FACTORS AFFECTING ICE NUCLEATION IN TOMATO PLANTS

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FACTORS AFFECTING ICE NUCLEATION

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CHAPTER I

INTRODUCTION

Frost injury causes major losses of agricultural crops. Losses of one half billion dollars have occurred in the citrus industry in one year (42). Spring frosts can virtually wipe out entire crops of peaches and apricots due to the tender nature of expanded flowers (34). In vegetable production, where getting an early crop is desirable, entire fields have to be replanted following a late frost. Increased expenses and reduced revenue result in hardship to the grower and ultimately increase cost to the consumer.

Plant response to low temperature stress varies with the type of plant. Plants are usually grouped into two categories, the hardy plants, which can tolerate freezing temperatures, and tender plants which cannot tolerate freezing temperatures. Hardy plants are able to acclimate to withstand temperatures as low as -196°C (35). On the other hand, tender plants do not have the ability to acclimate to freezing temperatures and cannot tolerate the formation of ice within the plant (12). Survival in tender plants is dependent upon avoidance of freezing (18). Tender plants avoid freezing mainly through supercooling,

the cooling of tissue water below the thermodynamic melting point without freezing. Under laboratory conditions tender plants have cooled to -10°C without freezing, but under natural conditions they rarely supercool below -3°C (23).

Supercooling is limited by the activity of ice nucleators, the agents that trigger freezing (18,19,21). Certain particles such as kaolinite and silver iodide have exhibited ice nucleation activity, but these particles are not very abundant in the atmosphere (30,40). Several organic compounds have been shown to be ice nucleators but they are only active in the crystalline form which rarely occurs in nature (32). These ice nucleators are not sufficient to explain the incidence of frost damage to plants at temperatures as warm as -2°C in the field.

Recently a bacterial source of ice nucleation has been found. Certain bacteria have been found to increase ice nucleation activity of plant tissues at temperatures associated with frost damage in the field (4,5,23,26,27). The most common bacterial ice nucleators are Pseudomonas syringae and Erwinia herbicola (5,26,27,33). Other bacteria found to be active in ice formation are P. fluorescens (27) and P. viridiflava (33). The most common species, P. syringae and E. herbicola are known to be widely distributed (21,23,33). The relatively warm ice nucleating temperatures and widespread distribution of these common INA bacteria could account for the extensive

occurrence of frost damage in the range of -2° to -5°C (21).

Other factors, in addition to INA bacteria, have been found to have an effect on ice nucleation in plants. In studies with tomato (1), bean, cotton, corn, and soybean (8), increases in plant mass resulted in warmer freezing temperatures in the absence of INA bacteria. The observed increase in freezing temperature could be a result of more sites being available for ice nucleation in larger plant samples.

Work by Cary and Mayland has demonstrated that plants exhibiting symptoms of water-stress will supercool more than well-watered plants of the same species (14). Studies have indicated that the presence of water on plant surfaces can also cause an increase in the freezing temperature of plants (8,14). Since dew frequently forms on the surfaces of leaves prior to freezing this factor could be important in frost damage. INA bacteria are currently thought to be one of the primary agents responsible for limiting supercooling (5,22,27,33). However, several criteria must be met in order for INA bacteria to play an important role in plant frost damage. INA bacteria must be efficient ice nucleators, they must be widely distributed, and plants must be able to supercool in their absence. Many studies have presented evidence supporting the first two criteria (18), but more work needs to be done on distribution studies. Population levels of INA bacteria, mainly on

perennials, have been determined in previous studies (15,21). However, information on the rate of colonization on transplants is lacking. Knowledge of colonization rates could affect management practices aimed at controlling INA bacteria.

My first objective was to determine the rate of colonization by ice nucleation active bacteria on tomato plants. Since previous work had indicated that the presence of water on leaves could also be a factor in ice nucleation, my second objective was to determine the effect of surface moisture on the freezing of plants.

CHAPTER II

LITERATURE REVIEW

Studies have shown that bulk water samples and plants do not always freeze at 0°C (10,18). Drops of water up to 2.5 cm diameter can supercool in the range of -10° to -20°C (10). Some hardy plant cells can supercool up to 40°C below the melting point of water in the absence of ice nuclei (12). Under laboratory conditions plants frequently supercool to -10°C (5) yet in the field plants are killed by temperatures as warm as -2°C (29,31). Supercooling is limited by the activity of ice nucleators, the agents that trigger freezing (18,19,21).

The search for ice nuclei responsible for freezing at relatively warm temperatures in the field has led to further research into naturally occurring agents. Atmospheric scientists launched a search for sources of naturally occurring ice nuclei that may be important in precipitation processes. Particles of dust and substances such as kaolinite had been thought to be responsible for ice nucleation in clouds, however kaolinite was found to be active at temperatures below -9°C (30). Work by Marcellos and Single (28) has shown that atmospheric particles probably do not contribute to ice nucleation in plants.

Silver iodide is active at temperatures above -8°C but is not very abundant in nature (40). A number of organic compounds such as amino acids, proteins, steroids, and terpenes have been shown to have ice nucleation activity at temperatures above -5°C but they are only active in the crystalline form (32). When these compounds are dissolved in water they lose ice nucleation activity (18). Research by Schnell and Vali (37) with leaf litter demonstrated the presence of ice nucleators able to initiate freezing at temperatures in the range of -2° to -5°C. These ice nucleators were later found to be of bacterial origin (26,39).

Additional studies discovered other bacterial species responsible for initiating ice formation. The most common bacterium found to be an ice nucleator was Pseudomonas syringae (5,26,27,33). Other pseudomonads, such as P. fluorescens (27) and P. viridiflava (33) have also been found to be active in ice formation. Another common ice nucleation active bacterium is Erwinia herbicola (22,33). The two most frequently encountered species are P. syringae and E. herbicola, but not all strains of these bacteria have ice nucleation activity (33).

Occurrence of the ice nucleation phenotype varies among a particular species of bacteria. For example, in a study by Paulin and Luisetti, 106 of 134 strains of P. syringae were active in ice nucleation while only one strain among those tested of E. herbicola was active (33).

In another study, 168 of 175 bacterial isolates incited frost damage (23). These included 43 of 48 isolates of E. herbicola and 124 of 126 isolates of P. syringae (23).

Both P. syringae and E. herbicola are reported to have a widespread distribution. This could account for the widespread occurrence of frost damage in the range of -2° to -5°C (21). The population levels, however, are variable in regard to climate, plant material, and time of year (15,19,21). Climatic conditions have an effect on the distribution with the highest levels occurring in cool wet areas and the lowest in hot dry regions (15,21). In drier portions of Washington state INA bacteria are not as common as in other wetter portions of the U.S. (15). Certain plants such as conifers and the cole crops have been found to harbor very few if any INA bacteria while on other plants they are very common (21). The population level of INA bacteria present during the year is also variable on a given species of plant. In woody perennials the levels are highest in the spring while populations increase through the season on annuals (21).

Freezing efficiency of ice nucleation active bacteria is determined by ice nucleation frequency and effective temperature range (17,19,23). Ice nucleation frequency is defined as the ratio of ice nuclei to bacterial cells at a given temperature. The nucleation frequency is variable within a species (17,23). At a temperature of -2.3°C an isolate of P. syringae had a nucleation frequency of 10^{-7}

nuclei per bacterial cell while an isolate of E. herbicola had a nucleation frequency of 10^{-8} nuclei per bacterial cell at -2.6° (23). Temperature also plays a role in nucleation efficiency. Nucleation frequency increases from 10^{-7} to 10^{-2} nuclei per cell as test temperatures decrease from -2.3° to -4.0° in P. syringae. In an isolate of E. herbicola, nucleation frequency increased from 10^{-8} to 10^{-5} nuclei per cell with a decrease in temperature from -2.6° to -4.0° (23). In general, P. syringae initiates freezing at a higher temperature and has a slightly higher nucleation frequency in its effective temperature range than E. herbicola (19,23).

Efficiency of nucleation has been determined for plant-borne bacteria as well as suspensions. Bacterial populations must be present at a threshold inoculum level to increase plant frost damage (27). Threshold levels of 4×10^5 cells/ml of P. syringae have been required to influence freezing at -5°C in zinnia (4) and tomato (5). In flower buds of Prunus sp. inoculated with P. syringae, nucleation frequency ranged from 8.7×10^2 cells per -5° ice nucleus in peach buds to 3.9×10^3 cells per -5°C nucleus in sweet cherry buds (16). On peach an isolate of E. herbicola required at least 10^3 cells per gram fresh weight to significantly increase freezing nuclei (3). In another study, at -2.5° nucleation frequency ranged from 10^3 to 10^7 cells per ice nucleus on oat leaves (17). With

E. herbicola on corn, 10^5 cells per gram fresh weight were required to significantly increase freezing (22).

For a particular strain the conditions in which the bacteria are cultured have an effect on the efficiency of ice nucleation (25,27). Culture conditions such as media composition (4,21,31), aeration (26), and temperature (25,27) affect the efficiency. A growth medium containing a carbon source such as dextrose or glycerol has been found to be needed for most bacteria to exhibit ice nucleation properties (4,22,25,33). In addition, aeration of liquid medium has increased ice forming activity of bacterial cultures (26). Growth temperatures have been shown to have a profound effect on bacterial ice nucleators. In a study by Lindow, cultures of P. syringae, isolate 31, grown at 24°C had the greatest nucleation frequency at -5° as compared to those grown at 12° and 32° (25). Another study showed the highest activity of another isolate of P. syringae when grown at temperatures between 4° to 10°C (27). In addition to temperatures during active growth of bacterial cultures, preconditioning of cultures in the stationary phase has had an effect on subsequent freezing temperatures. During preconditioning a stationary phase bacterial culture (one that is no longer multiplying) is exposed to a test temperature for a time period prior to ice nucleation assays. Exposure to a temperature of 4°C for 3 hours prior to ice nucleation assays optimized ice nucleation activity by P. syringae (5) and P. viridiflava

(2). With increase in incubation temperature the median ice nucleation temperature decreased (2).

Other factors, in addition to INA bacteria, have been found to have an effect on ice nucleation in plants. In studies with tomato (1), bean, cotton, corn, and soybean (8), increases in plant mass resulted in warmer freezing temperatures. Therefore, small samples (leaf discs, homogenates) do not accurately predict freezing in intact plants (8). In a study with peach stem sections, samples weighing less than 1 gram froze at -4°C or colder while 20 g samples froze at -2°C. The 20 g samples were found to be more indicative of what occurs in the tree as a whole (7). The effect of sample mass is not unique to plants. In autoclaved soil an increase in freezing temperature from -10.5° to -1.9°C was observed with increasing mass from 0.2 mg to 100 g (8). The freezing process is stochastic in nature. As sample size increases, the probability of a freezing event occurring also increases. With increasing sample size more sites are likely to be available for ice nucleation.

The water status of a plant can have an effect on the ability of a plant to supercool. Exposing frost-sensitive citron to water-stress conditions has been shown to improve freeze survival (41). Work by Cary and Mayland has demonstrated that plants exhibiting symptoms of water-stress will supercool more than well-watered plants of the same species (14). This held true for a 3-5 hour period,

but for longer periods well-watered plants had a better survival rate. The effect of water stress on supercooling may be from less water being available for ice formation.

Surface moisture also plays a role in the ability of a plant to supercool. Some studies have indicated that the presence of water on leaves can cause an increase in the freezing temperature of plants (8,14,38). Differences of 3°C in freezing temperatures were observed on misted cotton, soybean, and bean plants as compared to dry controls. Temperature measurements suggested that the effect was not due to evaporative cooling (8). In Eucalyptus, the surface moisture effect was also exhibited but differences between genotypes were observed that were dependent on the degree of waxiness on the leaves. With increasing glaucousness (waxiness) the plants supercooled more due to less water in contact with the leaf surface (38). The surface moisture effect could prove to be important since plant surfaces frequently reach dewpoint in the field.

Current methods of frost protection are effective to temperatures as low as -3°C (18), but a major limitation of these methods is the high cost. In orchards, heaters have been used effectively in the past (11). Wind machines have worked when a warm layer of air exists above a planting (11). Water applications are only effective when a few degrees of protection are required during a radiational

freeze (11). Covers used on vegetable crops are too labor intensive and expensive (11).

The current methods of frost protection do not take into consideration the implications of factors affecting ice nucleation in plants. INA bacteria have been shown to be an important factor in frost damage to plants in the laboratory (4,5,17,22,23,26). Control of frost damage by limiting populations of INA bacteria is an attractive approach.

Possible methods of frost control involve use of antibiotics, nucleation inhibitors, and competitive bacteria (19,20,24). Antibiotics are most effective when applied before bacterial populations have become established on the plants (24). All of the substances found to be effective nucleation inhibitors, chemicals that inactivate the ice nuclei associated with INA bacteria, are too phytotoxic to the plants (20). The use of competitive bacteria as a biological control of frost damage has the most promise (19), but further research is needed. Plants are inoculated with non-INA bacteria which compete for the available nutrients on plant surfaces and reduce the number of INA bacteria. Because of their small size, young seedlings appear to be the best candidates for frost avoidance. Greenhouse-grown seedlings are generally free of INA bacteria upon transplant to the field (1,6,24). Further research into other factors affecting ice

nucleation could lead to ultimately reducing the probability of frost damage.

CHAPTER III

COLONIZATION OF TOMATO PLANTS BY INA BACTERIA

Spring frosts can be devastating to tender plants such as tomato. Freeze damage results in increased expenses due to replanting costs and delayed harvest. Tomato plants can supercool (remain unfrozen) to temperatures as low as -8°C in the absence of external agents capable of triggering freezing (5). When ice nucleating agents, such as INA (ice nucleation active) bacteria, are present plants can freeze as warm as -2° (1,18). Management practices aimed at limiting populations of INA bacteria are potential methods of providing frost protection. If colonization of tomato transplants by INA bacteria can be delayed for a few weeks the probability of frost damage would be decreased. Although some distribution studies have been done on perennial plants (15,21), no information is available on the rate at which annuals are colonized by INA bacteria. My objective was to determine the rate of colonization of tomato plants by INA bacteria.

Tomato seeds (Lycopersicon esculentum Mill. 'Supersonic') were planted in 7.5 cm peat pots filled with commercial potting mix. Seedlings were grown in a

greenhouse maintained at a minimum temperature of 18°C. Plants were fertilized biweekly with a water soluble 18N-7.9P-17.4K fertilizer and watered as needed. After 6 weeks, water was withheld for 5 days prior to transfer to a screenhouse. The plants were hardened off under ambient conditions for 7 days. On April 22, 1987, the tomatoes were transplanted in the field 1 m apart with a double row of border plants on the ends of the plot and a single row of border plants on the sides.

Plants were sampled on the final day in the greenhouse and the day before transplanting to the field plot. Field sampling was conducted on days 1-5, 7, 9, 12, and 15 after transplanting. On each date 9 plants were chosen at random and dug with the roots and adhering soil intact. Of the 9 plants, 5 were assayed for populations of INA bacteria and the remaining 4 were used to determine freezing temperatures.

Population levels of INA bacteria were determined using procedures described previously (1) except each plant was homogenized in 180 ml of sterile deionized water. After 3-4 days at 21°C counts of total and fluorescent colonies were made directly from Pseudomonas agar F plates supplemented with 100 ug/ml cycloheximide. INA bacteria were detected by the plate harvesting technique (1) using 5 ml of sterile deionized water instead of liquid medium. Tubes were examined for freezing at -2°, -3°, -5°, and

-7°C. Tubes freezing at -5° or warmer were considered to contain detectable levels of INA bacteria.

Freezing temperatures were determined in a low temperature incubator (model CEC23, Rheem Scientific, Asheville, NC). Plants were buried in a container of vermiculite to the same depth as in the field to prevent freezing of soil moisture. Thermocouples were attached to the stems above the second node. Plant temperatures were recorded at 1 min scanning intervals using a datalogger (Model 21X, Campbell Scientific, Logan, UT). The chamber was cooled to -15°C at a rate of 3°C/hr with freezing temperatures determined by exotherm analysis (9). Plant and soil moisture contents were obtained after thawing. Water content was expressed as g water/g dry weight.

Data were analyzed using the General Linear Models Procedure (36). The model employed was: FT = WT WC INA, where FT = plant freezing temperature (°C), WT = plant fresh weight (g), WC = plant water content (g water/g dry wt), and INA = population of INA bacteria (log cells/g fresh wt).

Plants from the greenhouse harbored total bacterial populations of 2.0×10^4 cells/g fresh weight. Fluorescent bacteria were present on 40% of the plants sampled with a mean population of 7.9×10^2 cells/g fresh weight when present. After a week of hardening off in a screenhouse, total bacterial populations remained at about 2.5×10^4 cells/g fresh weight. All 5 plants yielded fluorescent

bacteria with a mean population of $6.3x10^2$ cells/g fresh weight. No INA bacteria were detected on plants sampled from the greenhouse or screenhouse.

After transplanting, mean total bacterial populations ranged from $1.9x10^4$ to $5.0x10^5$ cells/g fresh weight. Fluorescent bacteria were present on all plants sampled from the field with populations ranging from $7.9x10^2$ to $1.6x10^4$ cells/g fresh weight. INA bacteria were detected on at least one plant from the first day after transplanting through the remainder of the sampling period. Although the fraction of plants infested increased with time in the field, some plants did not have detectable levels of INA bacteria after two weeks. Mean populations were variable, ranging from $6.3x10^0$ to $1.3x10^4$ cells/g fresh weight.

The mean freezing temperature of plants from the greenhouse was -7.5°C (Table 1). After a week in a sheltered outdoor screenhouse a mean freezing temperature of -5.7° was observed while mean freezing temperatures of plants from the field ranged from -3.6° to -6.4°. Mean freezing temperatures of field plants were between -5.4° and -6.4° for the 9 days after transplanting with the exception of the first day (-3.6°). Freezing temperatures of about -4° were observed on days 12 and 15 after transplant.

The relatively warm mean freezing temperature observed on the day after transplant did not appear to be related to

population level of INA bacteria. The source of ice nucleation in 2 of the plants at about -2°C was not known. Since different plants were used for bacterial assays and freezing temperature determinations, the presence of large populations of INA bacteria on the 2 plants freezing at -2° could not be ruled out.

Studies have shown that moisture status (14), sample mass (1,8), and exposure duration (1,8) affect plant freezing temperatures. Plant water content significantly affected plant freezing temperatures in the present study (Table 2). However, the relationship may have been coincident, rather than real. Plants from the greenhouse and screenhouse had the highest water contents, yet the coldest freezing temperatures. Previous work has indicated that water-stressed plants tend to supercool to lower temperatures (14). No relationship between water content and freezing temperature was observed once plants were placed in the field. Plants were well watered, avoiding stressed conditions.

Plant mass, on the other hand, had a marked effect on freezing temperature. As plant weight increased, freezing temperatures became warmer. In fact, most of the observed increase in freezing temperatures during the course of this study could be attributed to growth of the plants.

Ice nucleation active bacteria were detected on tomato one day after transplant. Most plants sampled harbored INA bacteria from 3 days after transplant, however some plants

appeared free of INA bacteria as late as 15 days after transplant. Mean population levels of INA bacteria were variable rather than increasing steadily with time. Population levels may increase at a greater rate and reach higher numbers under more favorable environmental conditions. Mean air temperatures were extraordinarily warm for this time period [28°(max)/12°C(min)] with virtually no rain. Among the main effects no significant interactions were observed. The increase in mean freezing temperature of tomato transplants from -7.5° to -4° during the course of the study was due primarily to increasing plant size rather than colonization by INA bacteria.

Table 1. INA bacterial populations, freezing temperatures, and fresh weights of tomato seedlings sampled before and after transplant to the field.

Date[z]	Number of plants with INA bacteria[w]	INA bacterial population[y] (X ± SD)	Freezing temp. °C (X ± SD)	Fresh wt. (g) (X ± SD)
Apr. 13	0	ND[x]	-7.5 ± 1.2	14.1 ± 1.9
Apr. 21	0	ND	-5.7 ± 0.5	18.1 ± 3.4
Apr. 23	1	0.8 ± 0	-3.6 ± 2.5	19.4 ± 5.9
Apr. 24	2	0.8 ± 0	-6.4 ± 0.7	20.8 ± 1.2
Apr. 25	3	1.2 ± 0.6	-5.7 ± 0.7	19.9 ± 4.3
Apr. 26	3	2.7 ± 0.1	-6.0 ± 1.0	18.6 ± 5.1
Apr. 27	5	2.8 ± 1.4	-5.4 ± 1.4	23.7 ± 2.3
Apr. 29	3	1.6 ± 1.1	-5.7 ± 0.8	19.2 ± 5.9
May 1	5	2.3 ± 1.3	-5.4 ± 0.9	26.4 ± 7.4
May 4	5	1.1 ± 0.8	-4.1 ± 0.8	47.0 ± 7.4
May 7	4	1.5 ± 1.6	-4.0 ± 0.5	41.4 ± 19.4

[z]Plants moved from greenhouse to screenhouse for hardening on 14 Apr. and transplanted to field on 22 Apr.

[y]Log cells/g fresh weight

[x]None detected

[w]Total of 5 plants on each date

Table 2. General Linear Models Procedure results for the effects of fresh weight (WT), water content (WC), and INA bacterial population (INA) on plant freezing temperature.

Source	Mean square	F value	Pr>F
WT	15.4	10.4	0.0025
WC	10.0	6.8	0.013
INA	2.0	1.4	0.25

CHAPTER IV

THE EFFECT OF SURFACE MOISTURE ON FREEZING TEMPERATURES OF PLANTS

Several factors have been shown to increase frost damage to tender plants. These include INA bacteria, plant mass, water status of the plant, and surface moisture (1,8,13,14,38,41). In a previous study with tomato, increasing plant mass resulted in warmer freezing temperatures (1). Work by Cary and Mayland has demonstrated that plants exhibiting water-stress symptoms will supercool more than well-watered plants of the same species (14). Studies have indicated that the presence of water on leaves can result in an increase in the freezing temperature of plants (8,14). Plant surfaces frequently reach dewpoint before freezing temperatures are attained in the field. Since the presence of dew on plant surfaces may have an effect on increased frost damage it could be an important factor in frost control. My primary objective was to determine the effect of surface moisture on the freezing temperature of tomato plants. A secondary objective was to determine the effect of water-stress on ice nucleation in tomato plants.

Tomato seedlings were grown for 3 to 4 weeks in a greenhouse before freezing assays were performed. On each

date the plants were of relatively uniform mass. Plants were placed in a styrofoam sub-chamber that was placed into an environmental chamber for cooling the plants. The sub-chamber was partitioned into 2 equal sections, one side for misted plants, the other side remaining dry. Each side contained 2 plants, one well-watered, the other with water withheld for the previous 5 to 7 days. The tomato seedlings were placed in the chamber with the pots buried in vermiculite in order to insulate the root systems. Thermocouples were placed on the stem as described in the previous section. Wetness sensing grids were placed in each side of the styrofoam chamber to be certain that dewpoint had not been reached on dry controls and to document saturated moisture levels in the misted section throughout the treatment period. Plants were cooled at a rate of 1°C per hour. A scanning interval of 60 seconds was used to monitor temperatures. Plant freezing temperatures were determined as described previously. To rule out the possibility that INA bacteria were present (and confounding results), plants were assayed for INA bacteria using procedures described in the previous section. The experiment was replicated 18 times.

Well-watered plants with dry surfaces exhibited freezing temperatures ranging from -4.9° to -10.1°C with a mean of -8.0°C (Table 3). The freezing temperatures of water-stressed plants with dry surfaces ranged from -5.9° to -9.7°C with a mean of -7.6°. In contrast, misted plants

froze over a warmer temperature range than dry plants. Well-watered misted plants froze in the range of -1.7° to -5.6°C with a mean of -3.5°C. Freezing temperatures of plants that were misted and water-stressed ranged from -2.7° to -5.7°C with a mean of -3.7°C. Therefore, plants that were misted froze at warmer temperatures than plants with dry surfaces.

Considerable variation occurred in the moisture levels of the potting mix. Soil moisture levels of well-watered plants ranged from 2.7 to 5.7 g water/g dry weight with a mean of 4.5g water/g dry weight in both unmisted and misted plants. Water-stressed treatments had a range of soil moisture levels from 1.2 to 3.3 g water/g dry weight. Mean moisture levels of 2.0 g water/g dry weight in unmisted and 1.9 g water/g dry weight in misted treatments were observed.

In spite of plants of uniform mass on each date, plant fresh weights ranged from 0.8 to 9.3 g over the entire study. Although no significant differences in mean plant fresh weights were noted between treatments, mass had a significant effect on plant freezing temperatures with smaller plants supercooling to a greater extent. These results further substantiate the effect of plant size on plant freezing temperatures.

Bacterial populations were determined to rule out the possibility of INA bacteria populations affecting freezing temperatures. Total bacteria populations ranged from

$1.9x10^3$ to $2.8x10^5$ cells/g fresh weight. Fluorescent bacteria were not always detected on the plants but ranged from $6.7x10^0$ to $7.1x10^3$ cells/g fresh weight when they were present. None of the plants sampled harbored detectable levels of INA bacteria.

Previous studies had indicated the possible role of surface moisture in reducing supercooling of plants in the absence of INA bacteria (8,14,38). Since dew frequently forms on plants surfaces prior to freezing temperatures occurring, the warmer freezing temperatures experienced when plant surfaces are wet could be important in frost control. The results of this study indicated that misted plants froze warmer than dry plants. The persistence of surface water until freezing of the misted plants was confirmed by a wetness sensing grid. A wetness sensing grid on the dry side of the sub-chamber confirmed that dewpoint was not reached during the course of the treatments. The absence of detectable levels of INA bacteria clarifies the effect of surface moisture on plant freezing.

Plant water status has been shown to have an effect on freezing temperatures . Exposing frost sensitive plants to water-stress conditions has been shown to increase supercooling compared to well-watered plants of the same species (14,41). The effect of water stress on super-cooling was not apparent in this study, contrary to the work by Cary and Mayland (14). The tomato seedlings used

in this study were not at the wilting point at the start of treatments, however, the plants used in the study by Cary and Mayland were exhibiting severe water-stress symptoms. Therefore, extreme levels of tissue hydration may need to occur before measurable effects on freezing are observed.

It is doubtful that the effect of misting can be attributed to increases in plant water content resulting from the application of water on the plants. Changes in plant water content reflected by soil moisture content, did not have an effect on plant freezing. Another explanation for the effect of misting could be that potential ice nucleation sites, that are normally not in contact with the tissue water, make contact via the surface water. However, work by Ashworth et al (8), did not support this possibility since submerged shoots did not exhibit the misting effect.

The results of this study show that the persistence of surface moisture on plant surfaces significantly increased the plant freezing temperatures. No significant differences in freezing temperatures were observed between plants that were at field capacity and those that were water-stressed. If the plants had been severely water-stressed the effect may have been more evident. Factors such as the presence of surface moisture need to be taken into consideration in frost control.

Table 3. Tomato plant freezing temperatures, fresh weights, and soil moisture contents.

	Dry		Misted	
	FC[z]	WS[y]	FC	WS
Freezing temp. (°C)	-8.0 ± 1.5[x]	-7.6 ± 1.1	-3.5 ± 1.1	-3.7 ± 0.9
Fresh weight (g)	3.1 ± 2.5	2.8 ± 1.9	3.4 ± 2.6	3.1 ± 2.0
Soil moisture (g H_2O/g dry wt)	4.5 ± 0.8	1.8 ± 0.6	4.5 ± 0.9	1.9 ± 0.7

[z]Field capacity

[y]Water-stressed (water withheld for 5 days)

[x]mean ± standard deviation

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VITA 2

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