NOTES

VISUALIZATION OF FREEZING PROGRESSION IN TURFGRASSES USING INFRARED VIDEO THERMOGRAPHY

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ABSTRACT

Freezing injury can be a significant problem in turfgrasses. Understanding how freezing develops and ramifies throughout the plant could assist in the development of improved management or screening processes for cultivar improvement. The development of freezing injury is not well understood due partly to lack of technology to view freezing origin and progression of whole plants in real time. Perennial ryegrass (*Lolium perenne* L.) and supina bluegrass (*Poa supina* Schrad.) plants were incubated in either cold-acclimating or nonacclimating temperatures. Droplets containing ice-nucleating bacteria (*Pseudomonas syringae*) were placed on turf leaves, crowns, and roots. Plants were then subjected to progressively decreasing freezing temperatures in a controlled environment. An infrared thermal imaging radiometer (camera) was used to view ice initiation and propagation in whole plants in real time. Freezing always originated in roots, spreading acropetally and basipetally throughout connecting root tissues until it contacted the crown. Freezing was slow in the crown, then occurred rapidly upwards into shoots, then leaves. The time (i.e., temperatures) required for freezing was similar between the two species of nonacclimated plants. In acclimated plants, supina bluegrass roots froze earlier than roots of perennial ryegrass, though freezing times were similar for crown and leaf tissues. Ice-nucleating bacteria did not incite freezing of turf tissues. The project demonstrated the utility of infrared imaging for detecting freezing events in whole turfgrass plants. Results suggest that root tissue in the vicinity of the crown can be a source of ice which propagates into the crown and kills the plant.

Turf in cold climates can be injured or killed by subfreezing temperatures. The most common form of freezing injury occurs when intercellular water is frozen and the resulting decreased water potential draws water from adjacent cells, causing dehydration-related events (Jones, 1992). Intracellular ice formation or formation of intercellular ice crystals which puncture cells occurs rarely and usually only in nonacclimated or cold-sensitive plants (Jones, 1992). Less well understood is the process and sensitivities of freezing in turf tissues. Turfgrass crowns are the most critical part of the plant to protect from freezing because both roots and shoots originate from crown meristems. On the basis of the location of necrotic tissue following freezing, Beard and Olien (1963) reported the lower crown and associated roots of *Poa annua* L. plants were more susceptible to nonequilibrium freezing processes than the upper crown. Gusta et al. (1980) conducted electrolyte leakage tests of 'Fylking' Kentucky bluegrass (*Poa pratensis* L.) and determined leaf tissues were most cold tolerant (−40°C) followed by crowns (−28°C) and roots and rhizomes (−20°C). A subsequent study using three turfgrass species and 10 cultivars was unable to show roots had different freezing temperatures than crowns because electrolyte leakage from roots occurred continuously across the range of temperatures utilized (Rajashekar et al., 1983).

The origin and progression of freezing in turfgrasses is unknown and the information could be useful for developing improved management practices or cold-tolerant cultivars. The development of freezing in plants has been difficult to document because of the dynamic nature of freezing and lack of technology to observe freezing processes. Differential thermal analysis is the most widely utilized method for tracking freezing processes in plants and employs an array of thermocouples attached to plant tissues (Quamme, 1995). Scanning and freeze-fracture electron microscopy have been used to identify sites of ice occurrence in plants (Pearce and Willison, 1985; Malone and Ashworth, 1991). Nuclear magnetic resonance has been utilized to indirectly track ice formation and propagation in winter wheat (*Triticum aestivum* L.) by revealing unfrozen water in plant tissues (Millard et al., 1995). None of these techniques allow tracking freezing processes in whole tissues in real time while monitoring temperature.

Thermal imaging has recently been used to observe ice nucleation and freezing propagation in bean (*Phaseolus vulgaris* L.), barley (*Hordeum* spp.), and woody plants in vivo by sensing the latent heat of fusion developed as water froze (Wisniewski et al., 1997; Workmaster et al., 1999; Pearce and Fuller, 2001). Thermal imaging uses color to denote temperature differences, with black and purple representing the coldest temperatures and red and white representing the hottest temperatures. Our objective was to determine if infrared thermal imaging could be used to determine ice initiation and propagation in turfgrass plants.

MATERIALS AND METHODS

Plant Material

Fifteen-month-old field-grown plants of perennial ryegrass 'SR4200' and supina bluegrass 'Supranova' were collected from field plots in September 1998 at the O.J. Noer Turfgrass

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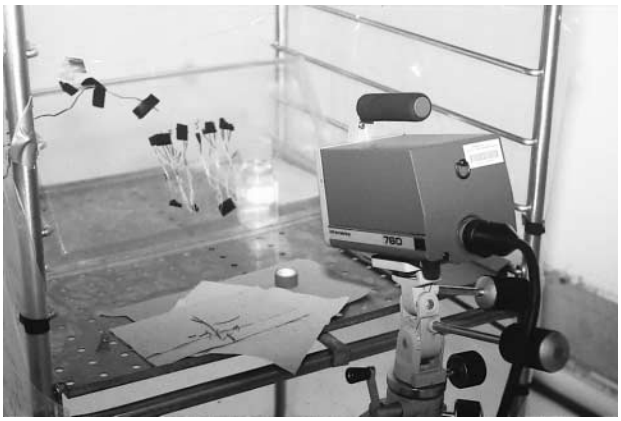


Fig. 1. Infrared thermal imaging radiometer and turfgrass plants in a controlled environment room.

Research Facility in Verona, WI. Field soil and debris were washed from the plants and roots were excised. Controlled environment rooms at the University of Wisconsin Biotron were used for acclimating and freezing plants. Plant crowns were immersed in an aerated, hydroponic system consisting of half-strength modified Hoagland's solution (Hoagland and Arnon, 1950). The plants were incubated at 10°C with a 12-h photoperiod (700 μmol m^{-2} s^{-1}) for 24 d. Following acclimation and root regrowth, half the plants were placed in a nonhardening regime (20/15°C, day/night) or a cold-hardening regime (5/2°C) for an additional 24 d.

Thermal Imaging

A sheet of transparent plexiglass was mounted vertically on a cart and placed in a controlled environment room. Perennial ryegrass and supina bluegrass plants from within an acclimation regime were removed from the hydroponic solution, rinsed with distilled water, and blotted dry with paper towel immediately before freezing tests. Plants were secured to the plexiglass with electrical tape and with roots exposed for viewing (Fig. 1). Transparent polyethylene sheeting was placed on the sides and behind the plant samples to minimize background temperature fluctuations caused by room air currents.

Plants were acclimated at 0°C for 20 min then inoculated with ice-nucleating bacteria (*P. syringae* strain Cit7). Axenic bacterial cultures on agar media were flooded with sterile distilled water and mixed with a sterile toothpick. Three drops (5 μL) of the bacterial suspension were placed on both leaf and root surfaces of each plant. Freezing tests began immediately following inoculation by decreasing cold room temperature from 0°C to as low as −28°C in 0.5°C increments at 3-min intervals. Three plants of both species were used during each test. Freezing tests were replicated four times. Plants were potted in a commercial potting medium and placed in the greenhouse to check for recovery.

An infrared thermal imaging radiometer (model 760, Inframetrics, North Billerica, MA) was used to observe ice nucleation and propagation of freezing processes in turf plants. The camera was placed in the controlled environment room to record and transfer images to a remote control and TV/VCR unit (Fig. 2). The temperature sensing range (color palette) of the camera was set at 2°C to provide maximum resolution. The color palette was adjusted to correspond with changes in room temperature. Prior to freezing plant tissue, the camera temperature was calibrated against a thermocouple across a range of cold room temperatures (0 to −11°C). A thermocouple was placed adjacent to the plants during freezing to provide reference temperature data for verification of camera readings. Videotapes were reviewed to determine time required for freezing processes and sequence of freezing events. Each plant was viewed individually to capture the sometimes subtle freezing events.

Statistical Analyses

Freezing times were analyzed using ANOVA in a completely randomized design with four replications. A factorial arrangement was used with species as the main plot and tissue types as subplots (MSTAT-C, 1988). Least significant difference values were calculated when F-values were significant for treatment effects.

RESULTS AND DISCUSSION

Room air temperatures sensed by the camera were within 1 to 2°C of temperatures measured with a thermocouple (Fig. 3). The camera understated temperatures above −4°C and overstated temperatures below −6°C. Camera response to changes in room air temperature was about 2 min slower than thermocouple response (data not shown). Plant temperatures, as indicated by the color palette of the camera, were generally within 2°C of the air temperature sensed by the thermocouple. Temperatures of unfrozen tissues typically ranged within 1°C of each other though some root tissues had temperatures below the visible range prior to and following freezing.

Thermal imaging allowed detection of most freezing events within turf tissues. Temperatures of objects lower than the camera range appeared black while temperatures above the camera range appeared white. Initial colors depended partially on distance of the plant organs from the camera, while the change in color of plant organs and tissues was used to detect freezing events and ice propagation. Freezing events in roots usually had lower color intensity compared with tissues with greater water contents such as leaf blades. The high water content of leaves provided dramatic color shifts from purple to yellow or orange, corresponding to a plant temperature shift of ≈2°C. Freezing events in roots were sometimes hard to detect as the color shift was often subtle, changing from purple to light blue or blue to green, corresponding to a plant temperature shift of only 0.25 to 0.5°C. When all freezing was finished in a particular tissue, the color display of the tissue reverted back to its nonfrozen color: black, violet, or blue. Transient (≤30 s) changes in air temperature of 0.5°C occasionally occurred as cooling coils of the chamber switched on and off. Since the resolution of the camera was 0.25°C, ephemeral freezing events that occurred at plant temperature changes of 0.25 or less were not readily detected during abrupt air temperature changes.

Initiation of Freezing Events, Ice Propagation, and Barriers to Propagation

Thermal imaging allowed observation of the differential freezing sensitivity among plant tissues (Fig. 4a–e). Roots always froze first, followed by crowns and lower shoots, with crown and leaf tissues having greater freezing resistance compared with roots (Table 1). For sur-

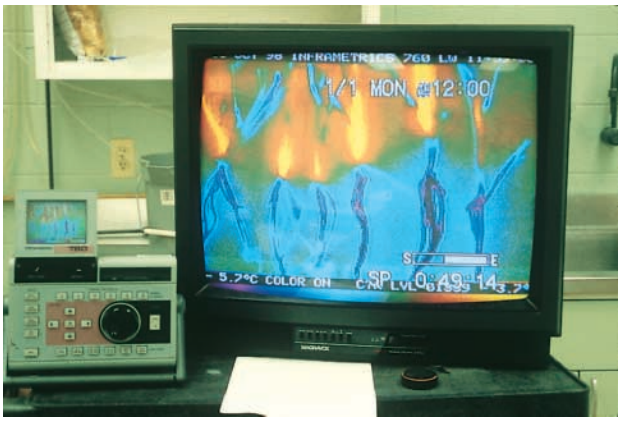
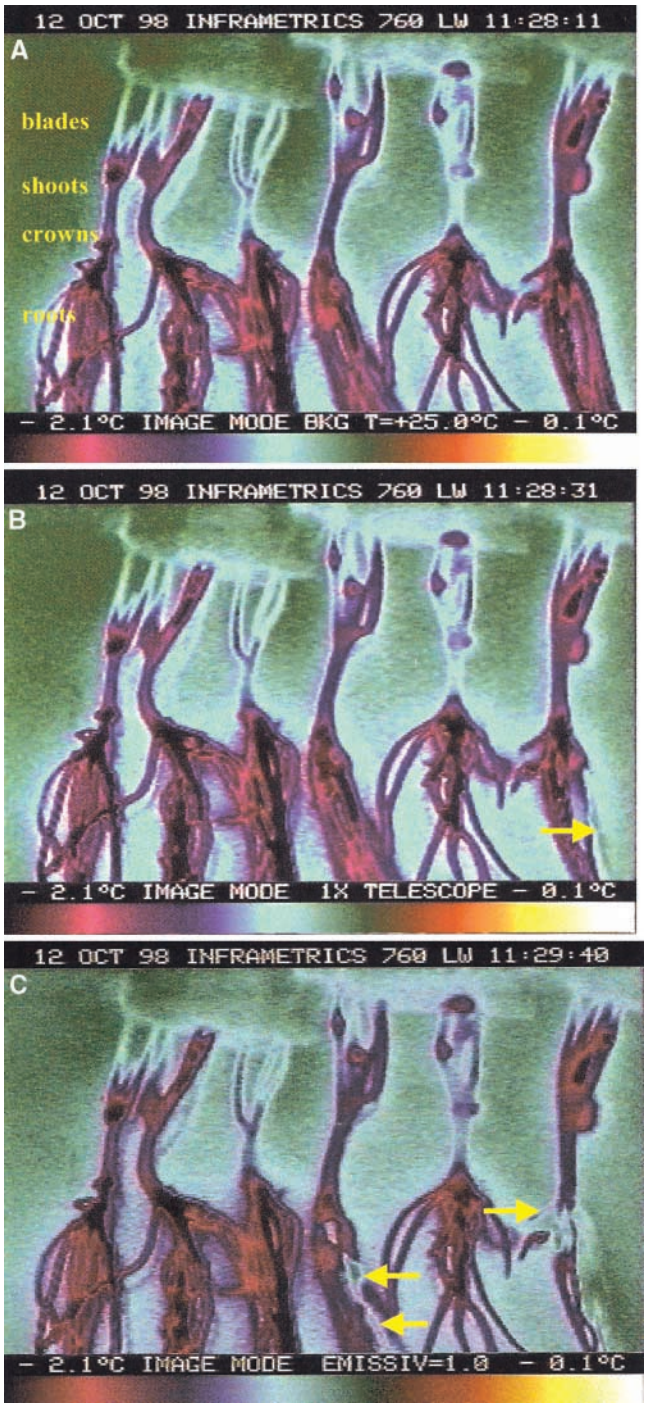


Fig. 2. Radiometer control (lower left) and television display (right) of freezing events of six turfgrass plants in real time. Colors represent heat, with violet representing lower heat than yellow. Black represents temperatures lower than the range settings on the radiometer while white represents temperatures above the radiometer settings. In the image, freezing of leaf blades appears orange to yellow due to the heat of fusion as water freezes. Freezing of roots is in decline (blue color) or have finished freezing (violet and black).



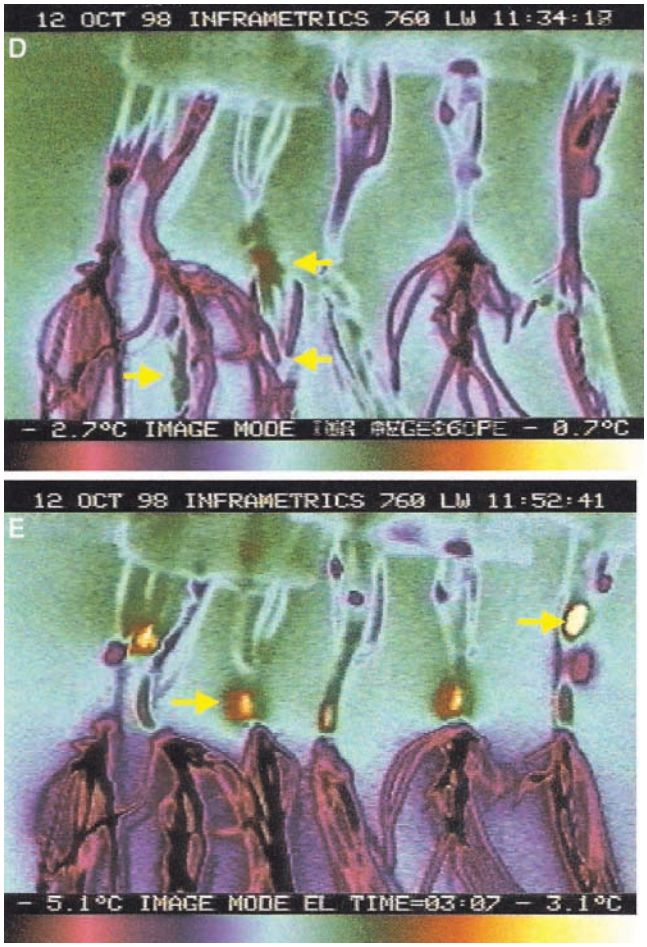


Fig. 4. Progression of freezing events in turfgrass plants viewed with infrared thermal imaging radiometer (camera) in real time. Plants were placed into a controlled environment room, held at 0°C for 20 min, then inoculated by placing water droplets containing ice-nucleating bacteria (*Pseudomonas syringae* strain Cit7) on leaf and root surfaces. Air temperature was reduced 0.5°C at 3-min intervals. Time is shown in the upper right corner of the image while temperature range being sensed by the camera is shown on the bottom of the image. Only selected freezing events are indicated in the figures. Fig. 4A shows three perennial ryegrass plants on the left and three supina bluegrass plants on the right as their images appear at an air temperature of −1°C 6 min after beginning the freezing test. Twenty seconds later (B), heat emission as water freezes inside plant tissues is indicated by the color change of roots on the sixth plant from the left. In another 69 s, freezing progressed acropetally toward the crown on the sixth plant, then basipetally into other roots, and the root system of the third plant began to freeze (C). When room temperature reached −2°C 12 min after the test began, freezing had started in roots of the second plant, proceeded acropetally from roots on the third plant into the crown, and was progressing through roots of Plants 4 and 6 (D). Note the red color of the crown in the third plant from the left, indicating greater heat loss due to higher moisture content compared with roots. At a room temperature of −5°C, 30 min after the test began, crowns and shoots of all plants were freezing, and freezing in root tissues was finished. The yellow circle on the sixth plant is a water droplet containing *P. syringae* freezing.

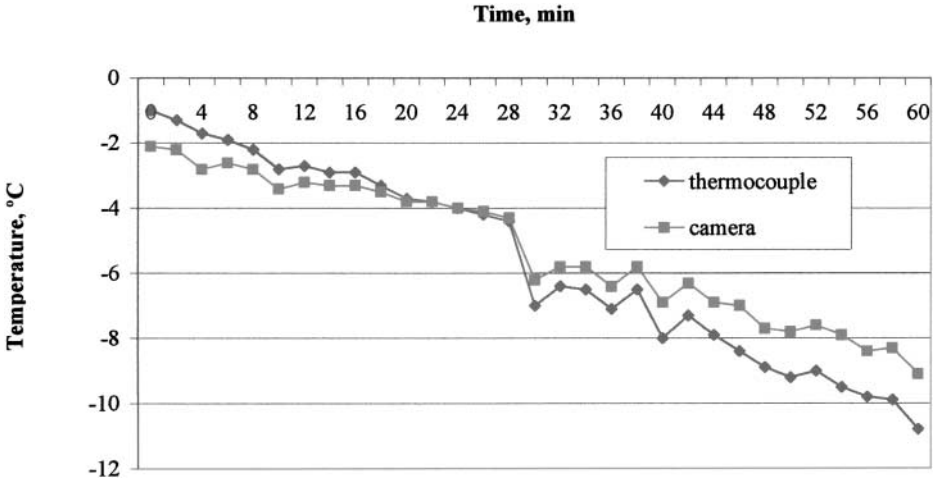


Fig. 3. Relationship of camera-sensed temperature to air temperature sensed with thermocouple: $°C_{thermocouple} = 1.16 + 1.27(°C_{camera})$.

vival reasons, these freezing differences are important as leaf tissue is typically exposed to colder air than crowns and roots, which are buffered by the soil environment.

Freezing propagated rapidly through roots, progressing several centimeters in <1 to 2 s. Freezing proceeded acropetally and basipetally in root tissues and ice crystals were propagated throughout connected roots within seconds. The crown consistently provided a barrier for ice propagation between roots which were not directly connected. The crown also provided a barrier which significantly slowed freezing into the upper crown and shoots. This phenomenon was more evident in crowns of supina bluegrass than perennial ryegrass.

Freezing occurred only slowly in the crowns, with one to several minutes required for the entire crown to freeze. Image colors gained intensity during freezing as additional water was frozen, ultimately displaying a vivid orange-red color. Once the entire crown was frozen, freezing propagated quickly up the shoots, though not as rapidly as in the roots. Freezing occasionally stopped or slowed at the leaf collar, suggesting some sort of barrier, then proceeded upwards to the leaf tips. Freezing proceeded only acropetally in shoots and leaves, contrary to freezing events in barley and velvetgrass (*Holcus lanatus* L.) in which basipetal freezing also occurred (Pearce and Fuller, 2001). The freezing observed in the plants destroyed sufficient meristematic tissue to kill all test plants. No plants recovered from the freezing tests when replanted in greenhouse conditions for up to 8 wk.

Table 1. Freezing rate differences among nonacclimated and acclimated† turf tissue types detected using infrared thermal imaging. Data are for the main effects of tissue type for nonacclimated tissue and for the interaction between tissue type and turf species of acclimated plants.

Tissue type	Time to freeze‡	
	min	
	Nonacclimated	
Roots	20.9	
Crowns, lower shoots	25.6	
Leaves	30.5	
LSD0.05	9.2	
	Acclimated	
	Perennial ryegrass	Supina bluegrass
Roots	19.1	13.4
Crowns, lower shoots	26.9	28.2
Leaves	34.5	33.3
LSD0.05	3.8§	

† Roots were removed from 15-mo-old, field-grown plants and regenerated in hydroponic culture fortified with half-strength Hoagland's solution to obtain soil-free plants. Plants were maintained in a controlled environment room at 10°C with a 12-h photoperiod (700 μmol m^{-2} s^{-1}) for 24 d during root regeneration. Nonacclimated plants were then incubated at 20/15°C (day/night) for 24 d and acclimated plants were incubated at 5/2°C prior to freezing tests.

‡ Freezing trials were conducted in a controlled environment room. Temperature declined from 0°C to −28°C at the rate of 0.5°C 3 min^{-1}.

§ The LSD value is for comparing means between species within a tissue type or among tissue types within a species.

Previous research has elucidated lethal freezing temperatures for discrete turf tissues: leaves, crowns, stolons, rhizomes, and roots (Gusta et al., 1980; Rajashekar et al., 1983; Maier et al., 1994), generally agreeing that roots have lower freezing tolerance than crowns and leaves. Results from our project indicate that crowns and roots should not necessarily be considered discrete organs when freezing studies are conducted. While crowns near the soil surface could freeze independently of roots buffered from low temperatures by soil, roots at a similar soil profile as the crowns could be a source of ice propagation into the crowns.

The preferred tissue path for ice propagation is unknown. Xylem tissues would seem to offer the most likely avenue for ice propagation due to their high water potential, particularly in nonacclimated plants. Microscopic investigations have correlated freezing injury with discolored tissue surrounding xylem (Beard and Olien, 1963; Ashworth et al., 1992). Rapid ice propagation would likely be slowed as xylem elements become dispersed and discontinuous in meristems (Zámećník et al., 1994), explaining the slowdown in ice propagation we observed in crowns and basal portions of leaves. However, Wisniewski et al. (1997) showed that ice propagation occurred more rapidly in cortical tissues of cross-sectioned woody stems than in xylem. Conflicting evidence in ice propagation among species indicate ice nucleation and freezing patterns vary among tissue types and plant species. Ice may also propagate through extracellular spaces (Pearce and Fuller, 2001).

Ice-Nucleating Bacteria did Not Initiate Freezing Events in Turf Tissues

Droplets containing ice-nucleating bacteria (*P. syringae*) froze but did not initiate freezing events in turfgrasses regardless of placement on leaves, shoots, or roots. The same strain had previously been shown to initiate freezing when placed on the calyx of cranberry fruits, cut stem surfaces, or adaxial leaf surfaces but not when placed elsewhere on the fruit or on the abaxial leaf surface (Workmaster et al., 1999). In order for bacteria to initiate ice nucleation events in turf, they would likely need contact with interior plant tissues which would normally only be achieved through leaf wounds or via direct contact with stomata. In turfgrasses, the stomata would be largely closed during freezing temperatures; furthermore, mowing wounds as a means of bacterial ingress are unlikely since turf is not generally mowed during freezing conditions. Lastly, there was no basipetally oriented freezing observed in either turf species in leaf or shoot tissue, suggesting the presence of ice-nucleating bacteria on turf leaf surfaces are not responsible for causing ice formation which could propagate into turf crowns. Leaf freezing from droplets placed at the leaf tips was not tested, however, which Pearce and Fuller (2001) found could incite ice propagation into a velvetgrass leaf.

Freezing Differences between Turf Species and Acclimation Regimes

In nonacclimated plants, freezing times were similar for plants of both species though there were differences in freezing times between tissues (Table 1). Crowns had freezing times similar to both roots and leaves (25.6 min compared with 20.9 and 30.5 min, respectively), though roots froze faster than leaves. In acclimated plants, roots froze before crowns which froze before leaves. There was also an interaction between species and tissues as supina bluegrass roots froze sooner than perennial ryegrass roots, while freezing times between the two species were similar for crown and leaf tissue.

The experiment was not designed to detect differences in freezing events between acclimation regimes though freezing times were similar. Other freezing studies have documented a greater freezing tolerance in cold-acclimated plants compared with nonacclimated plants (Rajashekar et al., 1983).

The freezing tolerance of perennial ryegrass is cultivar-dependent and ranges from −5 to −15°C (Gusta et al., 1980). Gusta et al. (1980) reported perennial ryegrass was less cold-tolerant than either creeping bentgrass (*Agrostis palustris* Huds.) or Kentucky bluegrass. The freezing tolerance of supina bluegrass, a perennial species native to the subalpine regions of Europe (Berner, 1980), is unknown. Supina bluegrass is a putative parent or close relative of *P. annua* (Heide, 2001) although it does not necessarily share similar environmental tolerances. Crowns of supina bluegrass appeared to slow ice propagation more than perennial ryegrass crowns, although the differences were too subtle to visualize to provide quantitative data for analysis. Freezing events in supina bluegrass usually exhibited greater color intensity and were longer lasting compared with perennial ryegrass, particularly in shoot and leaf tissues, perhaps due to higher water contents. Supina bluegrass is not as drought-tolerant as perennial ryegrass (J. Stier, 1999, personal observation) and consequently could maintain greater moisture content during acclimation than perennial ryegrass.

CONCLUSIONS

Shortcomings of the Technique

The major limitation to the technique was the inability to provide sufficient color contrast to detect ephemeral freezing events that occurred within 0.25°C of prefreezing temperatures during concomitant air temperature changes of ≥0.25°C. In tissue with little water, or when tissue was screened by other tissue (e.g., the back side of a root mass), color changes to indicate a freezing event were often subtle and could dissipate rapidly. This limitation mandated thorough, sometimes repetitive, screening of the video images to discern freezing events. A camera with sharper resolution might solve this problem though background temperature fluctuations would still have to be buffered.

The system cannot observe ice propagation in a turf plant in situ due to interference from soil and adjacent plants. Thus, the system as tested is artificial and may have produced artifacts which were not obvious to the authors. Use of a transparent growing medium (e.g., gel) with temperature-buffering capacities similar to soil could be useful to provide an in situ condition.

Successes

Infrared thermal imaging was useful for showing the sensitivity of tissues to freezing: Roots froze first, then crowns, then leaves. Future studies of freezing tolerance of crowns should consider pieces of attached roots since root tissues near the crown are likely a primary site of ice initiation which ultimately propagates into the crown. Root systems froze bidirectionally while freezing in shoots and leaves proceeded only acropetally. Freezing propagated quickly through tissues, particularly roots (several centimeters in 1 to 2 s). Tissues at the leaf blade collar and particularly the crown posed an intrinsic barrier to freezing. Ice-nucleating bacteria did not initiate freezing events in either perennial ryegrass or supina bluegrass.

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