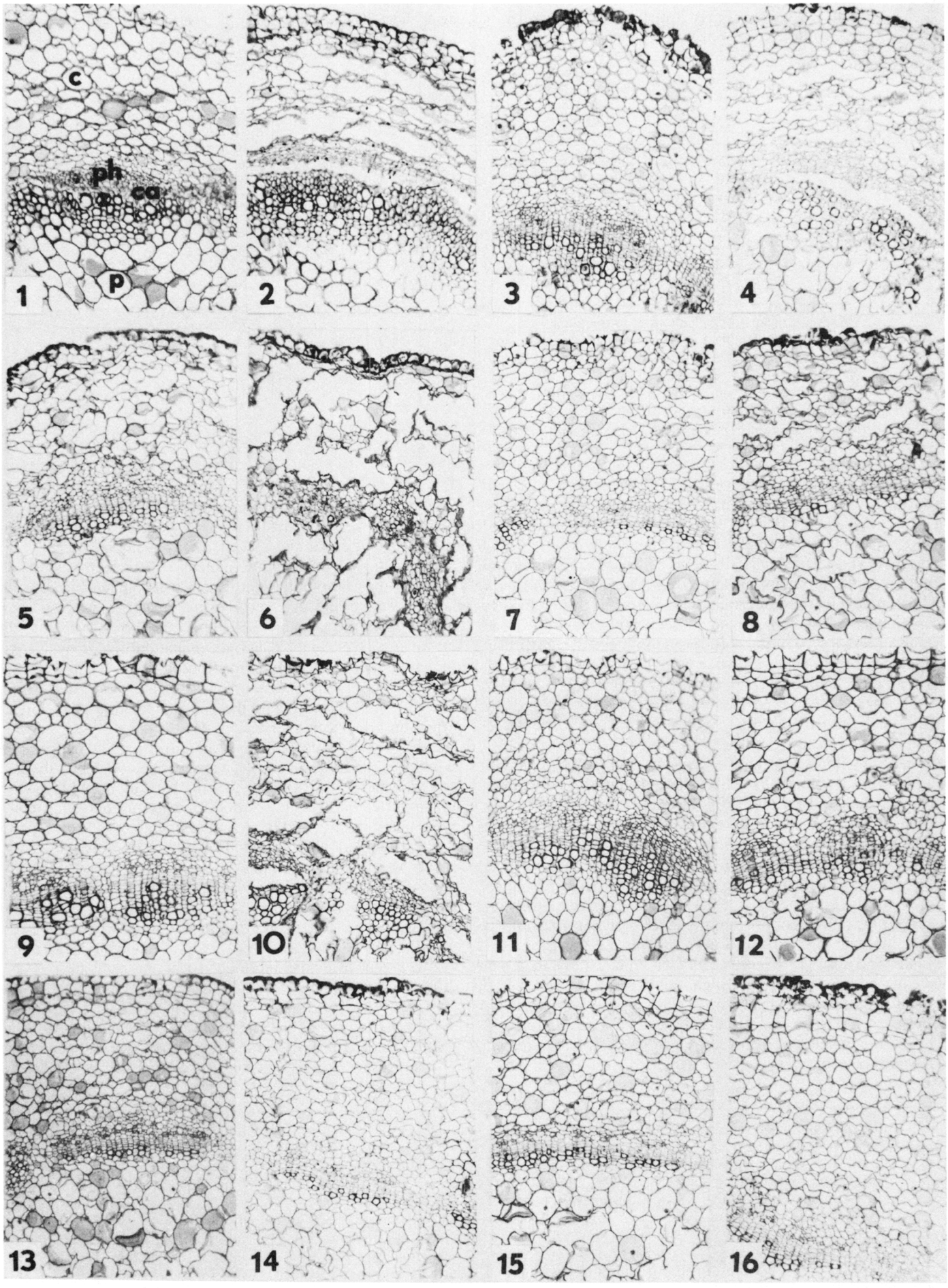

Table 1. Effects of photoperiod and temperature on root length, shoot length, shoot height, number of rhizomes, and rhizome dry weight of *C.* X *morifolium* 'Astrid'.[z]

Treatment		Root Length (cm)	Root dry wt (g)	Shoot ht (cm)	Shoot dry wt (g)	No. rhizomes	Rhizome dry wt (g)
Photo-period	Temp[x]						
LD	W/W	18.0a[w]	4.0a	12.0a	2.8a	6.6b	0.3a
	W/C	13.7c	2.9b	6.1b	0.9c	5.6b	0.1c
SD	W/W	14.4b	2.8b	5.7c	1.2b	9.1a	0.4a
	W/C	10.0d	1.2c	3.0d	0.5d	8.4ab	0.2b
LD	C/W	18.0a	4.4a	6.3a	2.3a	9.7ab	0.4b
	C/C	12.5c	2.4b	4.4b	1.0b	8.1b	0.2c
SD	C/W	15.7b	1.9c	3.8c	1.0b	9.9ab	0.5a
	C/C	12.0d	1.7c	3.1d	0.8b	10.3a	0.2c

[z]Values are means of 7 plants in each treatment.
[y]SD = 8 hr daylength; LD = natural daylength + 4 hr night interruption with incandescent light.
[x]W/W warm air, 24° ± 2°C; warm soil, 24° ± 2°
W/C warm air, 24° ± 2°; cool soil, 8° ± 2°
C/W cool 4° ± 1° night, 12° ± 2° day; warm soil, 24° ± 2°
C/C cool air and soil, 4° ± 1° night, 12° ± 2° day
[w]Mean separation in columns by Duncan's multiple range test, 5% level.

cortex and vascular cambium (Fig. 2, 4). Rhizome cells without the freeze treatment under W/C in both SD and LD are shown in Fig. 5 and 7. In LD with W/C, rhizome segments exposed to −8° were injured severely (Fig. 6) compared to those in LD with W/W (Fig. 2). Many cells were collapsed and obliterated resulting in the formation of large cavities. The cortical, pith, and vascular cells were freeze-injured. Segments from rhizomes grown under SD with W/C and subsequently exposed to −8° displayed less injury in the cortical and pith cells (Fig. 8) than did segments from plants grown in LD with W/C and W/W with SD and LD conditions (Fig. 6, 4, 2). Under LD with W/C conditions and −8° exposure, rhizome segments showed the greatest cellular damage (Fig. 6) of all treatments.

Fig. 9 and 11 show rhizome cells grown under C/W in both LD and SD without freezing stress. Rhizomes grown under LD with C/W and exposed to −8°C showed injury in the cortex, vascular cylinder, and pith (Fig. 10). Cells had collapsed, resulting in the formation of cavities. Separation of cells in the cambial region occurred. Less freezing injury occurred to cortical cells in rhizomes grown under SD with C/W (Fig. 12) compared to LD with C/W (Fig. 10). There was some separation of cells in the cortex and pith but the vascular tissue was uninjured (Fig. 12). Sections from rhizomes grown in LD with C/C and SD with C/C without freeze stresses are shown in Fig. 13 and 15. Those grown in the same environmental conditions and exposed to −8° had only slight injury (Fig. 14, 16). A few cortical cells were misshaped and small cavities were formed. There appeared to be less vascular tissue in rhizomes grown in cool soil compared to a warm soil regardless of whether the air was warm or cool.

The most rhizomes were initiated in SD and cool air but rhizome development was greatest with warm soil. Possibly warm soil affected both shoot photosynthesis and translocation to the rhizome explaining the extensive development in the warm soil. Similarly, the root environment under which 'Astrid' was grown had a very highly significant effect on photosynthesis (1).

Root length and dry weight were greater in warm soil and LD. Skene and Kerridge (19) reported a positive relationship between root elongation and accumulation of dry matter with increased root temperature. Greatest shoot dry weight and height occurred in LD and warm soil (Table 1). Other investigators agree that for some plants the soil temperature has a greater effect on growth than air temperature (10, 13, 14). It has been suggested that the decrease in shoot growth at low temperatures is not the result of mineral or water deficiency but of the level of endogenous growth substances (8, 13). Guinn and Hunter (8) found differences in the carbohydrate status of young cotton seedlings grown at different root temperatures. They reported that low temperature lowered the influence of growth-regulating substances such as cytokinin.

Some investigators (17, 18) reported a seasonal variation in tissue resistance to cold stress. Bark, cambium, and young xylem cells were considered to be the most cold-susceptible parts of growing plants (3). In 'Astrid' rhizome segments subjected to −8°C extent of damage to the cortical and cambial regions at different temperatures was correlated with photoperiods. The least injury to the tissues was apparent in SD and particularly in both SD and LD with cool air and soil. The most tissue injury resulted under LD with warm air regardless of soil temperature. This suggests that although soil temperature strongly influenced

Fig. 1–16 (facing page). Transverse sections of rhizomes from plants grown under LD and SD with different air and soil temperatures and −8°C cold stressed. (LD Fig. 1, 2, 5, 6, 9, 10, 13, 14; SD Fig. 3, 4, 7, 8, 11, 12, 15, 16; cold stressed Fig. 2, 4, 6, 8, 10, 12, 14, 16). ×87. **1.** LD with W/W temperature. **c** = cortex; **ph** = phloem; **ca** = cambium; **x** = xylem. **2.** LD with W/W and exposed to −8°. Cells in cambial region separated and some cells crushed. **3.** SD with W/W temperature. **4.** SD with W/W and exposed to −8°. Separation of cells in the cambial region and cortex. **5.** LD with W/C temperature. **6.** LD with W/C and exposed to −8°. Severe separation, shrinkage, and collapse of cells. **7.** SD with W/C temperature. **8.** SD with W/C and exposed to −8°. Separation of cells in cortex only. **9.** LD with C/W temperature. **10.** LD with C/W and exposed to −8°. Separation of cells in cambium and cortex and some cells collapsed. **11.** SD with C/W temperature. **12.** SD with C/W and exposed to −8°. Some cell separationn in cortex. **13.** LD with C/C temperature. **14.** LD with C/C and exposed to −8°. Trace of cell separation in cortex. **15.** SD with C/C temperature. **16.** SD with C/C and exposed to −8°. Only a trace of cell separation in cortex.

the degree of rhizome stress tolerance, it did not influence initiation of cold hardiness which depended on activity in the aerial portion. Similar conclusions based on regrowth and the 2, 3, 5-triphenyl tetrazolium chloride test have been reported (4).

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