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Degree of Undercooling and Injury of Whole Potato Plants following Exposure to -4C for 6 or 12 Hours

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Abstract. **Hardened and nonhardened whole plants of three potato species, *Solanum tuberosum* L., S. *acaule* Bitt., and *S. commersonii* Dun., and one interspecific cross, 'Alaska Frostless' *(S. tuberosum* x S. acaule) were placed in a low-temperature chamber capable of maintaining -4 ± 0.5C for 6 or 12 hours. The chamber was designed to control the root temperature independently from the rest of the plant. Cold acclimation did not affect the ability of any of the potatoes tested to undercool (supercool). *Solanum tuberosum* and 'Alaska Frostless' did not undercool for the times and temperatures tested and in all cases were killed. Whole plants of S. *acaule* and *S. commersonii* undercooled, in some cases, for up to 12 hours. When plants of S. *acaule* froze, they were severely injured, although their hardiness levels were reported to be lower than the temperature to which they were exposed in this study. Whenever leaves and stems of *S. commersonii* were frozen they were not injured. Once the soil was allowed to freeze, all plants, in all cases, were frozen.**

The cold-hardiness level of many potato species, crosses, and cultivars has been reported (Li, 1977; Richardson and Weiser, 1972). Most of these levels are based on laboratory examinations in which excised leaves were provided with ice nucleators to prevent undercooling (supercooling) and were then exposed to various subzero temperatures (Sukumaran and Weiser, 1972). This, however, may not accurately reflect the hardiness level of potatoes under field conditions.

Many plant species undercool (Burke et al., 1976). Hudson and Idle (1962) reported that under laboratory conditions, whole plants of *S. tuberosum* and *S. acaule* undercooled to -6C. *Solanum tuberosum* was always killed at - 3C or below when ice was formed within its tissues. When undercooling occurred, *S. tuberosum* leaves survived exposure to -11.5C for at least 45 min and -4C for at least 5 days (Lindstrom and Carter 1983, 1985). Therefore, when freezing was avoided, no damage resulted. Dearborn (1969) reported that 'Alaska Frostless' possesses higher frost resistance than *S. tuberosum.* In laboratory tests, however, Chen et al. (1976) found frozen leaves of 'Alaska Frostless' to be killed at the same temperature as *S. tuberosum.*

Our experiment was designed to compare the undercooling of whole potato plants in the laboratory under conditions that more closely simulate field conditions. Some potatoes may have a greater ability to undercool under natural conditions than others. In most hardiness tests, ice nucleators are added to the excised leaves to initiate freezing, but in nature certain plants may have less efficient or fewer nucleators than others (Amy et al., 1976; Lindow et al., 1978), enabling those plants with fewer or less efficient nucleators to undercool to a greater extent. Since 'Alaska Frostless' has been reported to be hardier than *S. tuberosum* under field conditions but does not show any freezing tolerance under laboratory conditions, we also tested the hypothesis that 'Alaska Frostless' may possess a greater ability to undercool than *S. tuberosum.* Neither of these species is able to tolerate freezing below -2 or -3C, but they may avoid injury at lower temperatures by undercooling.

Plant material. Three potato species, *S. tuberosum, S. acaule,* and *S. commersonii,* and one interspecific hybrid, 'Alaska Frostless' were grown under hardened and nonhardened conditions. *Solanum tuberosum* and 'Alaska Frostless' were propagated from tubers, and *S. commersonii* and *S. acaule* were propagated by division of a parent plant. The divided plants and tuber sections were planted in 0.2-m' pots containing a mixture of 3 soil : 2 sand : 2 peat (by volume). The plants were placed in growth chambers under a 14-h photoperiod with 20/15C day/night cycles. A photon flux density of 450 $mol \cdot m^{-2} \cdot s^{-1}$ photosynthetically active radiation was supplied to the plants. The plants were watered as needed and a nutrient solution (20N-20P-20K) was applied to the soil. Plants grown under the above conditions were termed "nonhardened." After 1 month, some of the nonhardened plants were exposed to a constant 2C day/night treatment for 2 weeks. All other environmental factors remained the same. Not all of the potato species and crosses gained cold resistance; however, all plants grown under the low-temperature regime will be referred to as cold-hardened.

Low-temperature chamber. The low-temperature chamber was designed with a double side wall with holes on the inside to allow cool air to filter over the plants (Fig. 1). A

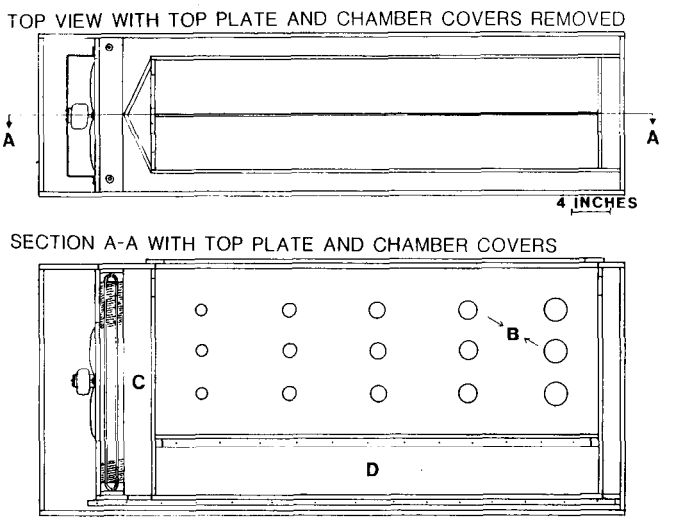

Fig. 1. Freezing chamber. The top view of the chamber shows the heat exchanger on the left and the ducting system within the box. Section A-A shows a cross section of the same chamber showing the vent holes (B), heat exchanger (C), and the lower removable compartment (D) in which the soil and roots of the plants were placed.

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Table 1. Freezing patterns and resulting injury of hardened (H) and nonhardened (NH) material of *Solanum acaule* and *S. commersonii* when exposed to −4 ± 0.5C for 6 or 12 h when soil was nonfrozen. Three patterns of freezing and injury were observed: 1) plants froze but remained alive (FA); 2) plants froze but were severely injured or killed (FI); and 3) plants undercooled but remained alive (UA). The values are expressed as a percent of the total number of plants (16) in the treatment that fell into each of the three freezing pattern/injury categories.

Plant material	Hours at −4C	Freezing pattern/injury (%) FA	FI	UA
S. acaule				
H	6	0	57	43
NH	6	0	54	46
H	12	0	91	9
NH	12	0	82	18
S. commersonii				
H	6	25	0	75
NH	6	37	0	63
H	12	100	0	0
NH	12	NT[z]	NT	NT

heat exchanger was placed at one end of the chamber and a fan was positioned so air could be blown through the exchanger and then be diverted along the sides of the box, passing out through the holes on the inside wall of the box. The air entered the inside box, flowed over the plant material, passed out an exhaust duct, and then was directed back to the air intake. A removable false bottom, consisting of a wooden piece split lengthwise down the center, was placed in the box so that the roots and soil could be kept from freezing, if desired. Foam rubber was placed on the sides of each piece so when fitted together, a stem could pass through the floor and be resealed to prevent the exchange of air between the two chambers. A controlled-temperature bath was used to pump an antifreeze solution through the heat exchanger.

Experimental methods. Sixteen plants of each genotype and each cold hardiness level were randomly placed in the low-temperature chamber. All plants were placed in a walk-in cooler (4C) for 4 h before they were transferred to the low-temperature chamber. This ensured that all plants were at the same temperature when placed in the low-temperature chamber. Each combination of genotype and hardiness level was exposed to -4 ± 0.5C for two time periods (6 or 12 h) and two soil conditions (frozen and nonfrozen).

Treatment monitoring. The temperature of the chamber, leaves, stems, and soil was monitored directly by thermocouples using a Honeywell 24-point recorder (Honeywell, model 112). The thermocouples were placed on all plants while they were in the walk-in cooler, so the temperature of the plants and soil was monitored from the time they were in the low-temperature chamber until the experiment was completed. This procedure allowed us to determine the temperature when plant tissue froze. The chamber, leaf, and stem were all at the same temperature after 1 h. The temperature of the soil reached a stable level for all treatments ≈2 to 3 h after the beginning of the low-temperature exposure. When the soil was allowed to freeze, it froze at -1C and remained at -1C for up to 12 h. The freezing point of the soil was determined by observing the rise in temperature associated with the freezing event.

Analysis of freezing injury. The freezing of a plant was verified by: 1) monitoring leaf and stem temperature and observing the momentary rise in temperature when tissue water froze; 2) observing ice crystals on the leaf and stem tissue surface; and 3) noting the "stiffness" of the leaf and stem before removal from the low-temperature chamber.

After the cold treatments, the whole plants were placed at room temperature for at least 24 h and were visually analyzed for freezing injury. The plants that were killed by freezing were wilted and had water-soaked leaves (Chen et al., 1976) and were easily distinguished from living plants.

The plants recorded as severely injured had nearly 100% of their leaf and stem tissue killed, while those plants recorded as alive had no observable damage. Three groups of freezing pattern/injury categories were observed: 1) plants that froze but remained alive; 2) plants that were frozen and were severely injured or killed; and 3) plants that undercooled and remained alive. Since plants within each of these three groups were either all dead or all alive and no variation existed, the values are expressed as a percent of the total number of plants in each treatment that falls into each of the three freezing pattern/injury categories.

Solanum tuberosum *and 'Alaska Frost*less'. All leaves and stems of all plants were frozen and severely injured, regardless of soil condition, acclimation state, or duration of exposure. The tissue froze at -3 ± 0.5C.

Solanum acaule. Two types of freezing patterns were observed in *S. acaule:* all leaves and stems on a single plant froze, or all leaves and stems on a single plant undercooled. Injury was related to freezing in two ways. Regardless of the plant material, hardened or nonhardened, when the plant tissue froze, the leaves and stems were killed; but when the plant tissue undercooled the leaves and stems were not injured (Table 1).

The soil condition influenced the freezing pattern of *S. acaule.* The leaves and stems of all plants froze, regardless of treatment, when the soil was allowed to freeze, but when the soil was kept from freezing, the leaves and stems of some plants undercooled (Table 1). *Solanum acaule,* hardened and nonhardened, undercooled to -3 ± 0.5C before ice formed on leaves and stems that did freeze.

Solanum commersonii. Two types of freezing patterns were observed: either all of the leaves and stems on a single plant froze, or all the leaves and stems on a single plant undercooled. In all cases, regardless of the treatment or whether the leaves and stems were frozen or undercooled, the leaf and stem tissue was not injured (Table 1). The soil condition also influenced the freezing patterns of *S. commersonii.* The leaves and stems of all plants froze when the soil was allowed to freeze. When the soil was kept from freezing, the leaves and stems of some plants undercooled (Table 1). *Solanum commersonii,* hardened and nonhardened, undercooled to -3 ± 0.5C before ice formed in plants that did freeze.

A plant can resist injury at subzero temperatures in two ways: by avoiding the freezing of water within its tissues, or by tolerating the frozen water within its tissues (Levitt, 1980). From previous research it has been established that both hardened and nonhardened plants of *S. tuberosum,* 'Alaska Frostless', and nonhardened *S. commersonii* cannot tolerate freezing, whereas hardened and nonhardened *S. acaule* and hardened *S. commersonii* can tolerate freezing (Chen et al. 1976; Chen and Li, 1980). For those plants that can tolerate freezing, the initiation of freezing must occur at or above a critical temperature, or the plants are killed at the point of freezing. Lindstrom (1981) and Lindstrom and Carter (1983) found that leaves of hardened *S. commersonii* survived freezing to -11C when the freeze was initiated at -5 ± 1C or higher, whereas, if the freezing was initiated at -5 ± 1C or lower, the leaves were killed at the point of freeze initiation. Rajashekar et al. (1983) showed similar results in *S. acaule.* They found that leaf disks of both hardened and nonhardened material were killed when the freezing was initiated at -2C or lower. When Chen and Li (1980) studied the hardiness of *S. acaule* and *S. commersonii* they initiated the freezing of tissues at about -1.5C. They reported nonhardened and hardened *S. acaule* to survive exposure to -6 and -9C, respectively, while *S. commersonii* survived exposure to -4.5C and -11.5C for the nonhardened and hardened material, respectively.

In our studies we found that whenever leaves and stems of nonhardened and hardened plants of *S. tuberosum, S. acaule,* and 'Alaska Frostless' froze they were severely injured in all cases. However, plants of *S. acaule* that undercooled showed no injury. *Solanum commersonii* leaves and stems were not injured, regardless of whether their tissue froze or undercooled.

Promotion of undercooling is a viable strategy to reduce injury in those plants that cannot tolerate any freezing within their tissues. For example, since *S. tuberosum* and 'Alaska Frostless' cannot tolerate freezing even when the freezing is initiated at -1 or -2C, the only way to increase their ability to resist freezing injury is to encourage undercooling. Although neither *S. tuberosum* nor 'Alaska Frostless' showed any great ability to undercool, their tissues did undercool to -3 ± 0.5C before the freezing of tissue water was initiated. Thus, they do possess some ability to undercool. From our data, we cannot say for how long they could remain undercooled at -3C.

Promotion of undercooling would not be a reasonable frost protection strategy for plants with behavior similar to *S. acaule.* In our studies, leaves of *S. acaule* were killed in all cases when they were frozen. Chen and Li (1980) reported the leaves to be hardy to

-6C for the nonhardened plants and -9C for the hardened plants. In their studies, freezing of the plant tissues was initiated at about -1.5C. We observed in our study that the plants undercooled to -3 ± 0.5C before tissue water was frozen. In this case, the potential hardiness in *S. acaule* was not achieved because the whole plant undercooled before it froze. This result is in agreement with the findings of Rajashekar et al. (1983). Therefore, for *S. acaule*, it would seem best to promote freezing at a higher temperature to maximize its hardiness potential.

In contrast to the other plant taxa, *S. commersonii* tissues survived ice formation at -3C, and, regardless of whether the leaf tissues froze or undercooled, they were not injured. That the leaves tolerated this freezing is consistent with the findings of Lind-Strom (1981) and Lindstrom and Carter (1983). Therefore, in this study, we observed that the hardiness potential of *S. commersonii* was not reduced by the undercooling to -3C that occurred in leaves and stems that froze.

Cold acclimation had no effect on the undercooling ability of any plant materials tested. In contrast, Rajashekar et al, (1983) found that cold acclimation lowered the ice nucleating activity of small leaf disks of S. *acaule* and *S. tuberosum.* Although our study did not show great differences between hardened and nonhardened material with regard to their ability to undercool, we cannot challenge their results since the resolution of our experiment was not sufficient to detect small differences. It is likely, however, that the differences between the two studies are due to the size of the tissue analyzed (Ashworth and Davis, 1984; Ashworth et al., 1985).

The soil condition affected the undercooling ability of the potato plants studied. When the soil was frozen, all leaves and stems of all plant material were frozen. When the soil was kept from freezing, only then could some of the plants undercool. When the soil column was frozen, we could not state whether the roots were completely frozen since we observed only the post-treatment condition of the leaves and stems. The ice crystals in the soil or roots had to be in the proper location and in sufficient number to provide nucleation for the undercooled tissue water.

Dearborn (1969) reported that 'Alaska Frostless' posssesses some degree of frost resistance. In contrast, Chen et al. (1976) found that 'Alaska Frostless' possessed no more frost tolerance than *S. tuberosum.* Our results support the work of Chen et al.

There are conflicting reports on the hardiness of potato species. Richardson and Weiser (1972), in a summary on the frost resistance of potato species, showed that within a species the reported levels of cold hardiness varied dramatically. For example, *S. acaule* and *S. commersonii* were reported to exhibit hardiness between -2 and -10C. Upon reviewing the literature, neither the temperature at which freezing was initiated nor the state of acclimation of the plants was consistently mentioned. The variability in the literature is likely due to the occurrence of undercooling in some of the tissues and not in others. Based on our experiments and the published literature, we think more variables should be considered before a final decision is reached about the degree of frost resistance a plant possesses. It is important to examine whole plants since they may respond differently to subzero temperatures than do small leaf sections or disks, as shown by *S. acauIe* in our experiments. The plant should also be evaluated for its ability to withstand freezing when the freezing is initiated at several progressively lower subzero temperatures. Finally, the actual plant temperature should be reported so the temperature of ice formation is known.

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