2. Benson, B.L. and F.H. Takatori. 1975. Asparagus research in 1974–1975. Univ. of Cal., Div. Agr. Sci. and Mgmt., Veg. Crops Series 173:1–9.
3. Benson, B.L. and F.H. Takatori. 1978. Meet UC157. Amer. Veg. Grower 26:8–9.
4. Benson, B.L. and F.H. Takatori. 1980. Partitioning of dry matter in open-pollinated and $F_1$ hybrid cultivars of asparagus. J. Amer. Soc. Hort. Sci. 105:567–570.
5. Benson, B.L. and J.E. Motes. 1981. Influence of harvesting asparagus the year following planting on subsequent spear yield and quality. HortScience 16:58. (Abstr.).
6. Blasburg, C. 1932. Phases of anatomy of *Asparagus officinalis*. Bot. Gaz. 94:206–214.
7. Currence, T.M. and A.L. Richardson. 1937. Asparagus breeding studies. Proc. Amer. Soc. Hort. Sci. 35:554–557.
8. Ellison, J.H. and D.F. Scheer. 1959. Yield related to brush vigor in asparagus. Proc. Amer. Soc. Hort. Sci. 73:339–344.
9. Ellison, J.H., D.F. Scheer, and J.J. Wagner. 1960. Asparagus yield as related to plant vigor, earliness, and sex. Proc. Amer. Soc. Hort. Sci. 75:411–415.
10. Ellison, J.H. and L.G. Schermerhorn. 1958. Selecting superior asparagus plants on basis of earliness. Proc. Amer. Soc. Hort. Sci. 72:353–359.
11. Jones, H.A. 1932. Effect of extending the cutting season on asparagus. Calif. Agr. Expt. Sta. Bul. 446.
12. Morris, D.L. 1948. Quantitative determination of carbohydrates with Dreywood's anthrone reagent. Science 107:254–255.
13. Norton, J.B. 1913. Methods used in breeding asparagus for rust resistance. Bur. Plant Ind. Bul. U.S. Dept. Agr. 263.
14. Shelton, D.R. and M.L. Lacy. 1980. Effect of harvest duration on yield and on depletion of storage carbohydrates in asparagus roots. J. Amer. Soc. Hort. Sci. 105:332–335.
15. Tiedjens, V.A. 1924. Some physiological aspects of *Asparagus officinalis* L. Proc. Amer. Soc. Hort. Sci. 21:129–140.
16. Tiedjens, V.A. 1928. Does root selection accomplish its purpose in asparagus culture? Proc. Amer. Soc. Hort. Sci. 25:37–40.
17. Young, R.E. 1937. Yield-growth relations in asparagus. Proc. Amer. Soc. Hort. Sci. 35:576–577.

*J. Amer. Soc. Hort. Sci.* 108(6):1030–1034. 1983.

# Ice Nucleation Active (INA) Agents in Freezing of Young Citrus Trees

**G. Yelenosky**
*Agricultural Research Service, U.S. Department of Agriculture, 2120 Camden Road, Orlando, FL 32803*

*Additional index words.* supercooling, freeze avoidance

***Abstract.*** **Aqueous suspensions of ice nucleation active (INA) bacteria [*Pseudomonas syringae* van Hall and *Erwinia herbicola* (Löhnis) Dye] and suspensions of nonbacterial agents, silver iodide, phenazine, and flurophlogopite, were used to induce freezing in young citrus trees, 'Valencia' orange [*Citrus sinensis* (L.) Osbeck] on sour orange (*C. aurantium* L.) rootstock. Trees sprayed with INA agents froze at higher temperatures than unsprayed trees. INA bacteria-induced freezing was significant only when leaf surfaces were allowed to dry prior to freeze tests. Leaves with dry surfaces supercooled 1° to 3°C more than wet leaves, which started to freeze, and about 1° sooner with than without INA agents. Differences between INA bacteria and nonbacterial agents were not significant in the moment of freeze of wet leaves. INA agents induced freezing in citrus leaves usually before −5°, and in water drops, before −4°. Freezing was easier to induce on the underside (abaxial) than top (adaxial) surfaces of leaves. Sucrose, proline, and expressed sap were nonINA on citrus leaves and in drops of water.**

Agricultural losses due to freezes are in millions of dollars annually in the United States and billion-dollar crops such as citrus are especially vulnerable (25). Protection systems, such as the burning of petroleum fuels, rapidly are becoming economically prohibitive and the search for low-energy systems continues. Supercooling is probably the most energy-efficient freeze avoidance mechanism that assures plant survival (4, 7). In some instances, artificially induced freezing to minimize supercooling is beneficial, taking full advantage of large ice crystals and equilibrium freezing as in the protection of peach buds (13).

Supercooling is largely an unexplained and unpredictable event in the freezing of plants, but it is associated with numerous factors ranging from diameter of xylem vessels (3) and degree of vascular development (2) to the presence of heterogeneous nucleators (8, 16, 18). Temperature- and water stress-induced cold hardening increases supercooling in citrus (20, 27), but varietal differences in supercooling do not necessarily reflect differences in cold hardiness (21).

INA agents offer some means of control. Elimination of INA nuclei may increase freeze avoidance or minimal supercooling may avoid lethal intracellular stresses. Ice nuclei, including those associated with cloud seeding (12), are being reinvestigated in crop protection systems. The most efficient and natural external ice nucleators of plants are probably INA bacteria (1, 11, 17), which have been implicated in freeze damage to crops, including citrus (18). The overall INA concept has both meterological and agricultural implications in modeling systems to promote an adequate and nutritional world diet.

This study was conducted under controlled-temperature regimes to determine the activity of INA agents in a variety of conditions commonly associated with the freezing of citrus trees. Relative effectiveness of different INA agents on citrus leaves and potential problems in citri-culture were major concerns.

## Materials and Methods

Test trials were varied to include different situations and INA agents. Trees were either self-grown or from commercial nur-

Received for publication April 30, 1983. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that also may be suitable. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

series. Self-grown trees were 'Valencia' orange (*Citrus sinensis*) budded on 10-month-old sour orange (*C. aurantium*) rootstock grown from open-pollinated seed. Individual trees were grown in 2.5-liter plastic pots containing equal parts of sphagnum peatmoss, vermiculite, and perlite. Trees were watered as needed under natural light in a greenhouse. Maximum light approached $1 \times 10^3$ μmol $s^{-1}m^{-2}$ (PAR) with 35°C and minimum 35% relative humidity during days, and 20° minimum and 97% maximum relative humidity during nights. Trees of uniform growth and appearance were randomly selected for tests.

INA bacteria used were slant cultures of *Pseudomonas syringae*, *Erwinia herbicola*, and lyophilized *P. syringae* (courtesy of S. Lindow, Univ. of California, Berkeley) and frozen broth cultures of *E. herbicola* (courtesy of Microlife Technics, Sarasota, Fla.). Slant cultures stored at 5°C were subcultured periodically on nutrient agar containing 2.5% glycerol. Bacteria populations were grown in nutrient broth with continuous agitation at 30° for 8 hr and stored at 4° for 20 hr. Turbid broth cultures were centrifuged at 5000 $\times g$ and bacteria pellets suspended in either 150 ml sterile water, 0.1 M phosphate buffer (pH 7.0), or in nutrient broth. Spray concentrations were based on pour plate dilutions. Estimates of bacterial populations prior to test sprays were the result of immersing single leaves, 3 to 5 per tree, each into 10 ml of sterile $H_2O$, shaking for 30 minutes on a vortex shaker, and a series of pour plate dilutions. Similar samples were taken after test sprays immediately before freeze tests. Frozen broth cultures of *E. herbicola* (Microlife Technics) were stored at −60° and contained $2 \times 10^{10}$ colony-forming units (CFU) per ml. These were diluted as needed with sterile water. Antagonistic or nonINA bacteria (Microlife Technics) were treated similarly and used in conjunction with agricultural streptomycin sulfate (17% streptomycin) to inhibit INA bacteria. Lyophilized *P. syringae* at $3.6 \times 10^{10}$ active ice nuclei per gram was used in 1% (w/v) freshly prepared aqueous suspensions. Other suspensions were of nonbacterial INA agents, silver iodide, phenazine, and flurophlogopite (a synthetic mica, courtesy of C. Rajashekar, Univ. of Minnesota, St. Paul).

Spray volumes per tree ranged from 15 ml to runoff. Spraying was done between 8 and 10 AM and occurred between 0.5 to 36 hr before freeze tests. Freeze tests were conducted in the dark and 50 ± 5% relative humidity in a controlled-temperature facility (23). Induced freezing was determined visually as water-soaking in the leaves (26). The moments of freezing or threshold temperatures were based on instant release of latent heat of fusion during exothermic scans using 36-gauge, copper-constantan thermocouples. Thermocouple leads were connected to digital multimeters (1 uv per digit resolution). Accuracy was rated ± 1 digit. Reference junction was an insulated ice bath stable at 0 ± 0.1°C. Variable speed strip-chart recorders, 0 to 100 mv, were connected to digital multimeters.

## Results and Discussion

Most noticeable differences in tests with INA bacterial isolates from California were in trials using relatively high concentrations of *P. syringae* (Table 1). INA-bacteria-induced freezing was significant only when citrus leaf surfaces were allowed to dry before trees were frozen. Wet leaf surfaces started to freeze about 1°C sooner with than without INA agents. Differences in freezing of wet leaves were not significant between INA bacteria and nonbacterial agents. Nonbacterial agents apparently are as effective as INA bacteria in inducing early freezing in citrus leaves with wet but not dry surfaces. Leaves with dry surfaces supercooled to −4.4°, whereas wet leaves started to freeze at −2.2° regardless of INA bacteria. The number of trees starting to freeze after 15 min at −4.4° averaged one tree every 6 min for INA-bacteria-sprayed trees, and one tree every 18 min for unsprayed trees. This 3-fold frequency increase attributed to INA bacteria is considered significant in the freezing of citrus trees

Table 1. Number of potted 'Valencia' orange trees freezing with and without INA bacteria sprayed on the leaves.[z]

| | | No. trees freezing | | | |
|---|---|---|---|---|---|
| | | *Pseudomonas syringae* | | Water controls | |
| Temp (°C) | Time (min) | Wet leaves[y] | Dry leaves[x] | Wet leaves | Dry leaves |
| −1.1 | 120 | 0 | 0 | 0 | 0 |
| | | (temp decreased abruptly) | | | |
| −2.2 | 0 | 0 | 0 | 0 | 0 |
| | 15 | 0 | 0 | 0 | 0 |
| | 30 | 3 | 0 | 2 | 0 |
| | 60 | 7 | 0 | 8 | 0 |
| | 90 | 4 | 0 | 4 | 0 |
| | 120 | 2 | 0 | 2 | 0 |
| Total (−2.2°C) | | 16 | 0 | 16 | 0 |
| Percentage of population | | 100 | 0 | 100 | 0 |
| Percentage of leaf kill/tree | | 18 ± 16 | 0 | 18 ± 15 | 0 |
| −4.4 | 0 | --- | 0 | --- | 1 |
| | 15 | --- | 0 | --- | 1 |
| | 30 | --- | 2 | --- | 0 |
| | 60 | --- | 4 | --- | 1 |
| | 90 | --- | 6 | --- | 2 |
| | 120 | --- | 4 | --- | 1 |
| Total (−4.4°C) | | | 16 | | 5 |
| Percentage of population | | | 100 | | 31 |
| Percentage of leaf kill/tree | | 16 ± 10 | | 11 ± 6 | |

[z] $4 \times 10^7$ CFU/ml (Calif. isolate), 100 ml/tree.
[y] Sprayed 0 hr before freeze.
[x] Sprayed 15 hr before freeze.

Table 2. Number of potted 'Valencia' orange trees freezing with and without INA bacteria sprayed on the leaves.

| | No. trees freezing | | | | | | |
|---|---|---|---|---|---|---|---|
| | Time at −3.3°C | | | | | | |
| Spray[z] (20 ml/tree) | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | Population (%) |
| *E. herbicola* (INA)[y] | 0 | 2 | 8 | --- | --- | --- | 100 |
| NonINA bacteria[x] | 0 | 0 | 1 | 0 | 0 | 0 | 10 |
| *E. herbicola* (INA)/nonINA | 0 | 0 | 10 | --- | --- | --- | 100 |
| NonINA/*E. herbicola* (INA)[v] | 0 | 4 | 4 | 2 | --- | --- | 100 |
| Culture media | 0 | 0 | 0 | 1 | 0 | 0 | 10 |
| Water | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

[z] 3 days prior to freeze.
[y] Isolate No. 26 ($2 \times 10^8$ CFU/ml), Microlife Technics.
[x] Isolate M232A ($2 \times 10^8$ CFU/ml), Microlife Technics.
[w] M232A sprayed immediately after Isolate No. 26.
[v] Isolate No. 26 sprayed immediately after M232A.

Table 3. Accumulative number of 6-month-old 'Valencia' orange trees freezing at different temperatures with and without INA bacteria sprayed on the leaves.

| Spray | No. trees freezing −1.1°C | −2.2° | −3.3° | −4.4° | | | | Frozen (%) |
|---|---|---|---|---|---|---|---|---|
| | 2 hr | 2 hr | 2 hr | 1 hr | 2 hr | 3 hr | 4 hr | |
| *Erwinia herbicola*[z] | 0 | 0 | 2 | 8 | 12 | 13 | 15 | 100 |
| *E. herbicola* + agricultural streptomycin sulfate[y] | 0 | 0 | 1 | 6 | 11 | 12 | 15 | 100 |
| Nutrient broth | 0 | 0 | 0 | 0 | 1 | 3 | 7 | 47 |
| Water | 0 | 0 | 0 | 0 | 3 | 4 | 6 | 40 |

[z]2 × $10^7$ CFU/ml, 30 ml per tree sprayed on foliage 2 days prior to freeze, 15 trees per treatment.
[y]200 ppm (AI) streptomycin, about 50 ml per tree one hr after INA bacteria spray.

at a constant temperature. All trees sprayed with INA bacteria were freezing after 2 hr at −4.4° in contrast to 31% of the trees without INA bacteria. Experience suggests that most of the remaining 69% of the unfrozen trees would start to freeze with longer duration at −4.4°, with some trees supercooling to −5.5° and even to −6.7° (21). Freeze avoidance beyond −6.7° is infrequent and unpredictable in citrus trees. These observations only suggest that INA agents such as bacteria on leaf surfaces and/or other heterogenous nucleators may be inhibiting supercooling in citrus during natural freezes.

Similar results to those in Table 1 also were evident with *E. herbicola* isolates from California. No work was done in comparing the INA *E. herbicola* with *P. syringae* in any one test. Overall results from 10 different tests and 500 trees indicated considerable variability in minimizing supercooling in citrus leaves. Equal concentrations of bacteria in sprays did not necessarily produce similar results in different tests. Variable results were especially evident to tests where concentrations were 2 × $10^7$ CFU/ml or less, but in some instances, higher concentrations of INA bacteria in sprays failed to induce earlier freezing in citrus leaves. Some of this variability is attributed to growing bacterial cultures at 30°C, as well as to the culture media used in this study. Both growth temperatures and culture media influence the INA factor (1, 9, 10, 11) and, in this study, 30° may have reduced relative ice nucleation activity 10% to 25%. Temperatures in the field would affect bacterial colonization of citrus leaves and critical threshold concentrations for ice nucleation activity.

We found no significant differences in activity in our work whether bacteria were suspended in sterile $H_2O$, 0.1 M phosphate buffer (pH 7.0), or retained in nutrient broth culture in making different spray concentrations. We also noted no rapid growth of INA bacteria on leaves of citrus trees under greenhouse conditions, unless trees were continuously misted. Before sprays, bacteria concentrations on leaves of greenhouse trees ranged from 2 × $10^2$ to 2 × $10^6$ CFU/leaf. These concentrations of unknown bacteria were not INA when applied to leaves of 'Valencia' orange trees and supercooled equally with glass-distilled $H_2O$ during supercooling tests of different INA agents in 0.5-ml drops of aqueous suspensions.

Spray concentrations of 2 × $10^8$ CFU/ml of INA *E. herbicola* were sufficient to cause 98% leaf kill on 15 six-month-old 'Valencia' orange trees with none of the trees avoiding injury during −4.4°C for 3 hours, 3 days after sprays. In contrast, at least 80% of the trees sprayed with media or water control solution avoided freeze injury as a result of greater supercooling and averaged less than 20% leaf kill. Neither sprays of antagonistic (nonINA) bacteria (Table 2), nor sprays of streptomycin (Table 3) significantly reduced the number of INA-bacteria trees freezing at different temperatures and durations. Apparently, antagonistic bacteria are more likely to inhibit further increases in the colonization of INA bacteria than inhibit the ice nucleation activity (8) and similarly, streptomycin can kill INA bacteria without eliminating the ice nucleation activity (12).

The ice nucleation activity of lyophilized *P. syringae* compared favorably with that of nonbacterial agents, silver iodide, phenazine, and flurophlogopite (Tables 4 and 5). Lyophilization has some economic advantages in the preparation, storage, and use of INA material and INA bacteria probably can substitute for nonbacterial agents in a variety of uses. In one test (Table 6), it was found that INA bacteria can be substituted for nonbacterial INA agents in practical citriculture where young trees are protected with latent heat of fusion during freezing of water in plastic bags (21). The nonbacterial agent, phenazine, is known to be an effective ice nucleator in cloud seeding (5), and phenazine concentrations in fluorescent pseudomonads (*P. syringae*)

Table 4. Number of 8-month-old 'Valencia' orange trees starting to freeze at different temperatures maintained for 2 hr each with and without INA agents sprayed on the leaves to runoff one day before cold treatments.

| INA agent | Concn | No. trees starting to freeze −2.2°C | −3.3° | −4.4° | −5.5° | −6.7° | Population (%) |
|---|---|---|---|---|---|---|---|
| *Erwinia herbicola* | 2 × $10^8$ CFU/ml | 0 | 2[z] | 8 | --- | --- | 100 |
| *Pseudomonas syringae* (lyophilized) | 1% (w/v) | 0 | 1 | 5 | 4 | --- | 100 |
| Silver iodide | 1% (w/v) | 0 | 2 | 6 | 2 | --- | 100 |
| Phenazine | 1% (w/v) | 0 | 0 | 4 | 6 | --- | 100 |
| Flurophlogopite | 1% (w/v) | 0 | 1 | 4 | 5 | --- | 100 |
| Nutrient broth | 1% (v/v) | 0 | 0 | 0 | 3 | 7 | 100 |
| Water | --- | 0 | 0 | 0 | 4 | 6 | 100 |
| Control | --- | 0 | 0 | 0 | 2 | 8 | 100 |

[z]Trees identified at the end of each temperature level, 10 trees per treatment.

Table 5. Number of 8-month-old 'Valencia' orange trees freezing at −4.4°C for 4 hr with and without INA agents sprayed on the leaves to runoff one day before cold treatment.

| | | No. trees freezing (−4.4°C) | | | | | |
|---|---|---|---|---|---|---|---|
| INA agent | Concn | 0 hr | 1 hr | 2 hr | 3 hr | 4 hr | Frozen (%) |
| *Erwinia herbicola* | 2 × $10^8$ CFU/ml | 0 | 5[z] | 2 | --- | --- | 100 |
| *Pseudomonas syringae* (lyophilized) | 1% (w/v) | 0 | 3 | 2 | 2 | --- | 100 |
| Silver iodide | 1% (w/v) | 0 | 3 | 3 | 1 | --- | 100 |
| Phenazine | 1% (w/v) | 0 | 2 | 2 | 3 | --- | 100 |
| Flurophlogopite | 1% (w/v) | 0 | 4 | 1 | 2 | --- | 100 |
| Nutrient broth | 1% (v/v) | 0 | 0 | 0 | 0 | 1 | 14 |
| Water | --- | 0 | 0 | 0 | 0 | 1 | 14 |
| None | --- | 0 | 0 | 0 | 0 | 0 | 0 |

[z]Trees identified at the end of each hour, 7 trees per treatment.

can be manipulated with stress and media nutrition (6). The role of phenazine concentrations in INA-bacteria-induced freezing is not known. The other nonbacterial agent, flurophlogopite, also is effective in minimizing supercooling in plants other than citrus (15).

INA bacteria and nonbacterial agents equally induced early freezing in citrus leaves in droplet freezing trials. Drops froze as early as −2.2°C with water soaking more pronounced on the abaxial than the adaxial surfaces of leaves. Ease of nucleating leaves with frozen drops at −4.4° during 1 hr averaged 23% when drops were placed on the adaxial surfaces of leaves and 83% when drops were suspended on the abaxial. The cuticular layer of citrus leaves apparently offers some resistance to ice nuclei on leaf surfaces.

The most effective INA agent in droplet freezing during nonplant freeze trials was flurophlogopite with an average supercooling level of −2°C (Table 7). None of the drops with INA agents supercooled much beyond −3.7° in contrast to −6.1° for water. Differences in moment of freezing between INA agents and water alone probably would have been greater if a more inert surface than copper were used. Factors that are implicated in the cold hardening of citrus trees such as sucrose, proline, and concentration of expressed sap appear to promote freeze avoidance with supercooling to −7.6° for 1% (w/v) solutions of proline.

Results of this study express the vulnerability of citrus leaves to different INA agents under a variety of conditions and differences in ice nucleation activity between INA bacteria and nonbacterial agents. The role of INA agents, bacterial and nonbacterial, seemingly is significant in applied agriculture through systems management in freeze avoidance and tolerance. Whether INA bacteria pose a serious problem in freezing of citrus trees during natural freezes cannot yet be satisfactorily answered in Florida which produces most of the citrus in the United States. Concerns that INA bacteria contribute significantly to citrus freeze damage need support in determining population dynamics of INA agents not only on leaves but also on wood (14), efficacy of antagonistic bacteria and bactericides, amount of supercooling under natural conditions, and in more detailed studies on the mode and mechanism of freezing in citrus tissues (19, 22, 24).

Table 6. Ice nucleation in plastic bags containing 100 ml of water with and without INA agents.

| INA agent | Concn | Amount per bag | Lowest temp. (°C) | Bags nucleated (%) |
|---|---|---|---|---|
| *Erwinia herbicola* | 2 × $10^8$ CFU/ml | 1 ml | −2.2 | 100 |
| Silver iodide | | 10 μg | −2.0 | 100 |
| Phenazine | | 10 μg | −2.0 | 100 |
| Nutrient media | | 1 ml | −6.1 | 0 |
| Water | | --- | −6.1 | 0 |

[z]Temperature decrease of 4°C per hour from 1.5° to −6.1°, 7 trees per treatment.

Table 7. Moment of freezing in 0.05-ml droplets with and without INA agents.[z]

| Comparisons | Agent | Concn | Moment of freezing | ΔT |
|---|---|---|---|---|
| 1 | Flurophlogopite (INA) | 1% (w/v) | −2.0 | −3.9** |
| | Water | 000 MOSM | −5.9 | |
| 2 | *Erwinia herbicola* (INA) | 2 × $10^8$ CFU/ml | −3.1 | −2.7** |
| | Water | 000 MOSM | −5.8 | |
| 3 | *Lyophilized Pseudomonas syringae* | 1% (w/v) | −3.2 | −2.6** |
| | Water | 000 MOSM | −5.8 | |
| 4 | Silver iodide (INA) | 1% (w/v) | −3.7 | −2.5** |
| | Water | 000 MOSM | −6.1 | |
| 5 | Phenazine (INA) | 1% (w/v) | −3.7 | −2.1** |
| | Water | 000 MOSM | −5.8 | |
| 6 | Nutrient broth | 1% (v/v) | −5.9 | −0.0 |
| | Water | 000 MOSM | −5.9 | |
| 7 | Sucrose | 1% (w/v) | −6.8 | +0.9** |
| | Water | 000 MOSM | −5.9 | |
| 8 | Proline | 1% (w/v) | −7.6 | +1.7** |
| | Water | 000 MOSM | −5.9 | |
| | *Expressed sap* | | | |
| 9 | Unhardened 'Valencia' leaves | 424 MOSM | −5.8 | −0.1 |
| | Water | 000 MOSM | −5.9 | |
| 10 | Cold-hardened 'Valencia' leaves | 642 MOSM | −7.2 | +1.2** |
| | Water | 000 MOSH | −6.0 | |

[z]Comparison of means, n = 5; temperature decrease 5°C per hour, continuous temperature monitoring of drops on suspended 45.7$^2$ cm × 0.6 mm thick copper sheet in controlled-temperature facility used to freeze whole plants.

**Significant at 1% level.

## Literature Cited


1. Anderson, J.A., D.W. Buchanan, R.E. Stall, and C.B. Hall. 1982. Frost injury of tender plants increased by *Pseudomonas syringae* van Hall. J. Amer. Soc. Hort. Sci. 107:123–125.
2. Asworth, E.N. and D.J. Rowse. 1982. Vascular development in dormant *Prunus* flower buds and its relationship to supercooling. HortScience 17:790–791.
3. Bilanski, W.K. and R.C. Tzeng. 1980. Supercooling as related to diameter of conducting vessels in plants. Trans. Amer. Soc. Agr. Eng. 23:1334–1336.
4. George, M.F., M.R. Becwar, and M.J. Burke, 1982. Freezing avoidance by deep undercooling of tissue water in winter-hardy plants. Cryobiology 19:628–639.
5. Head, R.B. 1962. Ice nucleation by α-phenazine. Nature 196:736–738.
6. Leisinger, T. and R. Margraff. 1979. Secondary metabolites of the fluorescent pseudomonads. Microbiol. Rev. 43:422–442.
7. Levitt, J. 1980. Avoidance of freezing, p. 119–125. In: Responses of plants to environmental stress, 2nd Edition. Academic Press, New York.
8. Lindow, S.E. 1982. Population dynamics of epiphytic ice nucleation active bacteria on frost sensitive plants and frost control by means of antagonistic bacteria, p. 395–416. In: P.H. Li and A. Sakai (eds.). Plant cold hardiness and freezing stress: mechanisms and crop implications, Vol. 2. Academic Press, New York.
9. Lindow, S.E. 1982. Epiphytic ice nucleation-active bacteria, p. 335–362. In: Phytopathogenic prokaryotes, Vol. 1. Academic Press, New York.
10. Lindow, S.E. 1982. Relationship between ice nucleation frequency of bacteria and frost injury. Plant Physiol. 70:1090–1093.
11. Lindow, S.E., D.C. Arny, and C.D. Upper. 1982. Bacterial ice nucleation: a factor in frost injury to plants. Plant Physiol. 70:1084–1089.
12. Maki, L.R. and K.J. Willoughby. 1978. Bacteria as biogenic sources of freezing nuclei. J. Appl. Meteorl. 17:1049–1053.
13. Proebsting, E.L. and P.K. Andrews. 1982. Supercooling and *Prunus* flower bud hardiness, p. 529–539. In: P.H. Li and A. Sakai (eds.). Plant cold hardiness and freezing stress: mechanisms and crop implications, Vol. 2. Academic Press, New York.
14. Proebsting, E.L., Jr., P.K. Andrews, and D. Gross. 1982. Supercooling young developing fruit and floral buds in deciduous orchards. HortScience 17:67–68.
15. Rajasekar, C., P.H. Li, and J.V. Carter. 1982. Frost injury in supercooled leaves of tuber-bearing *Solanum* species, p. 379–393. In: P.H. Li and A. Sakai (eds.). Plant cold hardiness and freezing stress: mechanisms and crop implications, Vol. 2. Academic Press, New York.
16. Schnell, R.C. and G. Vali, 1972. Atmospheric ice nuclei from decomposing vegetation. Nature 236:163–165.
17. Yankofsky, S.A., Z. Levin, T. Bertold, and N. Sandlerman. 1981. Some basic characteristics of bacterial freezing nuclei. J. Appl. Meteorol. 89:1013–1019.
18. Yankofsky, S.A., Z. Levin, and A. Moshe. 1981. Association with citrus of ice-nucleating bacteria and their possible role as causative agents of frost damage. Current Microbiol. 5:213–217.
19. Yelenosky, G. 1982. Seasonal variations in physiological factors implicated in cold hardiness of citrus trees, p. 561–573. In: P.H. Li and A. Sakai (eds.). Plant cold hardiness and freezing stress: mechanisms and crop implications, Vol. 2. Academic Press, New York.
20. Yelenosky, G. 1979. Water-stress-induced cold hardening of young citrus trees. J. Amer. Soc. Hort. Sci. 104:270–273.
21. Yelenosky, G. 1979. Self-heating tree wraps in freeze protection. Proc. Fla. State Hort. Soc. 92:25–27.
22. Yelenosky, G. 1979. The potential of citrus to survive freezes. Proc. Intern. Soc. Citricult. 1:199–203.
23. Yelenosky, G. 1975. Cold hardening in citrus stems. Plant Physiol. 56:540–543.
24. Yelenosky, G. and C. Horanic. 1969. Subcooling in wood of citrus seedlings. Cryobiology 5:281–283.
25. Yelenosky, G. and R. Young. 1981. Citrus freeze problems in Florida. Citrus Ind. Mag. 62:7–11.
26. Young, R. and A. Peynado. 1967. Freezing and water-soaking in citrus leaves. Proc. Amer. Soc. Hort. Sci. 91:157–162.
27. Young, R. and A. Peynado. 1965. Changes in cold hardiness and certain physiological factors of Red Blush grapefruit seedlings as affected by exposure to artificial hardening temperatures. Proc. Amer. Soc. Hort. Sci. 86:244–252.