Developmental Consequences of Cold Temperature Stress at Transplanting on Seedling and Field Growth and Yield. I. Watermelon

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ABSTRACT. Watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai) seedlings transplanted before the last frost date may be exposed to temperatures alternating between freezing and optimal until field temperatures finally stabilize. Cold stress may ultimately reduce growth and yield. To simulate such temperature alternations that occur naturally after field transplanting, diploid ‘Carnival’ watermelon seedlings were exposed immediately before field planting to cyclic cold temperature stress at 2 ± 1 °C then transferred to a greenhouse at 29 ± 5 °C. In 1997, transplants were exposed to 2 °C from 3 to 81 hours and in 1998, exposure ranged from 9 to 81 hours. Cold-stressed seedlings were field planted after all potential risk of cold stress in the field had passed. In 1997, cold stress decreased seedling shoot and root fresh and dry weights, leaf area, chlorophyll and carbohydrate contents but not seedling height. In 1998, all seedling growth variables decreased in response to longer durations of cold stress. Plants cold stressed for up to 81 hours transpired more for 1 week after transplanting than those exposed to shorter periods of cold stress. In both years, vining (date first runner touched the ground), flowering, and fruit set were delayed significantly as cold stress hours increased. Although early yields were unaffected, total yields decreased linearly in both years with increasing hours of cold, with 38 to 40 hours of cold stress reducing yield 10% in both years. Data indicate that ‘Carnival’ watermelon transplants exposed to cold stress soon after transplanting may suffer yield reductions.

Exposure of plants of a tropical or subtropical nature, such as members of the Cucurbitaceae, to chilling temperatures may stunt plant growth, induce wilting and leaf necrosis, and increase disease susceptibility (Rikin et al., 1976; Sasson and Bramlage, 1981). The optimum air temperature for watermelon (*Citrullus lanatus*) plant growth ranges from 20 to 32 °C with 18 °C and 35 °C as the minimum and maximum, respectively (Bates and Robinson, 1995). Growth may be reduced as a consequence of impaired photosynthesis, respiration, membrane integrity, and hormonal balance (Graham and Patterson, 1982).

One-half of the annual supply of the watermelons in the United States is consumed during June and July (Mizelle, 1988), and prices usually decrease linearly during this time (Mizelle, 1985). In order to capture this market window, many growers in the coastal southeast United States, field plant in early March, sometimes weeks before the last killing frost date. These seedlings may be exposed to temperatures cycling between chilling and optimal for long time periods before the temperatures finally stabilize. Thus, we hypothesize that exposure of watermelon seedlings to low temperatures may delay flowering, fruiting, and possibly reduce early and total yields and fruit quality.

Limited research is available describing long-term effects of short-term cold stress on vegetable transplants. Low temperatures have been reported to reduce growth of tomato (*Lycopersicon esculentum* Mill.) (Pardossi et al., 1988), corn (*Zea mays* L.) (Hetherington et al., 1983; Stamp, 1987), muskmelon [*Cucumis melo* L. (Reticulatus Group)] (Risse et al., 1978), watermelon (Bradow, 1990; Hassell, 1979) and cotton (*Gossypium hirsutum* L.) (Stewart and Guinn, 1969). Therefore, the objectives of this

study were to 1) determine the long-term effects of short-term cold stress at transplanting on watermelon seedling growth, early field growth, early and total yields, and fruit quality and 2) establish the hours of cold stress necessary before significant yield reductions occur.

Materials and Methods

COLD STRESS VS. TRANSPLANT GROWTH. Diploid ‘Carnival’ watermelon seeds (Rogers Brothers Seed Co., Salinas, Calif.) were sown with the micropyle pointing down in 5.4 cm deep cells (57 cm³) (Hummert Intl., Earth City, Mo.) filled with Metro Mix 300 growth medium (Grace Sierra Co., Milpitas, Calif.) on 22 Apr. 1997 and 21 Apr. 1998. The flats were covered with plastic to retain moisture and placed in a germination room at 32 °C for 2 d then moved into a greenhouse where they were kept under natural photoperiod and day/nights of $29 \pm 5/25 \pm 5$ °C. Seedlings were watered as needed with tap water and fertilized twice with 50 mg·L⁻¹ of a 20N–8.6P–16.6K water soluble fertilizer.

Before the onset of cold stress, the cell flats were cut into quarters with each section containing eight transplants for transplant growth analysis and eight seedlings for field planting. Each quarter section represented one replication. The seedlings were 18-days-old at the time of first cold stress imposition. To obtain the required cumulative hours of cold stress ranging from 3 to 81 h, plants were exposed to 2 °C of varying duration and frequency. Cold stress started 9 and 8 May (17 d after seeding) and ended 19 and 18 May (27 d after seeding) in 1997 and 1998, respectively. Plants were moved into dark walk-in coolers during the night, exposed to cold temperatures for the prescribed time, and then returned to a warm greenhouse. Cold stress durations in 1997 and 1998 were 3 h (from 2100 to 2400 HR), 6 h (from 2400 to 0600 HR), and 9 h (from 2100 to 0600 HR). Cold stress frequencies or repetitions of exposure were 1, 3, 6, or 9 d in 1997 and 3, 6, or 9 d in 1998. The combination of duration and frequency produced

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12 and 9 cumulative cold stress treatment combinations in 1997 and 1998, respectively. All cold stress treatments were timed to terminate on the same day to provide uniform transplant data collection and same-day field planting. Additionally, two control treatments were included: 1) noncold stressed plants of the same chronological age as cold stress plants (older control); and 2) noncold stressed plants at the ideal transplant size at the time of transplanting (second true leaf stage), but 9 d younger than the older control (young control). To produce the second control, two flats of seeds were sown 9 d later than the first control on 2 May 1997 and 1 May 1998 using the same procedures as mentioned previously. Since it was expected that the older control would become overgrown and leggy, the purpose of the younger control was to compare performance of an ideal-sized transplant to the older, noncold-stressed transplant. The experimental treatments were replicated four times in 1997 and six times in 1998 with all trays arranged in a randomized complete block design in the greenhouse.

On 19 May 1997 and 18 May 1998, eight plants from each treatment were harvested for transplant growth data. The following growth variables were measured: leaf area per seedling (including petioles) as measured with a leaf area meter (LI-3100; LI-COR, Lincoln, Nebr.), seedling height (measured from the medium surface to the approximate apical meristem tip), shoot and washed root fresh weight (FW) (cut at the medium surface) and dry weight (DW) (dried at 65 °C for 48 h). Leaf chlorophyll content of the youngest fully expanded leaf on each plant was determined using a SPAD chlorophyll meter (SPAD 502; Minolta Co., Osaka, Japan) and expressed in SPAD units defined by the manufacturer as “1” equivalent to very pale green coloration (chlorotic) and “50” equivalent to very dark green coloration. Sucrose content of dried watermelon seedlings was determined on ground samples using the Anthrone method as described by Morris (1948).

Data were subjected to analysis of variance (ANOVA) to determine the significance of each cold stress factor (duration, frequency, and their interactions). To determine effects of cumulative hours of cold temperature stress on watermelon seedling growth, data were pooled over duration and frequency treatments. Polynomial regression analysis was performed between seedling growth variables and cumulative hours of cold exposure to determine the significance and strength of relationships. Only data from the older controls were included in the regression analysis excluding young control data. Data not meeting assumption of normality were transformed using the logarithmic transformation before analysis. Orthogonal contrasts were used to determine growth differences between the young and old controls.

COLD STRESS VS. EARLY FIELD GROWTH, EARLY AND TOTAL YIELD, AND FRUIT QUALITY. ‘Carnival’ watermelon seedlings were grown as described previously and subjected to the same cold stress treatments. Eight transplants from each treatment replication were hand-transplanted into the field at the Clemson University Coastal Research and Education Center, Charleston, S.C., on 19 and 18 May in 1997 and 1998, respectively, after all risk of frosts had passed. The last average frost date for the area is 21 Mar. The soil type was Yonges loamy fine sand, an Aquic Hapludult. Soil tests from respective fields were taken each year before planting and the fields were then fertilized with N,P, and K at 175, 75, and 146 kg·ha⁻¹, respectively, according to soil test recommendations. The field was limed with Ca and Mg at 146 and 88 kg·ha⁻¹, respectively, from dolomitic limestone in 1997 per soil tests, but

no liming was necessary in 1998. Beds on 1.8 m centers were fumigated with 75% methyl bromide and 25% chloropicrin at the rate of 450 kg·ha⁻¹ and covered with 0.032 mm black plastic. The treatment plots consisted of one row, 9.6 m long and contained eight plants. Each treatment plot was replicated four times in 1997 and six times in 1998 and arranged in a randomized complete block design. The plots were irrigated as needed. Tensiometers (20 cm deep) were used to determine 15% soil moisture depletion to signal the start of a 1-h irrigation cycle. Pesticide applications were based on standard commercial recommendations (Cook, 1996).

Effects of cold stress on early stand establishment in the field were monitored. Transpiration was recorded twice in 1997 (1 or 4 d after transplanting) and three times in 1998 (2, 4, or 7 d after transplanting) with a steady-state autoporometer (LI-1600) during the warmest time of a clear day (between 1200 and 1400 HR). Transpiration was measured on the last fully expanded leaf of the second and third plant within each treatment plot. Days to vining (date first runner touched the ground), detection of first male and female flowers, and first fruit set (>3 cm in diameter) were recorded daily for the second, third, and fourth plants of each test row.

Watermelons were harvested for the first time on 17 July 1997 and 9 July 1998 and these first harvests were classified as early yield. Second and third harvests were 22 and 29 July 1997 and 16 July and 23 July 1998, respectively. On the last harvest, all fruit were removed from the plants. Each fruit was weighed and graded as marketable or cull according to USDA standards (U.S. Dept. of Agriculture, 1978). The minimum marketable weight was 5 kg/fruit and misshapen fruit or fruit <5 kg were classified as culls.

To determine effects of cold temperature exposure on fruit quality, a sub-sample of three fruit in 1997 (one from each harvest) and five fruit in 1998 (one fruit from first harvest and two fruit from second and third harvests) from each treatment plot were randomly selected. These fruit were cut open and length, width, and rind thickness measured. Soluble solids were measured with a hand-held refractometer on a sample taken from the center of the each fruit. Data analysis was performed as described previously.

It was expected that cold stress would reduce watermelon yield significantly, and usually the researcher or industry sets a tolerance level of minimally acceptable yield reductions. In the present study, a 10% yield reduction was chosen as the maximum tolerable yield reduction and >10% losses were considered unacceptable. To calculate this, the intercept of the model equation determined from polynomial regression was equivalent to the yield of noncold stressed plants. If the yield of any treatment was <90% of noncold stressed yield, then that particular treatment suffered a 10% yield reduction. The same procedure was used to determine the tolerance levels for 15% and 20% if a 10% tolerance level for yield reduction is considered too stringent.

Results

COLD STRESS VS. TRANSPLANT GROWTH. ANOVA revealed that duration and frequency cold stress factors significantly affected every seedling variable. By termination of the experiment, duration, frequency or their interactions did not affect any yield variables. From these data, cumulative cold stress treatments were derived to produce the total cold stress hours experienced. Analysis of cumulative cold stress hours indicated significant yield reductions, indicating that total cold stress had a more potent

influence on growth and yield than how cold stress was derived by manipulating duration and/or frequency. Therefore, only cumulative cold stress data will be presented.

In 1997, most of the seedling growth variables were affected significantly by increasing hours of cold stress (Fig. 1). As cold stress increased from 0 to 81 h, shoot FW (Fig. 1A), leaf area (Fig. 1B), shoot DW (Fig. 1C), root FW (Fig. 1E), root DW (Fig. 1G) and sucrose content (Fig. 1H) decreased linearly. However, the relationship between seedling height and cold stress were inconsistent and nonsignificant (Fig. 1D). The response to cold stress was curvilinear for shoot FW (Fig. 1A). Shoot FW remained fairly stable up to 36 h of cold exposure, but decreased with continued exposure. Leaf chlorophyll content also decreased curvilinearly and leveled off at 54 h of cold exposure (Fig. 1F). As length of cold temperature exposure increased, the plants became progressively more chlorotic and sucrose content decreased (Fig. 1H).

Plants responded quite similarly in 1998 to the cold stress although there were some differences between the years. Shoot DW (Fig. 1C), root FW (Fig. 1E) and root DW (Fig. 1G) and

sucrose content (Fig. 1H) similar to 1997 all decreased linearly with increasing cold stress. Leaf area (Fig. 1B), chlorophyll content (Fig. 1F), and seedling shoot FW (Fig. 1A) decreased curvilinearly as cold increased to 54 h but this response leveled off with longer periods of cold stress. The relationship between cold stress and seedling height was completely different in 1998 than in 1997. Seedling height declined linearly with increasing hours of cold stress with plants 60% shorter than the older controls after exposure to 81 h of cold (Fig. 1D).

COLD STRESS VS. EARLY FIELD GROWTH, EARLY AND TOTAL YIELD, AND FRUIT QUALITY. Changes in transpiration patterns are considered markers of physiological activity and in this study, they were considered indicators of either recovery or biochemical disturbances. In both years, as cold stress increased, transpiration increased linearly (Fig. 2A). In 1998, the R^2 value for the relationship between cold stress and transpiration was lower than in 1997 probably due to climatic differences between the 2 years. Average leaf temperature during the transpiration measurements in 1997 was 29 versus 36 °C in 1998. Since transpiration rate is very closely related to temperature, these higher temperatures apparently increased transpiration rates.

Early plant growth in the field differed each year in response to cold stress. As cold stress increased from 0 to 81 h in 1997, days to vining (Fig. 2B) and first female flower (Fig. 2C) increased linearly and were delayed by 5 to 6 d, respectively. The response to cold stress on days to first male flowering was curvilinear, remaining fairly constant up to 36 h of cold stress, but then flowering delays increased as cold stress hours increased (Fig. 2D). Despite these differences, days to first fruit were unaffected by cold stress (Fig. 2E). In contrast, all these variables in 1998 increased linearly with increasing hours of cold stress. Plants exposed to 81 h of cold stress vined about 5 d later than the controls (Fig. 2B). Male (Fig. 2D) and female (Fig. 2C) flowers were 6 and 4 d later than the controls, respectively, but first fruit set (Fig. 2E) was delayed only 2 d.

In both years, as the growing season progressed, the effect of cold stress became less apparent as the plants developed towards fruit set (Fig. 2E). Especially during the second year of the study, the R^2 values became progressively smaller for later occurring growth events, indicating the effect of cold stress effects diminished and as a consequence, the cold-stressed plants eventually recovered. In both years, cold stress did not affect long-term, early melon production (data not presented) but, total yields decreased as a consequence of cold stress at

Fig. 1. Relationships between cumulative cold hours and watermelon seedling growth variables measured 27 d after seeding. (A) Shoot fresh weight (FW), (B) leaf area, (C) shoot dry weight (DW), (D) height, (E) root FW, (F) chlorophyll, (G) root DW, and (H) sucrose. Regression analysis was performed on individual seedling data ($n = 32$ in 1997 and 48 in 1998). NS, ***Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Fig. 2. Relationship between cumulative cold hours and watermelon early field growth. (A) Transpiration, (B) days to vining, (C) days to first female flower, (D) days to first male flower, and (E) days to fruit set. For transpiration data, $n = 16$ and 36 in 1997 and 1998, respectively. For flowering data, $n = 12$ and 18 in 1997 and 1998, respectively. NS, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01 , or 0.001 , respectively.

Extended periods of cold stress significantly delayed seedling growth suggesting the photosynthetic apparatus may have been damaged. Loss of photosynthate can be facilitated by reduced CO_2 uptake, loss of chlorophyll during and following cold temperature exposure or impaired oxidation-reduction chain linking Photosystem II to Photosystem I (Markhart, 1986). Hassell (1979) reported that 'Sugar Baby' watermelon seedlings, exposed continuously to 1 or 4.4°C for up to 60 h, became chlorotic and some seedlings died. Severe leaf yellowing and lowered chlorophyll levels observed in the present study suggest that increasing hours of cold stress might have interfered with chloroplast function.

Watermelon seedlings, subjected to long periods of cold stress, continued to transpire more soon after transplanting than the controls and those exposed to lesser cold stress hours. This suggests that the stomates may have been open during cold stress and had not yet fully recovered from cold stress even after 1 week in the field. Transpiration rate declines during low temperature stress due to rapid closure of stomates in an attempt to prevent water loss and wilting (Tognoni, 1990; Wilson, 1976). However, transpiration depends largely on the pre-chilling status of the stomates. Guye and Wilson (1987) reported that mung bean [*Vigna radiata* (L.) R. Wilcz.] stomates exposed to 0°C did not

close if they were already open at a temperature of 0°C , nor did they open if they were already closed. Irigoyen et al. (1996) reported that corn seedlings chilled at 5°C for 5 d, transpired 70% more than nonchilled control plants at the end of chilling period and for 5 d following chilling. They attributed the higher rates of transpiration of chilled plants to locking of stomates in the open position during chilling. In our study, cold stress affected field growth of watermelons differently by year. Excessive rainfall during flowering in 1997 (Table 1) probably inhibited bee activity, delaying pollination or causing abortion of female flowers and fruit, resulting in cold stressed plants setting fruit at the same time as the control plants. In contrast, the weather in 1998 was ideal for watermelon growth with timely rainfall and optimal temperatures for growth and development. Differences in plant growth induced by cold stress at transplanting still lasted to fruit set although these differences became progressively smaller as the growing season progressed.

Flower initiation in melons usually starts within 3 weeks after cotyledon expansion and is related to temperature. Warmer temperatures enhance flower development, but temperatures both below and above optimal, can delay flower initiation of many species (Moe and Heins, 1990). Since watermelon seedlings in our study were cold stressed during the second and third week of growth, low temperatures may have delayed flower primordia formation in the meristems. Hassell (1979) reported that exposing 'Sugar Baby' watermelon seedlings to days/nights of $21/10^\circ\text{C}$ for 8 d, delayed vine growth compared to seedlings exposed to $21/18^\circ\text{C}$. Our results agree with the aforementioned studies, but it is important to note that cold stress affected fruit set minimally.

Discussion

Cyclic cold stress significantly retarded watermelon seedling growth. Increasing the exposure to 2°C , progressively reduced watermelon seedling shoot and root growth, leaf area, and carbohydrate content in both years. Our findings are similar to those reported on relative growth rate of corn (Wolfe, 1991), cucumber leaf area (*Cucumis sativus* L.) (Bulder et al., 1987), watermelon shoot FW and DW (Bradov, 1990), cucumber and squash (*Cucurbita pepo* L.) root FW and DW (Reyes and Jennings, 1994), and muskmelon seedling height and sucrose content (Korkmaz and Dufault, 2001).

the time of transplanting (Fig. 3). In 1997, as the hours of cold stress increased, neither average fruit weight (data not presented) nor number of fruit produced per plot (Fig. 3A) decreased significantly; however, total yields decreased linearly (Fig. 3B). In contrast, average fruit weight in 1998 (data not presented) was unaffected by cold stress, but fewer fruit were produced per plot (Fig. 3A) and this effect significantly lowered yields linearly with increasing cold stress (Fig. 3C). The necessity to use a younger control versus an older control was not warranted since both controls yielded similarly and more than cold stressed plants (Fig. 3).

Yield of culls was unaffected by cold stress in both years (data not presented). Additionally, cold stress did not affect fruit quality, specifically fruit length, diameter, and rind thickness in either year (data not presented). Fruit soluble solids decreased linearly in 1998 as cold stress increased from 0 to 81 h, but this effect was considered negligible (data not presented).

Fig. 3. Relationship between cumulative cold hours and watermelon (A) total fruit number and total yield for (B) 1997 and (C) 1998. Only data from old control plants are included in the regression analysis; however, data for the young control plants were also shown for comparison purposes. Regression analysis was performed on replication means ($n = 9$ in 1997 and 7 in 1998). NS, ***, **** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

First fruiting of plants exposed to 2 °C for 81 h occurred only 2 d later than control plants and by fruit set, the effect of cold stress had mostly disappeared. Although cold stress did not affect early yield statistically, total yields decreased linearly with cold stress in both years, becoming more severe with increasing cold stress. For

example, total yields decreased 10% with 38 to 40 h of cold stress in 1997 and 1998, respectively (Fig. 3B and C). Additionally, a 20% reduction in yields occurred with 76 to 79 h of cumulative cold stress in 1997 and 1998, respectively. Yields were significantly higher for all treatments in 1998 than in 1997, due to more ideal growing conditions in 1998. Our study revealed that the older and younger control plants yielded similarly in both years and strictly requiring younger transplants for commercial watermelon production is not necessary. Vavrina et al. (1993) reported that transplant age of watermelon had no effect on early and total yields. In another study, NeSmith (1993) reported that 30-d-old squash transplants produced slightly higher yields than 10-d-old transplants.

Few studies describe the effects of low temperatures on watermelon growth and yield. Hassell (1979) found that exposing 2-week-old 'Sugar Baby' watermelon seedlings to 10 °C during a 9 h night for 8 d reduced yield 20% compared to plants exposed to 18 °C during the night. Hall et al. (1993) reported that 'Crimson Sweet' watermelons planted 28 Apr., yielded more fruit than those planted 1 Apr., due to 12 °C warmer soil temperatures during the later planting. They also suggested that the risk of cold injury with early plantings makes later transplanting of watermelons more desirable. Even though the relationship between total yield and cumulative cold hours was not very strong in the present study, 'Carnival' watermelon should not be planted very early in the field if there is great risk of low temperature injury. Since we only evaluated 'Carnival', it is unknown if other watermelon cultivars will behave similarly under the conditions of this research.

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Table 1. Summary of weather data for the 1997 and 1998 growing seasons of watermelon at the Clemson University Coastal Research and Education Center, Charleston, S.C.²

	May		June		July	
	1997	1998	1997	1998	1997	1998
Rainfall (mm)	3	6	343	76	216	173
Temperatures (°C)						
Extreme minimum	14.4	17.7	10.7	11.6	17.7	20.5
Mean minimum	18.1	21.9	19.4	22.4	22.2	23.8
Extreme maximum	30.5	33.8	32.7	37.8	35.5	36.6
Mean maximum	26.4	31.4	28.3	34.4	32.0	33.8
Mean	22.3	26.7	23.8	28.3	27.2	28.8

²Data represent only the days the experiment was conducted in the field.

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