Chilling injury is a physiological disorder of many agricultural crops, especially those species of tropical and subtropical origin. In those plants, exposure to nonfreezing temperatures below about 12 °C induces, among other symptoms, surface pitting, accelerated water loss, increased disease susceptibility, reduced growth, and loss of seedling vigor (Saltveit and Morris, 1990). The extent of injury is governed not only by the species and cultivar (Cabrera et al., 1992) and the temperature and duration of exposure, but also by the stage of tissue development and its prior exposure to stresses (Collins et al., 1993; Lafuente et al., 1991; Saltveit, 1991). For example, 5-mm cucumber seedling radicles are more chilling sensitive than shorter radicles (Rab and Saltveit, 1996), and a previous heat, cold, or ethanol shock increased the tissue’s tolerance to subsequent chilling (Jennings and Saltveit, 1994).

Gravitropic responses, which determine the direction of root growth, are of great importance in the spatial distribution of roots in the soil (Nakamoto, 1993) and in the ability of roots to extract available soil moisture and nutrients (Nakamoto et al., 1991). Despite the importance of gravitropic responses in stand establishment and plant growth, little attention has been given to the environmental factors that may affect the gravitropic response of young seedlings. Among the environmental factors that affect gravitropism, temperature (Fortin and Poff, 1991), soil water content (Nakamoto, 1993; Takahashi and Scott, 1991), and osmotic shock (Leopold and LaFavre, 1989) have received some attention.

The roots of young seedlings are exposed to a variety of abiotic stresses during their early stages of development (Bennett et al., 1992), and effective stand establishment depends on their ability to withstand and respond appropriately to these various stresses (Helm et al., 1989). Thus, it is important that the effects of stress on gravitropism be elucidated to understand the growth and development of stressed roots. The studies reported in this paper were conducted to determine the effect of chilling stress and of a prior heat shock on the gravitropic response of cucumber radicles.

**Materials and Methods**

**Plant material.** Seeds of chilling sensitive ‘Poinsett 76’ cucumber were imbibed in aerated water overnight at 25 °C. After imbibition, the seeds were transferred to moist paper toweling over a layer of capillary cloth that was sandwiched between two 12 × 29-cm Plexiglas plates held together with rubber bands. The seeds were oriented normally so that the emerging radicle would grow downward. The plates were held in a vertical position at 25 °C in a humid, ethylene-free atmosphere until the radicles had grown to the appropriate length, at which time selected seedlings were transferred to smaller 7 × 14-cm Plexiglas units assembled as before.

**Temperature treatments.** After the selected germinated seeds had been transferred to the freshly assembled 7 × 14-cm Plexiglas units, they were held for a few hours at 25 °C. The units were then rotated 0° or 90° and transferred to a new temperature ranging from 2.5 to 25 °C. It took about 60 min to cool the seeds in the assembled Plexiglas units from 25 to 2.5 °C or to warm them from 2.5 to 25 °C (Rab and Saltveit, 1996). The degree of curvature was periodically determined at the treatment temperature. In other low temperature experiments, germinated seeds were held for various durations at 2.5 to 12.5 °C in their normal vertical orientation before they were transferred to 25 °C and rotated 90°.

Germinating seeds and seedlings were heat shocked by carefully transferring them from the Plexiglas units to plastic 15 × 100-mm-diameter Petri dishes containing one layer of Whatman no. 1 filter paper moistened with 2 mL of water. The dishes were floated on 45 °C water for various durations to heat shock the germinating seed. It took about 60 s to heat the seeds from 25 to 45 °C or to cool them from 45 to 25 °C (Rab and Saltveit, 1996). The heat-shocked seeds were held at 25 °C for 2 h before they were transferred to a fresh 7 × 14-cm Plexiglas unit and held at 2.5 or 25 °C.

**Measurement of gravitropic response and chilling injury.** The length and curvature of the radicles were measured through the clear Plexiglas with a clear plastic ruler to the nearest millimeter and with a clear plastic protractor to the nearest degree immediately before and after chilling, and periodically during subsequent holding at 25 °C. The extent of chilling injury was measured as the inhibition of radicle growth and/or curvature at 25 °C subsequent
to chilling (Jennings and Saltveit, 1994; Rab and Saltveit, 1995).

Experimental design. All experiments were repeated at least three times with similar results. In each experiment, each treatment was applied to at least two plates containing 15 seeds each. Experiments used a completely randomized design, and data were analyzed by analysis of variance. When significant differences were detected, LSD values were calculated at the 0.05 level.

Results

The radicles began to curve almost immediately upon rotating the germinating seeds 90°, and the rate of curvature slowed as the radicle tip approached a vertical orientation (Fig. 1A). The curvature of radicles initially about 1 mm in length increased linearly for the first 6 h before abruptly changing to a slower, but still linear increase for the remaining 6 h (14° h) for the first 6 h, contributing 91% of the maximum response; then 1.4° h for the remaining 6 h, contributing the remaining 9% of the maximum curvature). In contrast, curvature of radicles initially 3 to 9 mm in length increased in a curvilinear fashion during the entire 12 h of the experiment. Radicles with initial lengths of 3, 5, 7, and 9 mm had rates of curvature during the first 2 h of 19.9°, 19.0°, 13.0°, and 11.5° h, respectively, and these curvatures contributed 49%, 50%, 37%, and 34% of the response at 12 h. The curvatures attained after 12 h incubation were significantly higher for the radicles initially at 1 mm (i.e., 93°) than for the 3- or 5-mm radicles (i.e., 82° and 76°, respectively). The difference between the 3- and 5-mm radicles, however, was not significant at either 2 or 12 h. Radicles initially at 7 and 9 mm had significantly less curvature than the 3- and 5-mm radicles at 2 and 12 h. The final degree of curvature at 12 h was related to the initial length of the radicle by the following linear equation: degree of curvature after 12 h = 93.6° – (3.18° x length) with an r² of 0.86.

The 90° rotation by itself did not significantly reduce the rate of radicle elongation (Fig. 1B). The differences in radicle length between the normally oriented and horizontally rotated radicles were insignificant for radicles with initial lengths of 1 and 3 mm. The differences became steadily greater as the initial radicle length increased from 5 to 9 mm, but it never became significantly different. An initial radicle length of 5 mm was selected for all subsequent experiments because the radicles exhibit maximum chilling sensitivity at that length (Rab and Saltveit 1995) and the rates of curvature for radicles around 5 mm were more uniform than longer radicles (Fig. 1A).

Insignificant levels of curvature were observed in radicles exposed to chilling temperatures of 2.5 or 5.0 °C for the 48 h duration of the experiment (Fig. 2A). In contrast, the curvature of radicles held at 10 to 25 °C were significant and increased linearly with increasing temperature. At these nonchilling temperatures, the curvature at 12 h is given by the following linear equation: curvature = –14.2° + (3.46° x temperature) with an r² of 0.97. The curvature at 48 h is given by this equation: curvature = –12.4° + (4.07° x temperature) with an r² of 0.95. The change in curvatures from 12 to 48 h are given for each temperature by the following equations: for 5.0 °C, curvature = 0.415° + (0.0075° x h) with an r² of 0.90; for 10 °C, curvature = 8.44° + (0.416° x h) with an r² of 0.98; for 15 °C, curvature = 18.7° + (0.713° x h) with an r² of 0.99; for 20 °C, curvature = 46.2° + (0.546° x h) with an r² of 0.98; and for 25 °C, curvature = 67.5° + (0.321° x h) with an r² of 0.87. As can be seen from an inspection of these equations and of Fig. 2A, the Y-intercept and the slope show an abrupt break between 5.0 and 10 °C, the approximate break previously shown to be between chilling and nonchilling temperatures for cucumber fruit and seedlings (Cabrera and Saltveit, 1990; Jennings and Saltveit, 1994).

Thermal time is the duration of exposure in hours at a specified temperature (T) times the specified temperature minus a base temperature (Tb); the units of thermal time are degree hours (Dahal et al., 1990). Data in Fig. 2A were plotted versus thermal time. A base temperature of 6 °C was used since it gave the greatest r² value for a plot of curvature versus thermal time for the 10 to 25 °C data (Fig. 2B, insert). The plot of curvature versus thermal time revealed two major periods of response. During the first 12 h at 10 to 25 °C, the degree of radicle curvature increase linearly (r² = 0.996) as a function of thermal time (Fig 2B; the line connecting the left-hand data point for each temperature). The rate of curvature after 12 h was then increased. The increase was roughly linear for each temperature whether plotted versus time (Fig. 2A) or versus thermal time (Fig. 2B).

Radicles that were initially 5 mm in length were exposed to chilling or nonchilling temperatures (e.g., 2.5 to 12.5 °C) for 0 to 72 h and their recovery was studied for an additional 36 h at 25 °C. The effect of chilling was most obvious during the first 12 h of recovery (Fig. 3A). The degree of curvature measured at this time decreased as the duration of exposure to all temperatures increased. The degree of curvature after 12 h at 25 °C was decreased.
by 29%, 36%, 20%, and 8% compared to the 25 °C control by 24 h exposure to 2.5, 5.0, 7.5, or 10 °C, respectively. Exposure to these same temperatures for 48 h reduced curvature recorded after 12 h at 25 °C by 62%, 66%, 46%, and 9%. The marginal reduction in curvature at 10 °C indicates that up to 48 h exposure to this temperature did not induce a significant level of chilling injury.

The linear equations describing the effect of duration of temperature exposure on subsequent curvature at 25 °C are given below. The degree of curvature with increasing duration of exposure to chilling temperatures = 57.0° – (0.75° × h exposure) with an \( r^2 \) of 0.97 for 2.5 °C, 59.3° – (0.78° × h) with an \( r^2 \) of 0.96 for 5.0 °C, and 60.7° – (0.57° × h) with an \( r^2 \) of 0.98 for 7.5 °C. At the marginal chilling temperature of 10 °C, the equation is 61.3° – (0.31° × h) with an \( r^2 \) of 0.78. While the Y-intercept, i.e., the curvature from unstressed seeds, remains close to the projected average of 60° ± 2° for all the temperatures used in this experiment, the slope describing the incremental contribution of each additional hour of exposure to the reduction in subsequent curvature declined from an average of 0.76°/h for 2.5 and 5.0 °C to 0.57°/h for 7.5 °C and to 0.31°/h for 10 °C. The \( r^2 \) also decreased from an average of 0.97 for 2.5 to 7.5 °C, to 0.78 for 10 °C.

The decrease in the slope indicates that increased durations of exposure were having less of an effect on reducing subsequent curvature as the nonchilling temperatures were approached, while the decreased \( r^2 \) value indicates that the response was becoming nonlinear. This increased variability was probably caused by the differential recovery from chilling, specifically because the reduction in curvature at 10.0 and 12.5 °C was only significant after 72 h of exposure. These data again suggest that 10 °C may be near the threshold chilling temperature for gravitropic curvature.

After recovering for 24 h at 25 °C, differences in the inhibition of curvature caused by 24 h exposure to 2.5, 5.0, and 7.5 °C became nonsignificant (Fig. 3B). Most of the curvature that would occur had occurred by 24 h since there was no significant difference between curvatures at 24 and 36 h for any duration of exposure or for any temperature, except for an increase in curvature for radicles exposed to 7.5 or 10.0 °C for 72 h (Fig. 3C). Radicles held at 10.0 or 12.5 °C for 72 h showed a significant decrease in curvature to 58% and 70% of the controls. After recovery at 25 °C for 36 h, the curvature of radicles was still significantly 10% to 12% less than the controls.

There was no elongation of the radicles at either 2.5 or 5 °C (Fig. 4A). Radicles elongated to 7.3, 9.7, 20.4, and 29.9 mm during 72 h at 7.5, 10.0, 12.5, and 15 °C, respectively. The linear regression equation for radicle length after 72 h at 7.5 to 15 °C is as follows: radical length (mm) = –18.5 + (3.14 × temperature), with an \( r^2 \) of 0.98.

**Fig. 2.** Effect of temperature on the gravitropic curvature of cucumber radicles. (A) Seedlings with 5-mm-long radicles were rotated 90° when transferred to 2.5, 5.0, 10, 15, 20, or 25 °C. The degree of curvature was measured every 12 h for 48 h. The vertical bar represents the 5% LSD value. (B) Relationship between the degree of curvature and the accumulated thermal time calculated as \( T – Tb \times \text{time} \), using \( Tb = 6.0 °C \). (Insert) Correlation coefficients versus the base temperature for calculated values of termal time.

**Fig. 3.** Effect of prior temperature exposure on subsequent curvature. Seedlings with 5-mm-long radicles were held in a vertical orientation at 2.5, 5.0, 7.5, 10.0, or 12.5 °C for 0, 24, 48, or 72 h. They were then rotated 90° after transfer to 25 °C. The degree of curvature was measured after 12 (A), 24 (B), and 36 (C) h at 25 °C. The vertical bar represents the 5% LSD value.
0.95. A similar trend was observed for the final length attained after an additional 12 h at 25 °C (Fig. 4B). The linear regression equation for radicle length after 72 h exposure to 7.5 to 15 °C and an additional 12 h at 25 °C is as follows: radical length (mm) = – 22.6 + (4.36 × temperature), with an $r^2$ of 0.97. The dramatic change in slope at 10 °C for the lines describing elongation as a function of temperature for the initial readings (Fig 4A) and the final readings (Fig 4B) again indicate that 10 °C is the division between chilling and nonchilling temperatures for cucumber radicles. The same break in the slopes at 10 °C can be seen for the 48- and 72-h treatments when differences between the final and initial lengths are plotted (Fig 4C).

When measured 12 h after chilling treatment, elongation and curvature were significantly reduced by 18 h of chilling (Fig. 5A). In contrast to radicle elongation, which was inhibited 46% by 18 to 24 h of chilling and 57% by 30 to 48 h of chilling, inhibition of radicle curvature continued to increase as the duration of chilling increased from 12 to 48 h. The linear equation describing the relation between subsequent curvature and chilling at 2.5 °C for 12 to 48 h is as follows: curvature = 75.2° – (1.41° × h chilling), with an $r^2$ of 0.90. As the tissue recovered from chilling, a different pattern was observed when the lengths and curvatures were measured after 24 h at 25 °C (Fig. 5B). While the longer periods of chilling from 24 to 48 h still caused a uniform reduction in growth (i.e., a 62% reduction from 3.01 to 1.13 cm), shorter periods from 0 to 24 h caused a progressive reduction in growth described by the following linear equation: length = 3.01 – (0.071 × h chilling) with an $r^2$ of 0.97. Curvature measured after 24 h of recovery at 25 °C was again unaffected by 0 to 12 h of chilling, while curvature was decreased by 18 to 48 h of chilling and is described by this linear equation: curvature = 72.8° – (0.98° × h chilling) with an $r^2$ of 0.90 (Fig. 5B). By 36 h after transfer to 25 °C, the tissue had partially recovered and the length and curvature appeared unaffected by 0 to 12 h of chilling (Fig. 5C). In contrast, increased durations of chilling from 12 to 48 h caused a progressive decline in length and curvature. The decrease in length is given by the following linear equation: length = 4.18 – (0.06 × h) with an $r^2$ of 0.96. The decrease in curvature caused by 12 to 48 h of chilling is given by this linear equation: curvature = 83.9° – (1.02° × h) with an $r^2$ of 0.98.

While subsequent radicle elongation was reduced by heat-shock treatments of up to 10 min exposure to 45 °C (data not shown), such treatments did not significantly reduce the subsequent curvature for nonchilled tissue held at 25 °C for 12, 24, or 36 h (Fig. 6A). The maximum curvature of around 68° ± 2.6° was attained after 36 h, but over 90% ± 5% of that curvature had occurred within the first 12 h (Fig. 6A).

Heat shocks of 4 to 10 min significantly reduced the inhibitory
effect of 72 h of chilling at 2.5 °C on gravitropic curvature measured 12, 24, and 36 h after chilling (Fig. 6B). While the curvature of chilled radicles was always significantly less than that of the unchilled controls, the extent of inhibition for radicles heat shocked for 4 to 10 min declined from 79% at 12 h (60.9° ± 3.3° to 12.7° ± 1.4°), to 47% at 24 h (66.8° ± 2.0° to 35.4° ± 2.0°), and to 35% at 36 h (67.9° ± 1.8° to 44.0° ± 1.4°). When the differences between chilled and unchilled radicles are compared over durations of heat shock and recovery (Fig. 6C), it was apparent that the beneficial effect of heat shock are expressed to a greater extent as the time after chilling increased.

### Discussion

The rapid and linear increase in curvature of gravistimulated 1-mm radicles was in contrast to the curvilinear increase exhibited by radicles initially 3 to 9 mm in length (Fig. 1A). The interaction between initial radicle length and gravitropic curvature is shown by observation that the curvature rarely reaches 90° in longer radicles. It was observed that the curvature of longer radicles usually settled somewhere between 60° and 75°, and that there was a decrease in the rate of curvature as radicles approached a vertical orientation (Fig. 1A). This decrease in curvature is to be expected since the gravistimulus is a function of time and mass acceleration, and a decreasing angle of stimulus would result in decreasing acceleration (Volkmann and Sievers, 1979). However, if the decrease in the rate of curvature as the radicle approached a vertical orientation were the only factor influencing gravitropism, the radicle would have reached 90° given enough time. Experimental evidence suggests that when maize roots are gravistimulated, the initial inhibition of growth on the lower side and promotion of growth on the upper side of the curve is gradually reversed (Ishikawa et al., 1991), probably due to decreasing hormonal gradients or to changes in the auxin sensitivity of the tissue on alternate sides of the curve (Evans, 1991). These changes in hormonal effects may explain why the curvature is always <90°. Such changes, however, seems to be related to certain developmental stages; otherwise, the 1-mm gravistimulated radicles would not have behaved differently (e.g., reaching 90°) than the 5- or 7-mm-long radicles (Fig 1A). It is also likely that a curvature <90° may be an optimal growth angle as roots get older. A curvature <90° may be advantageous in exploring the soil laterally from the plants. Determination of whether the decrease in curvature with increasing initial length was caused by decreased sensitivity of the tissue to hormonal gradients as proposed by Evans (1991), was beyond the scope of this study.

The initial response of 1-mm radicles to gravistimulation could also have been a manifestation of an altered pattern of response to stress since radicles about 1 mm in length grow slower than radicles ≥3 mm in length and are relatively less responsive to other abiotic stresses (Irvine and Price, 1983; Jennings and Saltveit, 1994).

The decrease in curvature at lower temperatures could simply be a function of reduced growth, and that if provided sufficient time the curvature at all temperatures would eventually reach values comparable to that at 25 °C. This hypothesis was tested by determining whether curvature would be similar after the same accumulated thermal time (degree-hours). Curvature during the first 12 h was linear with thermal time (with a base temperature of 6.0 °C; Fig. 2B). This linearity indicated that the rate of curvature was simply a consequence of heat-units accumulated at each temperature. In contrast, the different slopes for the curvature versus thermal time for each temperature, and the lack of linearity between curvature and thermal time for other duration of exposure indicated that temperature was having a more profound effect on the geotropic response than a simple temperature related reduction in growth. For example, 200 hour-degrees produced 29°, 34°, 54°, and 66° of curvature at 10, 15, 20, and 25 °C, respectively.

Chilling is hypothesized to cause membrane dysfunction (Lyons, 1973), microtubule depolymerization (Rikin et al., 1983), and calcium redistribution (Minorsky, 1985). Some of these changes (e.g., microtubule depolymerization and calcium redistribution) are also related to the gravitropic response (Pooviah and Fendren, 1987; Blancaflor and Hasenstein, 1993). It was therefore not surprising that chilling greatly reduced the degree of curvature.

Holding 5-mm cucumber radicles for up to 72 h at different temperatures revealed two types of responses. Radicles exposed to 2.5 to 12.5 °C for up to 72 h had subsequent curvatures that were positively correlated with the holding temperature and negatively correlated with the duration of exposure (Fig. 3). In the chilling range of 2.5 to 10 °C, there was a progressive decrease in chilling induced loss of gravitropic curvature (Fig 3 A and B). However, there was also a loss of curvature at 25 °C when seedlings were previously held at nonchilling temperatures. That this decrease in curvature was not due to chilling is evident from Fig. 4, where the...
length at the holding temperature (Fig. 4A) and the length after an additional 12 h at 25 °C (Fig. 4B) show a clear break at 10 °C. In experiments in which the rotated radicles were incubated for 72 h at 12.5 °C, the curvature of longer radicles was again found to be less than for smaller radicles. The decrease in curvature at 12.5 °C may be due to some factor, other than chilling, brought upon by growth of the seedling.

Chilling appeared to have a greater inhibitory effect on radicle elongation than on curvature. During the first 12 h, elongation was maximally inhibited by 18 h at 25 °C, while curvature continued to decline with up to 48 h of chilling (Fig. 5). At later sampling times, chilling inhibition of both elongation and curvature became similar. Curvature is the result of a series of events: perception of gravity by the tip, production of a signal by the tip, transmission of the signal to the subapical zone of cellular elongation, and differential elongation of cells on opposite sides of the radicle to produce curvature. However, cells in the subapical zone may also be important in sensing and responding to gravity (Ishikawa and Evans, 1993). Radicle elongation does not rely on so many events, but does rely on the radicle tip for hormones that govern cellular elongation. A level of chilling that would render apical cells dysfunctional and disrupt the supply of growth regulators to subapical tissue, thereby inhibiting elongation, would produce less chilling injury in subapical tissue, which is more chilling tolerant than apical tissue (Rab and Saltveit, 1995). However, if subapical cells could sense and respond to gravity by the redistribution of endogenous plant growth regulators, then a chilling duration that would severely affect the tip and elongation would have much less of an effect on the gravitistimulated curvature of subapical tissues. As the tip recovered from chilling and became the source for growth regulators, the processes of radicle elongation and curvature would become similar. Our results support this scenario.

Heat-shock proteins constitute an endogenous protective mechanism that various organisms have evolved against environmental stresses (Burke and Orzech, 1988). Heat-shock-induced chilling tolerance is cycloheximide dependent (Jennings and Saltveit, 1994) and requires the synthesis of heat-shock proteins (Lafuente et al., 1991; Collins et al., 1994). In contrast to the slight growth retardation by heat shock in nonchilled tissue (Jennings and Saltveit 1994), gravitropic curvature was unaffected by a heat shock (Fig. 6A). This suggests that there is some degree of independence between the physiological control of radicle elongation and gravitropic curvature. The ability of a heat shock to reduce the chilling-induced loss of gravitropic curvature is similar to its ability to reduce the chilling-induced reduction in radicle elongation (Jennings and Saltveit, 1994).

While the degree of curvature of nonheat-shocked radicles did not increase greatly over time in radicles chilled for 72 h, it did increase significantly after 24 h in the heat-shocked radicles so that the curvature of radicles heat shocked for 4 to 10 min was three times greater than that of the nonshocked radicles. The degree of curvature, however, was about half of that of the nonchilled group (Fig 6B). These results clearly demonstrate that a brief heat shock induces chilling tolerance in cucumber radicles, as it does in other species and organs (Collins et al., 1993; Lafuente et al., 1991; Rab and Saltveit, 1996).

Since gravitropic curvature is dependent on a number of components (e.g., sensing gravity, redistribution of hormone(s), and subsequent changes in growth rates on the upper and lower sides of the tissue), the question arises as to which component or components are affected by chilling. We previously reported that the subapical tissue of 5-mm radicles is relatively less sensitive to 96 h of chilling that is apical tissue, and that subapical tissue can fully recover from this level of chilling injury (Rab and Saltveit, 1995). Thus it is unlikely that the less-severe chilling regime applied in the experiments reported in this paper (i.e., 72 h of chilling) could have contributed to the loss of curvature by an inhibition of elongation by subapical tissue. The subapical portion of the radicle, where most cell elongation takes place, is thought to play an important role in gravitropism (Ishikawa and Evans, 1993). In contrast to subapical tissue, tissue in the radicle tip, where most cell division takes place, is the most chilling sensitive part in young seedlings, and is also the site of gravity perception and of the hormonal signal(s) (Sack, 1991). The influence of chilling on gravitropic curvature is thus likely to be a combination of effects on the radicle tip, which perceives gravity and initiates a signal, and on the subapical zone, which translates the signal into the differential growth that produces curvature. Since chilling initially reduced radicle growth to a greater extent than curvature, the observed chilling-induced reduction in gravitropic curvature probably acts through the disruption of the flow of growth regulators from the tip to the subapical cells. Reduction in the amount transported would inhibit elongation, while disruption in the spatial distribution of growth regulators during their transport to the less sensitive subapical cells would impair the ability of subapical tissue to perceive and respond to gravity.

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