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Light Stress Following a Frost Episode Influences the Frost Tolerance of a Wild Potato Species

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Abstract. **The effect of light stress following various levels of freezing stress on photosynthetic and respiratory functions in leaf tissue of *Solanum acaule* (Bitt.) was investigated under laboratory conditions. Terminal leaflets from plants grown at 18/16C (light/dark) and 400 $\mu mol \cdot s^{-1} \cdot m^{-2}$ photosynthetically active radiation (PAR) were slowly frozen in the dark to various minimum temperatures and then thawed on ice, also in the dark. Immediately after thawing, paired leaf disks were cut from a single terminal leaflet. Disks were then held at 1.0C for 3 hr, with one disk exposed to 800 $\mu mol \cdot s^{-1} \cdot m^{-2}$ PAR and the other disk of the pair was held in the dark. Our experimental approach allowed comparative studies on tissue from the same leaflet. Light during the post-thaw period, as compared to darkness, resulted in greater inhibition of photosynthetic processes, but had little effect on respiration. Our results demonstrate a) the importance of considering a light stress component during the assesment of relative frost tolerance in photosynthetic tissue and b) that chloroplast functions are much more sensitive than mitochondrial functions to a post-thaw light stress.**

Photosynthesis is a key process in the biosynthesis of organic molecules and in the generation of metabolic energy within photosynthetic tissues. The ability of plants to survive and recover from injury incurred during a natural frost episode may depend, in some instances, upon the sensitivity of photosynthetic processes to the associated stresses. With the exception of some studies on the effect of winter stress (freezing temperatures in the presence of light) on photosynthesis in pines (Öquist, 1981, 1983; Öquist and Martin, 1986; Strand and Öquist, 1986), nearly all experimental evaluations of the relative tolerance of photosynthetic tissues to subzero temperatures involve freezing and thawing in the dark. The effect of incident light energy following a freeze–thaw cycle on the ability of herbaceous plants to maintain metabolic functions and/or recover has not been investigated.

It is well-documented that, whenever the trapping of light energy within the chloroplast exceeds the capacity of metabolic processes to use (and thus dissipate) this energy, the photosynthetic apparatus can be injured (Powles, 1984). An imbalance in the flow of light energy through the chloroplast could arise during environmental conditions that result in an increase in incident light energy levels and/or an impairment of the enzymatic capacity to use trapped light energy (Osmond, 1981). A potentially damaging situation, therefore, exists on mornings following a radiational frost when skies are clear, resulting in relatively high incident light intensities. In addition, low morning air temperatures following a frost would decrease enzymatic reaction rates and thus the capacity to use trapped light energy in carbon fixation and reduction. Furthermore, any damage to the photosynthetic apparatus sustained during the prior freezing stress would reduce the capacity of photosynthetic tissue to use and therefore dissipate excess light energy.

The objectives of the present study were two-fold: a) to document the general relationship between incident light energy and herbaceous leaf tissue temperature following a natural frost, and b) to study the effect of light following a simulated freeze–thaw cycle on photosynthesis and respiration in *S. acaule* leaf tissue.

Materials and Methods

Plant material. Clonal material of *Solanum acaule* Bitt. (PI 472659), *S. commersonii* Dun. (PI 472834), and *S. tuberosum* L. cv. Red Pontiac were propagated as stem cultures on MS medium (Murashige and Skoog, 1962) before transfer to 8-liter pots filled with 1 peat : 1 vermiculite (v/v) (Jiffy Mix, JPA, West Chicago, Ill.). Plants were grown for 6 to 10 weeks at 18/16C (day/night) and 70% ± 5% RH with 14 hr of 400 $\mu mol \cdot s^{-1} \cdot m^{-2}$ PAR (400–700 nm) at the canopy level from cool-white fluorescent lamps (Sylvania/GTE, Danvers, Mass.). Modified one-half strength Hoagland's solution (Hoagland and Arnon, 1950) was supplied in excess four times daily.

All studies investigating the physiological response of photosynthetic tissues to light stress following a simulated freeze–thaw cycle were carried out with material from *S. acaule*. Studies to document the physical relationship between incident light levels and the temperature of herbaceous leaf tissue following a natural frost were carried out with material from *S. commersonii* and *S. tuberosum*. This relationship was observed to be independent of the plant material used.

Light and low-temperature treatment chamber. A two-compartment chamber for exposure of previously frozen leaf disks to high-light intensity at low temperature (Fig. 1 A and B) was constructed from 6-mm gray polyvinyl chloride (PVC) sheets. The internal dimensions of each compartment measured 16 mm high × 122 mm long × 114 mm wide with a volume of 2.225×10^5 mm^3. Each compartment was filled with 80 ml of buffer consisting of 0.5 mM $CaSO_4$ and 50 mM Hepes·KOH (*N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid) at pH 7.2, leaving a head space of $\approx 1 \times 10^5$ mm^3 (less leaf disk holders). For each compartment, leaf disk wells were made by cutting 17-mm-diameter holes in a 4-mm-thick, 107 × 111-mm square of high-density styrofoam (f in Fig. 1 A and B). Wells were

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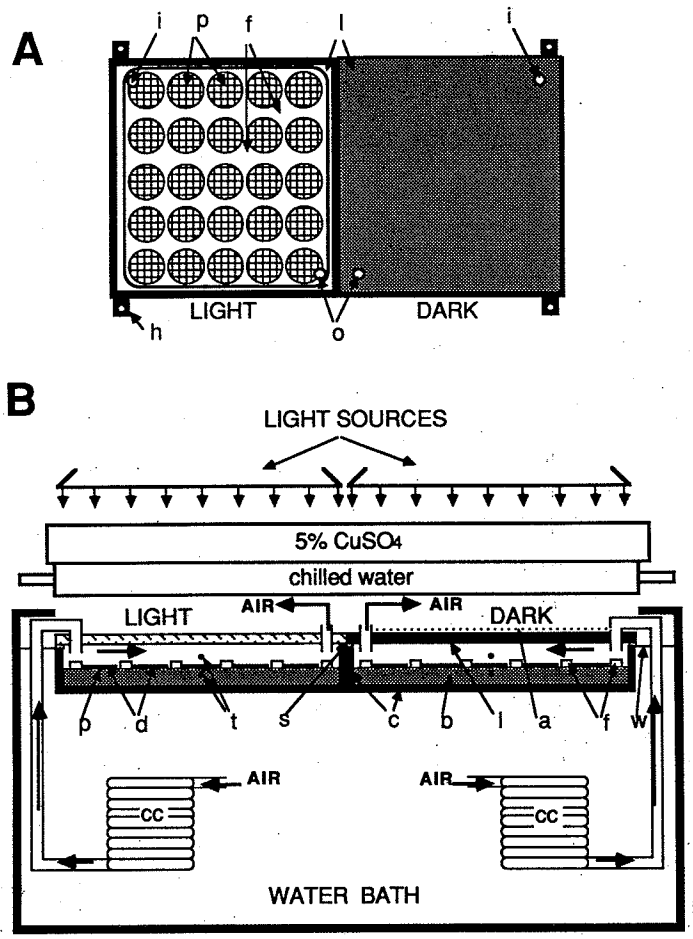

Fig. 1. (**A**) Chamber for high-light and low-temperature treatments. (**B**) Apparatus for providing high-light and low-temperature stress treatments. Symbols represent: a, aluminum foil; b, buffer solution; c, chamber wall; d, leaf disk; f, styrofoam wells, h, chamber supports; i, treatment chamber inlets for chilled air;, l, chamber lid; o, chamber air outlets; p, plastic screen; s, closed-cell foam seal; t, copper–constantan thermocouples; w, coolant level; heavy arrows indicate air flow through the treatment chambers.

backed with nylon screen (p in Fig. 1 A and B) and secured with nylon monofilament line. This approach allowed leaf disks to be floated on the surface of the buffer while maintaining disk spacing. Each compartment was closed with a 3-mm-thick closed-cell foam gasket to seal around lids of either 6-mm gray translucent PVC covered with aluminum foil in the dark compartment or 6-mm transparent plexiglass in the lighted compartment. Three-millimeter inlet and outlet holes were drilled into the corners of each lid to provide air flow over the disks. To maintain leaf disks at a controlled temperature during light treatments and to allow gas exchange, the air over the disks was constantly replenished with chilled air. Before entry into the disk chamber, the air was pumped via aquarium pumps (Thiberg Pumps, no. 1, Princeton N.J.) through 0.5 m of 5-mm (i.d.) copper cooling coils submerged in the cooling bath. Aquarium pumps, cooling coils, and the leaf disk chambers were connected with 2-mm (i.d.) Tygon tubing. The assembly was then suspended by adjustable brackets to within 4 mm of the tops of the compartment lids in a cooling bath (Lauda/Brinkman, Model K-4R, Westbury, N.Y.) set at −1.2C to maintain buffer temperature in the compartments at 0.5C. Light was supplied by two 250-W tungsten halogen lamps (Quartzline PAR flood lamp, General Electric Co., Cleveland, Ohio). Light intensity could be varied by raising or lowering the light assembly. Light was first filtered through a 2.5-cm-deep, 5% $CuSO_4$ solution and then through 2.5 cm of circulating chilled water to reduce the heat load. Thermocouples to monitor temperature were placed in the buffer and in the headspace, ≈3 mm above the leaf disks of each chamber.

Freezing and thawing (outside environment). To establish a relationship between the temperature of herbaceous leaf tissue and incident light levels, a single plant (two plants in one instance) of either *S. tuberosum* cv. Red Pontiac or *S. commersonii*, depending on availability of suitable material, was placed outside in Madison, Wis. on 3 days (9 and 14 Nov. and 14 Dec. 1986) when air temperatures were above freezing. Copper–constantan thermocouple wires (24-gauge) were inserted into the midvein of one leaflet in the upper canopy. This leaflet was positioned horizontally with wire supports and a Li 600 quantum sensor (LI-COR) was mounted immediately adjacent to the leaflet, perpendicular to the horizon. Air temperature was measured with a thermocouple wire placed 70 mm directly above this leaflet and shielded from direct radiation with a small piece of aluminum foil. Plants were allowed to freeze in the evening and then thaw the following morning. Leaf tissue temperature, air temperature, and incident light energy levels were monitored during the subsequent rewarming of tissue in the post-freeze period. Tissue temperature was also monitored at several locations within the plant to follow ice propagation (data not shown).

Freezing and thawing (laboratory conditions). Similar leaves, in which the terminal leaflets were 80% to 90% of full expansion and exposed to an incident light level of 400 ± 50 $\mu mol \cdot s^{-1} \cdot m^{-2}$ PAR at the time of sampling, were selected from *S. acaule* plants grown as described previously. Experimental units consisting of one terminal and two subtending lateral leaflets, all still attached to the same rachis, were excised and completely randomized before assignment to the various treatments. All assays on freshly harvested controls were conducted immediately after excision. Three replicates (n = 3) of the chilling pretreatment (0C) and each of the freezing temperature pretreatments (−3, −4, −5C) and six replicates (n = 6) of the freshly harvested controls (C), were used in the present experiment.

In the freezing pretreatments, excised leaflets were frozen using a procedure similar to the one used by Sukumaran and Weiser (1972). Leaf sections (one terminal and two laterals) for freezing were rinsed in distilled water and then placed basipetal end down in 25 × 200-mm pyrex glass tubes with stainless steel weighted caps. Constantan–copper thermocouple wires (24-guage) were inserted into each rachis to measure leaflet temperature. Tubes containing leaflets were placed in a glycol bath for freezing (Forma Scientific, Model 2325, Marietta, Ohio). Temperature was lowered to −0.5C and held for 45 min until all tissue was equilibrated to this temperature. Freezing was initiated by dropping ice crystals on the tissue and verified by noting the temperature rise in the tissue. Tissue temperature was then lowered at 1C/hr to -3, -4, or -5C and held at the indicated temperature for 30 min before a 2-hr thaw on ice in the dark. Chilled (0C) pretreatments were held in the dark on ice for the duration of the freeze–thaw treatments.

The two lateral leaflets from each leaf section were excised for measurement of percent electrolyte leakage. To facilitate the infiltration of tissue, leaf margins were trimmed from the two lateral leaflets that then were submerged in distilled deionized water and infiltrated under vacuum. Tissue was shaken for 1 hr

on a gyratory shaker at room temperature and the electrical conductivity of the leachate measured and expressed as a percentage of leakage from the same tissue following killing at 250C (Palta et al., 1977).

Post-thaw light stress (laboratory conditions). Paired leaf disks, 13.5 mm in diameter, were cut from across the midvein of *S. acaule* terminal leaflets previously frozen and thawed under laboratory conditions or chilled on ice in the dark for the duration of the freeze–thaw treatments. In the post-thaw period, one disk of each pair was subjected to a high-light and low-temperature stress, while the other disk was held in the dark at the same low temperature. The disks were supported in individual wells on the surface of the buffer in either the light or dark compartment of a high-light and low-temperature stress chamber (Fig. 1). Buffer temperature was maintained at 0.5C by placing the entire chamber in a refrigerated water bath and passing chilled room air through the compartments. The leaf disks were held in these compartments for 3 hr with one disk of a pair in total darkness and the other in the presence of 800 ± 100 $\mu mol \cdot s^{-1} \cdot m^{-2}$ PAR from 250-W tungsten lamps. Following the stress treatments, all disks were held at 0 to 4C in the dark until photosynthetic and respiratory assays could be conducted. Previous studies have shown that injured or healthy leaf tissue, when held in the dark at 0 to 4C for up to 30 hr, shows no recovery or further deterioration of photosynthetic or respiratory processes (Steffen, 1987).

Photosynthetic and respiratory assays. Measurements of photosynthesis and respiration were made on the paired disks from *S. acaule* leaflets following post-thaw light stress treatments. Paired samples were assayed within 35 min of one another. For these assays, leaf disks were infiltrated under vacuum with the same buffer used in light and dark post-thaw treatments, sliced into pieces ≈1.2 × 1.6 mm, and then placed in a water-jacketed oxygen electrode chamber at 18C in the presence of 20 mM $NaHCO_3$ (Steffen and Palta, 1986). Respiratory oxygen uptake was measured for 8 min with the chamber darkened, followed by an 8-min determination of light-limited photosynthesis in the presence of 114 $\mu mol \cdot s^{-1} \cdot m^{-2}$ PAR and then an 18-min determination of light-saturated photosynthesis in the presence of 1750 $\mu mol \cdot s^{-1} \cdot m^{-2}$ PAR (Steffen and Palta, 1986). Chlorophyll was then extracted from each leaf disk in 96% ethanol and quantitated spectrophotometrically according to Wintermans and Demot (1972).

Results

Incident light levels and tissue temperature (outside environment). Each day of outside monitoring of air and leaf temperature and incident light levels following a nocturnal frost episode varied somewhat in terms of minimum temperature attained, atmospheric clarity, etc. The data presented in Fig. 2 from *S. tuberosum* on 15 Dec. 1986 represent typical results obtained. Midday temperature reached 10.4C and peak incident light level was 860 $\mu mol \cdot s^{-1} \cdot m^{-2}$. Incident light levels, at the surface of the leaflet being monitored, reached 430 $\mu mol \cdot s^{-1} \cdot m^{-2}$ (50% of peak level) before tissue thawing was complete. Observations from 3 days on a single plant (two plants on one occasion) demonstrated that incident light levels reached 54%, 50%, 46%, and 39% of the daily peak level while tissue was still frozen. No species effects were observed in this relationship between incident light level and tissue temperature.

Ion leakage (laboratory conditions). In *S. acaule* tissue frozen and thawed in the laboratory to −3.0C, there was a small amount of ion leakage (10% of the total), although it represented nearly a 3-fold increase over freshly harvested controls and nearly

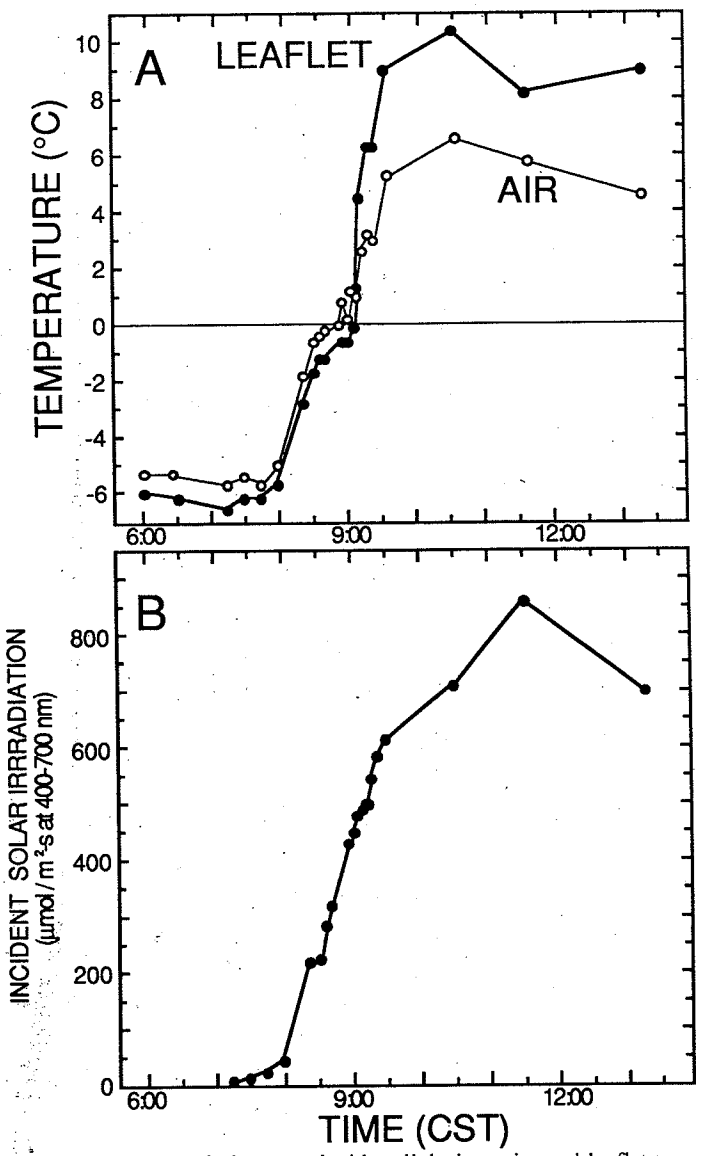

Fig. 2. Relationship between incident light intensity and leaflet temperature during the thawing of a potted *S. tuberosum* plant placed outside in Madison, Wis. on 14–15 Dec. 1986. All measurements presented here were monitored at the same leaflet. (**A**) Leaflet temperature and air temperature measured 7 cm above the leaflet. (**B**) Light intensity incident to the leaflet surface.

a 2-fold increase over tissue chilled at 0C for the duration of these experiments (Fig. 3). A more dramatic efflux equaling 24% of the total ions was observed following the thawing of tissue previously frozen to −4.0C and at −5.0C; 60% of the total ions had leaked from the tissue. Studies by Palta et al. (1982) have shown that potato leaf tissue can recover from a substantial freeze–thaw injury in which up to 30% of the total ions have leaked from the cell. Irreversible damage was usually indicated when ion leakage exceeded 30% of the total ions present within the cell. In the study presented here, the sharp increase in ion leakage between −4.0 and −5.0C suggests the difference between cell survival and cell death.

Light-limited photosynthesis (laboratory conditions). While the various freezing stresses and post-thaw treatments had no effect on chlorophyll content of tissue from *S. acaule* (data not shown), photosynthetic function was consistently and markedly

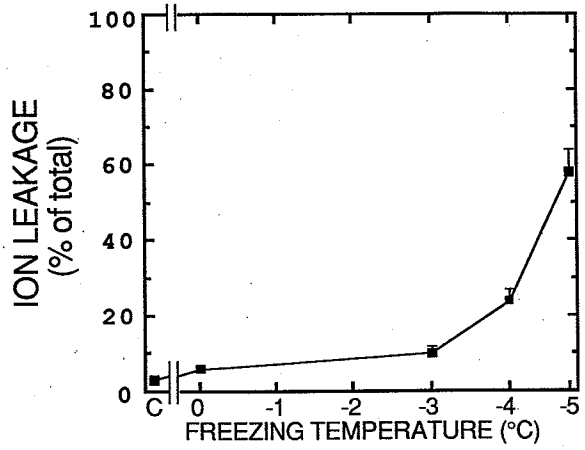

Fig. 3. Ion leakage from *S. acaule* leaf tissue after freezing to between −3.0 to −5.0C and thawing for 2 hr in the dark at 0C during laboratory simulations. C represents unstressed tissue and 0 represents leaf tissue held at 0C for the duration of the freeze–thaw treatments. Values presented are means of three replications, with the exception of six replications for the fresh controls (C), + SE. Errors are smaller than the symbols where error bars are not shown.

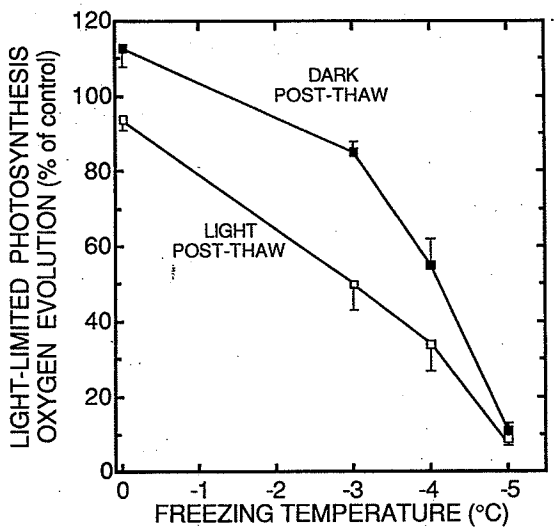

Fig. 4. Total light-limited (114 μmol·s^{-1}·m^{-2} PAR quanta) photosynthetic oxygen evolution from *S. acaule* leaf tissue following simulated freeze–thaw cycles with either light (open symbols) or dark (solid symbols) post-thaw treatments. Values are means ± SE, n = 3.

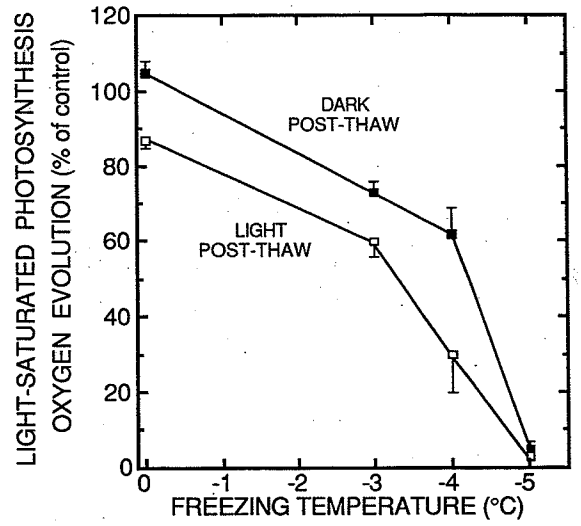

Fig. 5. Total light-saturated (1750 μmol·s^{-1}·m^{-2} PAR quanta) photosynthetic oxygen evolution from *S. acaule* leaf tissue following simulated freeze–thaw cycles with either light (open symbols) or dark (solid symbols) post-thaw treatments. Values are means ± SE, n = 3.

Table 1. Effect of light in the post-thaw period on the LT_{50} (temperature at which function is 50% impaired) of cellular functions in tissue from *S. acaule* following simulated freeze–thaw stress.

	Function			
			Photosynthetic oxygen evolution	
Post-thaw treatment	Ion leakage (%)	Respiration[z] (O_2 uptake)	Light-limited	Light-saturated
		LT_{50} (°C)		
Dark	−4.8	< −5.0	−4.1	−4.2
Light	ND[y]	< −5.0	−3.0	−3.3

[z]At −5.0C, the lowest temperature used in this study, respiration rates in both light and dark post-thaw treatments were still >50% of control values.

[y]Ion leakage was measured immediately after thawing. No data were taken on electrolyte leakage from light-stressed tissue because the loss of electrolytes to the bathing medium during the light treatments made the accurate determination of total electrolyte leakage impossible.

more damaged in the presence of light than in the paired dark-held tissue (Figs. 4 and 5, Table 1). Previously chilled tissue (Fig. 4) showed no detectable inhibition of light-limited photosynthesis (96% of fresh control) following the light stress treatment, while the dark-held tissue was slightly stimulated. In tissue frozen to -3.0C, however, the light stress caused a precipitous drop to 50% of fresh control, while the same tissue held in the dark declined only 15%. After a −4.0C freeze, the light-stressed tissue had a light-limited photosynthetic rate of 34% of control, while the same dark-held tissue gave 55% of control rates. At −5.0C, light-limited photosynthesis was almost completely eliminated in tissues from light and dark treatments (Fig. 4).

Light-saturated photosynthesis (laboratory conditions). In tissue from *S. acaule*, the relative effects of post-thaw light and dark treatments on light-saturated photosynthesis were similar to the effects on light-limited photosynthesis, although the kinetics were different (Figs. 4 and 5). Again, chilling at 0C resulted in a slight stimulation following a dark period at low temperature, while there was a slight inhibition resulting from the subsequent light stress (Fig. 5). At −3.0C, the post-thaw light stress caused a lesser decline in light-saturated vs. light-limited photosynthesis (to 60% vs. 50% of control, respectively) and dark-held tissue showed a greater decline in light-saturated than light-limited photosynthesis (73% vs. 85% of control, respectively). After a −4.0C freeze, light-saturated rates in light-stressed tissue were reduced to 30% of fresh control, while the dark held tissue was 62% of control (Fig. 5). Light-saturated photosynthesis following a −5.0C freeze was completely eliminated in tissue from light stress and dark treatments.

Respiration (laboratory conditions). When the previous freezing stress was −5.0C, respiration had declined to ≈60% of the freshly harvested control in light-stressed and dark-held tissue

of *S. acaule*. With the exception of the chilled (0C) treatment pairs, light treatments following the freezing stress caused no significant increase in damage to respiratory function when compared to dark post-thaw treatments (Fig. 6). In tissue that was not previously frozen but only chilled at 0C, light treatments caused an inhibition of respiration, while tissue held in the dark showed a slight stimulation (86% vs. 109% of the fresh control, respectively).

Comparison of some cellular functions. As the freezing stress of tissue from *S. acaule* was increased in 1C increments from −3.0 to −5.0C, there was a steady decline in all cellular functions measured (Figs. 4–6). This decline was apparent in both the presence and the absence of light in the post-thaw treatment period. Photosynthesis, both light-limited and light-saturated, was the most sensitive indicator of increased freezing stress, followed by ion leakage and then respiration (Table 1). The greater sensitivity of photosynthesis than respiration is even more apparent when considering the cumulative effect of the freezing and light stresses (Table 1).

Discussion

The monitoring of leaf temperature and incident light levels following a nocturnal frost episode demonstrated that photosynthetic tissue can be exposed to relatively high incident light levels while still frozen (Fig. 2). The present study clearly demonstrates the injurious effect of incident light on *S. acaule* leaf tissue following a simulated freeze–thaw stress. This post-thaw light stress causes a selective impairment of photosynthetic functions, but has no detectable effect on cellular respiration. The impairment of photosynthetic function resulting from the light stress appears to be additive to the injury caused by freezing and thawing in the dark. However, at −5C, a lethal freezing stress, this additive effect of light was not apparent. While the light level used in this study was nearly twice the level under which the plants were grown, we feel that the light stress intensity and duration is a good first approximation of the magnitude of this post-thaw stress in the natural environment. We have shown that leaf tissue can be exposed to light levels approaching one-half full sunlight, while still frozen (Fig. 2). In addition, the photosynthetic apparatus is usually exposed to several hours of relatively high light intensities and relatively low temperatures, during mornings following radiational frosts. These conditions have been shown to cause injury to the photosynthetic apparatus in the absence of freezing (Miedema, 1982). Furthermore, following a frost, there has very likely been an additional impairment in the capacity of the photosynthetic apparatus to make use of (and thus dissipate) incident light energy due to the freezing-induced injury. The results presented here may help to explain the common observation that freezing injury is not always apparent immediately following a frost, but develops over a period of 1 or 2 days.

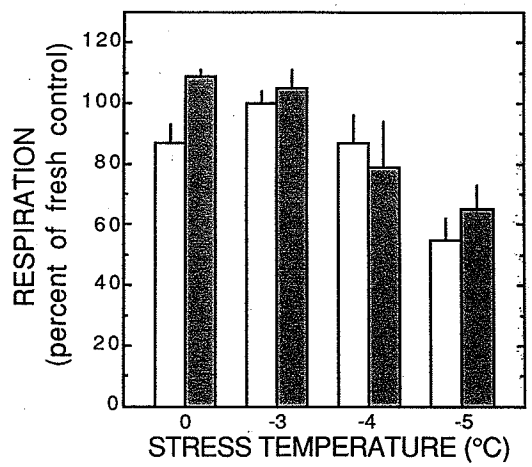

Fig. 6. Respiratory oxygen uptake from *S. acaule* leaf tissue following simulated freeze–thaw cycles with either light (open bars) or dark (solid bars) post-thaw treatments. Values are means + SE, n = 3.

These results also suggest that experimental evaluations of frost tolerance, in which all stresses are carried out in the absence of light, may overestimate the ability of photosynthetic tissue to tolerate a frost episode under natural conditions. Previous studies (Steffen and Palta, 1986) have shown that increasing light intensities from low to moderate levels (20 to 450 $\mu mol \cdot s^{-1} \cdot m^{-2}$ PAR) dramatically increases injury to the photosynthetic apparatus during growth at low, non-freezing temperature stress, and that injury increases with the duration of exposure. These studies have also shown that genetically related *Solanum* spp. vary in their ability to tolerate the light-stress component. Work by Wheeler and Tibbitts (1986) has demonstrated that, within the species *S. tuberosum*, there exists considerable variation in the ability to tolerate a continuous-light photoperiod. If freezing tolerance and the tolerance of light stress are independent, the screening for frost tolerance in the absence of light may lead to erroneous conclusions about the relative frost-tolerance of plant materials. Clearly, more work needs to be done in the illucidation of the relative impact of freezing stress and the post-thaw light stress on the ability of herbaceous plants to survive and maintain productivity following a natural frost episode.

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