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Use of a Hydrophobic Particle Film as a Barrier to Extrinsic Ice Nucleation in Tomato Plants

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Abstract. **Extrinsic ice nucleating agents (such as ice-nucleation-active bacteria, dew, etc.) significantly limit the ability of herbaceous plants to supercool. It is believed that with an absence of these extrinsic nucleating agents, a plant could supercool to less than –4 °C. Other evidence, however, indicates that intrinsic nucleating agents may limit the extent of supercooling. Infrared video thermography was used to study freezing in young, 'Rutgers' tomato (*Lycopersicon esculentum* L.) plants and to determine if a hydrophobic barrier on the plant surface could prevent extrinsic nucleating agents such as Ice$^+$ bacterial strain (Cit7) of *Pseudomonas syringae* Van Hall from initiating freezing within a plant. Freezing tests were conducted in a programmable freezing chamber, a radiative frost chamber, and outdoors. Freezing was visualized and recorded on videotape using an infrared radiometer. Freezing of the plants was induced extrinsically by application of droplets (5 to 7 μL) of water containing Cit7. To provide a barrier to the action of extrinsic ice nucleating agents, an emulsion of hydrophobic kaolin (aluminum silicate mineral) was applied to the plant surface before application of an extrinsic nucleating agent. Results indicate that dry, young tomato plants can supercool to as low as –6 °C whereas plants having a single droplet of Cit7 would freeze at –1.5 to –2.5 °C. Application of the hydrophobic barrier blocked the effect of Cit7 and allowed whole plants to also supercool to –6 °C, despite the presence of frozen droplets on the leaf surface. When whole plants were sprayed with water and Cit7 using an aerosol sprayer and exposed to –3 °C, plants coated with the hydrophobic particle film exhibited significantly less foliar injury then nontreated plants. Similar results were obtained using the radiative frost chamber. Experiments conducted under natural frost conditions also resulted in less injury to the coated plants. The hydrophobic kaolin particle film performed better at preventing plants from freezing due to extrinsic ice nucleation than nonaltered, hydrophyllic kaolin alone or an antitranspirant with putative frost protection properties.**

While the melting point of ice is 0 °C, the freezing temperature of water is not as defined (Ashworth, 1992) and, although it is not recognized commonly, pure water has a low probability of freezing at temperatures warmer than –40 °C (Franks, 1985). This is because a small ice crystal embryo is necessary for ice to form and grow to any substantial size. The process of initiating ice formation is referred to as ice nucleation. In nature, water exists mainly as an ionic or colloidal solution where ice nucleation is initiated on the surface of objects or on suspended particles (Ashworth, 1992). Heterogeneous ice nucleators are effective in inducing ice formation and are very abundant. That is why most freezing occurs at temperatures just slightly below 0 °C.

The role of heterogeneous ice nucleators in inducing ice formation in plants is important because if the activity of these agents could be manipulated, significant advances could be made in limiting frost injury to freezing-sensitive plants and/or plant parts. A major topic of research has been the relative importance of extrinsic ice nucleation agents, such as ice-nucleation-active (INA) bacteria (e.g., *Pseudomonas syringae*), and intrinsic nucleation agents synthesized by plants (Ashworth and Kieft, 1995). While all plants can supercool (i.e., have tissues below 0 °C without freezing) to some extent, the extent of supercooling varies between plant species and is influenced by the presence of ice nucleating agents which may be of plant or bacterial origin (Lindow, 1995).

Recently, infrared video thermography has been used to observe directly ice nucleation (i.e., initial ice formation) and propagation in plants (Carter et al., 2001; Ceccardi et al., 1995; Fuller and Wisniewski, 1998; Pearce and Fuller, 2001; Wisniewski, 1998; Wisniewski and Fuller, 1999; Wisniewski et al., 1997; Workmaster et al., 1999). Ice formation is an exothermic event and release of the heat of fusion as water changes phase from a liquid to a solid can be monitored and visualized.

In herbaceous plants, extrinsic nucleating agents can cause water present on the surface of plants to freeze at a warm (–1.0 to –2.5 °C), subzero temperature. Concomitantly, ice on the surface of the leaf induces freezing of the plant. Intitiation of ice within a plant appears to be induced by physical growth of an ice crystal into the internal portion of the leaf. Growth of an ice crystal may occur through a stomate, a crack in the cuticle, wound, broken epidermal hair, or other lesion. Internally, the ice crystal acts as a nucleus and induces extracellular water to freeze which in turn leads to the freezing of water present in xylem tissues. Once ice is present in the extracellular space, cells dehydrate in order to come into equilibrium with the vapor pressure of the ice in a temperature dependent manner (Ashworth and Kieft, 1995; Chen et al., 1995; Pearce and Fuller, 2001;Wisniewski and Fuller, 1999).

Results of our previous research on ice nucleation (Pearce and Fuller, 2001; Wisniewski and Fuller, 1999) has reinforced the concept that by somehow blocking activity of extrinsic nucleating agents one may allow plants to supercool to a lower temperature and thereby provide some frost protection. In the present study, infrared thermography was used to study freezing in young 'Rutgers' tomato (*Lycopersicon esculentum*) plants to determine if application of hydrophobic kaolin (M96-018) on the plant surface could act as a barrier preventing the action of extrinsic nucleating agents, such as INA (Ice$^+$) bacteria, from initiating freezing within a plant. Hydrophobic kaolin consists of kaolin particles that have been coated with a hydrophobicity-inducing substance. Hydrophobic kaolin products are added to plastic and rubber compounds to increase water repellancy and strength, and to improve electrical

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insulation properties. They are used in rubber coatings of wire and cable, other rubber electrical parts, plastic parts for automobiles, and polyester gel coats for fiberglass products. Currently, there are no agricultural uses of hydrophobic kaolin.

Materials and Methods

Plant culture and infrared thermography. 'Rutgers' tomato plants were grown in a greenhouse without supplemental lighting at days/nights of 22.5 ± 2.5/20 °C in individual pots containing Metro Mix 360 (Scotts-Sierra Hort. Products, Marysville, Ohio), growing medium, watered daily and fertilized weekly with 15N–13.2P–12.5K Miracle Gro water soluble fertilizer (Scotts-Sierra Hort. Products.) Plants were used when they were 4 to 6 weeks old. Freezing tests were conducted in a programmable freezing chamber (Tenney environmental test chamber model T20S; Lunaire, Williamsport, Md.). Freezing was visualized and recorded on videotape using a radiometer (model 760; Inframetrics, N. Billerica, Mass.). Use and interpretation of output from the infrared camera has been described previously in detail (Pearce and Fuller, 2001; Wisniewski et al., 1997; Workmaster et al., 1999). The camera was set on a two degree centigrade span which was adjusted continually downward as the temperature was lowered. Using an artificial color pallette, differences in temperature of the objects viewed are seen as different colors where cool colors (blue, violet, etc.) represent the lower range of the temperature span and warm colors (yellow, orange, red, etc.) represent the warmer range of the temperature span. Black and white represent temperature below and above the set temperature span, respectively. As used, the infrared camera can detect temperature changes with a sensitivity of 0.1 °C.

Hydrophobic particle film (M96-018). To provide a barrier to the action of extrinsic ice-nucleating agents, M96-018, a hydrophobic kaolin particle film (Engelhard, Islin, N.J.) was applied to the plant surface using a hand-operated aerosol sprayer before applying an extrinsic nucleating agent. Initially, several formulations and/or methods of application were assessed for coverage. They were M96-018 applied as a dust, 10% emulsion (w/v) in water, 10% emulsion (w/v) in water with 1% (w/v) cottonseed (*Gossypium hirsutum* L.) oil, and, 10% emulsion (w/v) in 10% (v/v) methanol. The methanol formulation provided the most effective and uniform coverage (data not presented).

Convective freezing experiments

Freezing individual leaves. Tomato leaflets were removed from plants and either coated with M96-018 or left noncoated. The coating was applied ≈2 h before the onset of the experiment. Silicone grease was applied to the cut end of the petiole of each leaflet to prevent desiccation. A single 5- to 7-µL droplet of water containing INA (Ice^+) bacteria (*Pseudomonas syringae*), strain Cit 7 (provided by S. Lindow), was applied to the upper surface of each leaf (coated and noncoated) to serve as an extrinsic ice nucleation agent. Although the concentration of bacteria was not assessed, in all cases the bacteria induced freezing of external droplets of water in the range of –1.7 to –2.5 °C. Leaves were placed in the programmable freezing chamber and equlibrated at 0.5 °C. The temperature was lowered at 1.5 °C/h from 0.5 to –2.0 °C, 2.5 °C·h^{-1} from –2.0 °C to –5.0 °C, and then at 3.5 °C·h^{-1} down to the final temperature of each specific experiment. In all cases, the temperature of the chamber was not lowered until the plant material (as visualized with the infrared camera) was equilibrated with the set temperature.

Comparison of hydrophobic kaolin with hydrophilic kaolin and Moisturin. In additional experiments, conducted in the same manner, the freezing response of M96-018-coated leaflets was compared to noncoated (dry) leaflets, various formulations (as described above) of normal (hydrophilic) kaolin-coated leaflets, and a commercial product, Moisturin (Burke's Protective Coatings, Washougal, Wash.), designed to protect plants from freezing. The various coatings were applied ≈2 h before the onset of the experiment.

Freezing whole plants. The freezing response of M96-018-coated and uncoated whole plants was monitored using infrared thermography. Coatings were applied either 2 h or 16h (night before) before start of the experiment. A fine spray of water (≈10 to 12 mL) containing Ice^+ bacteria was applied with a hand-operated aerosol sprayer to noncoated (control) tomato plants and plants that had been coated with the hydrophobic particle film. Experiments were performed either with single plants viewed with the infrared camera or with several treated and nontreated plants at the same time in which case only a visual determination of injury was made at the end of the experiment. When single plants were used, temperatures were lowered gradually to –5.0 to –6.0 °C as described previously. When groups of plants were used, the temperature was lowered to –3.0 °C at 2.5 °C·h^{-1}, held for at least 15 min and then warmed to 5 °C for 30 to 60 min. Plants were then placed in the greenhouse and observed for injury after 12 to 16 h at ambient temperature (20 to 25 °C). Plants were left at the final freezing temperature for at least 15 min before being warmed back to 5 °C for 30 to 60 min. Individual pots and the soil surface were insulated with plastic bubblewrap to ensure that freezing did not occur from the soil. Freezing injury was rated on a scale of 0 to 5 where 0 = no injury, 1 = slight injury to leaf margins, 2 = injury to leaf margins and a few leaves killed, 3 = a moderate number of leaves killed, 4 = only a few leaves uninjured, and 5 = the plant completely killed. About 50 plants of each treatment were subjected to –3 °C in groups of four to five plants per treatment. The effect of each treatment was recorded as percentage injury in each of the classes. Statistical analysis consisted of analysis of variance (ANOVA) and means were separated by Duncan's multiple range test. Experiments with whole plants where the extent of injury was categorized into one of five classes were analyzed by a paired *t* test of damage levels for the controls (noncoated) vs. the treated (coated) plants. Variances of each population were assumed to be equal and each individual was considered a random replicate. The probability of a difference was determined by a paired *t* test.

Simulated radiation and natural frosts. An experiment was conducted with 4 to 6 week old 'Rutger' tomato plants, grown and fertilized as described previously, utilizing a freezing chamber that has been designed to simulate radiative frost conditions (Fuller and LeGrice, 1998). The chamber was located at Seale-Hayne College of Agriculture, Newton Abbot, United Kingdom, in the laboratory of the third author. The chamber has a large cooling plate located above the plants, kept at –38 °C that simulates a cold night sky. The plate is cooled to –38 °C before the onset of the experiment. No active air movement occurs in the chamber and cooling plates located on the sides of the chamber reduce the potential of convective air currents. Chamber temperature is maintained by regulating the temperature of the side cooling plates. Plants were moved from the greenhouse directly into the chamber and allowed to equilibrate with the chamber temperature (2.0 °C). The chamber was cooled at 1 to 2 °C/h to –1.6 °C and held for at least an hour. Plants were then removed and assessed for injury after several hours at 20 °C. As described previously, plants were categorized from 0 to 5 depending on the extent of injury. Six plants coated with the methanol formulation of M96-018 and six noncoated plants were used in the experiment. The coating was applied ≈2 h before the onset of the experiment. Insulation was provided around the pots to prevent or delay

freezing of the soil. Temperatures were monitored using a data logger and thermocouples placed at several locations within and above the canopy, although only the temperature directly above the top of the leaf canopy is presented for clarity. Results were analyzed using a paired *t* test.

Natural frost conditions. This experiment was conducted on the grounds of Seale-Hayne College of Agriculture, Newton Abbot, United Kingdom on the night of 23 to 24 Feb. 2001. Six coated and six noncoated 'Rutgers' tomato plants, 4 to 6 weeks in age, were placed outside at 2000 HR, left overnight, and then placed at 20 °C at 0830 HR the following morning for several hours, after which injury was assessed. The coating was applied ≈2 h before the onset of the experiment. While outdoors, the plants were placed within a meter quadrant with equal numbers of plants of each treatment located on the outside edge of the quadrant and on the inside of the quadrant. The temperature 3 to 5 cm above the leaf canopy was measured using a data logger and thermocouples. Results were analyzed using a paired *t* test.

Results

Freezing individual leaves. A 5 to 7 µL droplet of Ice$^+$ bacteria was placed on tomato (*Lycopersicon esculentum)* leaves that were either noncoated or coated with the hydrophobic particle film (M96-018). In contrast to the drop on the noncoated leaf, the one on the coated leaf had less contact with the leaf surface (Fig. 1). The droplets of Ice$^+$ bacteria froze on both the coated and

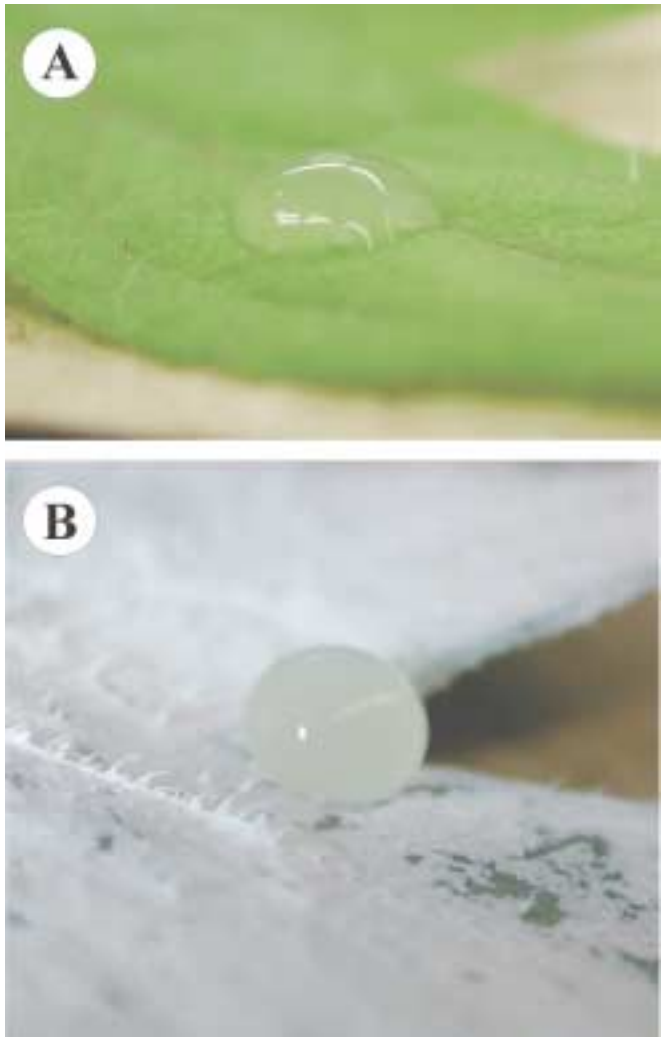

Fig. 1. Photographs of droplets of water containing Ice$^+$ bacteria, strain Cit7 of *Pseudomonas syringae*, on the surface of (**A**) a noncoated tomato leaflet and (**B**) a tomato leaflet coated with the M96-018 hydrophobic kaolin particle film. Note how the droplet on the coated leaflet in **B** is more spherical and has less contact with the leaf surface than the droplet on the noncoated leaflet in **A**.

Fig. 2. Exposure of noncoated and M96-018-coated tomato leaflets to freezing temperatures. A droplet of water containing Ice$^+$ bacteria was placed on the upper surface of each leaf. (**A** and **B**) Infrared images; (**C**) a photograph of the leaves after they were returned to room temperature and the hydrophobic particle film was removed. The droplet of Ice$^+$ bacteria froze at about –1.5 °C on both leaves. The frozen droplet induced the noncoated leave to freeze (**A, left**) while the coated leaf remained nonfrozen (**A, right**). The coated leaf was still nonfrozen at –5.5 °C (**B, right**). After removal from the environmental chamber and warming (C), the noncoated leaf appeared completely water soaked (**left**) while the M96-018-treated leaf (**right**) appeared completely turgid and noninjured.

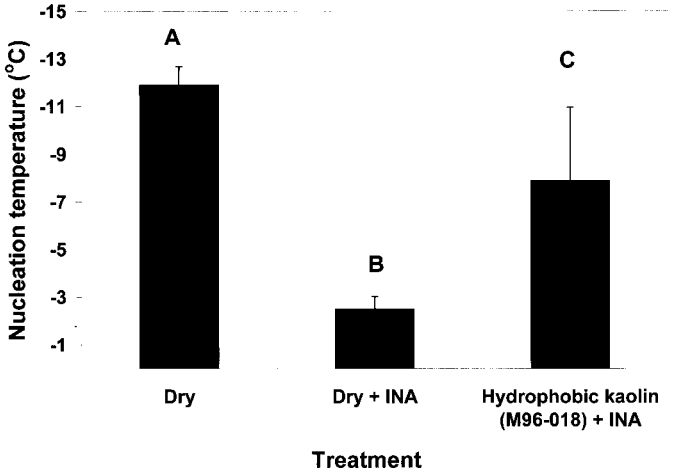

Fig. 3. Mean nucleation temperature of noncoated and M96-018-coated tomato leaflets. Treatments consisted of dry, noncoated leaves; dry, noncoated leaves with a single droplet of Ice$^+$ bacteria; and leaves coated with M96-018 hydrophobic kaolin particle film plus a droplet of Ice$^+$ bacteria. Treatment effects were significant at $P < 0.001$ by ANOVA. Treatment means ± SD with different letters are significantly different at $P = 0.05$ (n = 24).

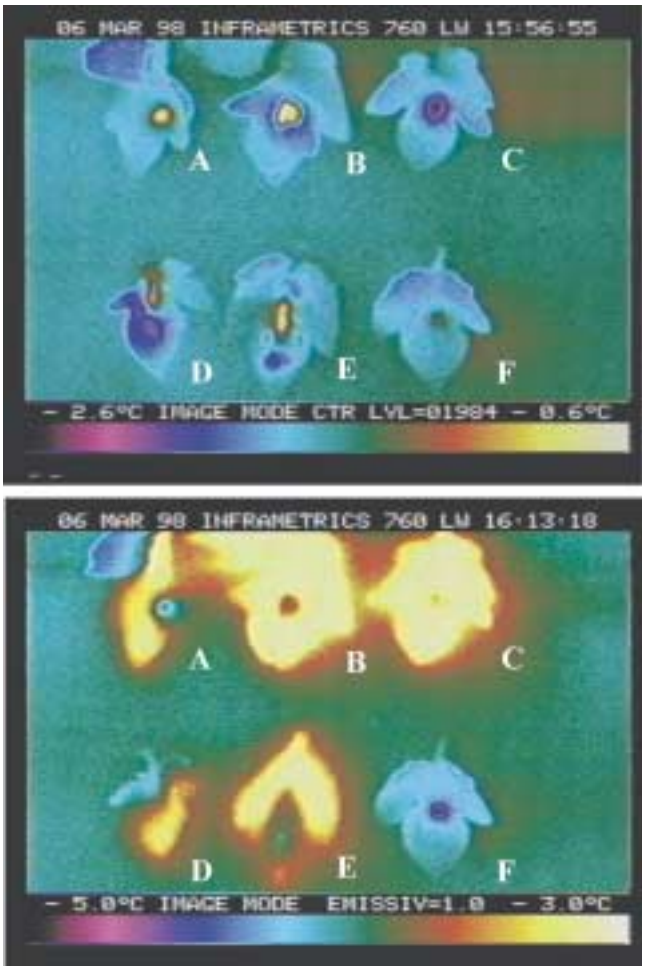

Fig. 4. Infrared thermography of tomato leaflets exposed to freezing temperatures. Treatments consisted of (**A**) noncoated leaflets and leaflets coated with, (**B**) Moisturin, (**C–E**) dust, water, or cottonseed oil formulations of the hydrophilic kaolin, respectively or, (F) M96-018 hydrophobic kaolin particle film. Each leaflet, in all treatments, received a single drop of Ice$^+$ bacteria to induce freezing of the leaves. In the top panel, all the Ice$^+$ droplets have frozen but the leaves are still unfrozen at about –2.2 °C. In the lower panel, the leaflets of all treatments except the one coated with the hydrophobic kaolin particle film (**F**) are frozen. Leaf temperature of treatment F is about –4.2 °C.

noncoated leaves at –1.8 ± 0.5 °C. The presence of the frozen droplet of ice induced freezing of the untreated, control leaf (Fig. 2A) whereas the treated leaf remained unfrozen at temperatures of less than –5.5 °C (Fig. 2B) and lower. This indicated that the hydrophobic particle film prevented induction of ice formation in the leaf by an extrinsic nucleating agent (the frozen droplet of Ice$^+$ bacteria). Nontreated and M96-018-treated leaves after removal from the environmental chamber and subsequent thawing are illustrated in Fig. 2C. The nontreated leaf was completely killed whereas the treated leaf was uninjured. Repeated comparisons of coated and noncoated leaves (where several leaves were exposed simultaneously) indicated that the mean freezing temperature of dry leaves (no Ice$^+$ bacteria) was –12 °C, whereas the freezing temperature of dry leaves plus the Ice$^+$ bacteria was –2.8 °C, and the hydrophobic-particle-film-treated leaves plus Ice$^+$ bacteria froze at –8 °C (Fig. 3).

Comparison of hydrophobic kaolin with hydrophilic kaolin and Moisturin. The ability of the hydrophobic particle film (M96-018) to block ice nucleation was compared to a hydrophilic kaolin material and to Moisturin, a commercial frost-protection product**.** Individual leaflets of tomato were left noncoated (Fig. 4A), treated with Moisturin (Fig. 4B), various formulations (dust, water, or cottonseed oil) of the hydrophilic (normal) kaolin (Fig. 4C–E), or the hydrophobic, M96-018 kaolin in 10% methanol (Fig. 4F). A 5 to 7 µL drop of water containing Ice$^+$ bacteria was placed on the surface of each leaf before cooling the leaflets to temperatures below 0 °C. Only the hydrophobic film provided a significant level of protection from freezing despite the presence of a frozen droplet on the surface of the leaf (Fig. 4F, lower panel). Results of repeated experiments comparing the freezing of leaflets coated with normal kaolin, hydrophobic particle film, or Moisturin, are presented in Fig. 5. Dry leaves without application of Ice$^+$ bacteria served as a comparison. As in the previous experiment with leaflets of tomato (Fig. 3), dry leaves froze at about –12.0 °C while Moisturin- and hydrophilic-kaolin-coated leaves, having a drop of Ice$^+$ bacteria on the surface, froze at about –2.5 to –3.0 °C. The hydrophobic-particle-film-treated leaves, also with a drop of Ice$^+$ bacteria on the surface, froze at an average

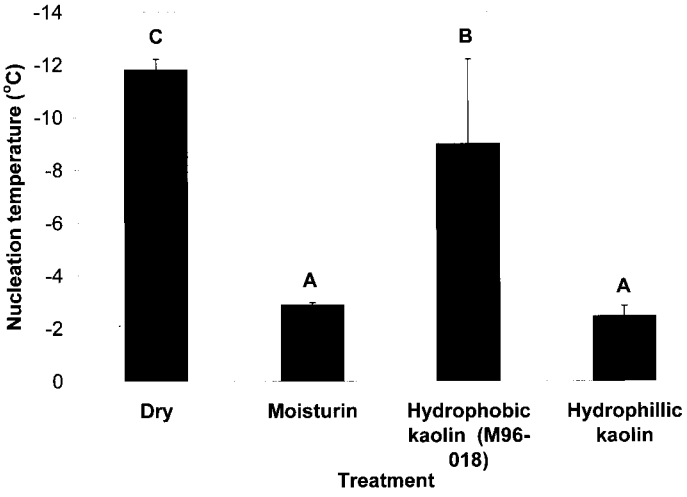

Fig. 5. Mean nucleation temperature of noncoated and M96-018-coated tomato leaflets. Treatments consisted of leaftlets left dry (noncoated), coated with Moisturin, coated with M96-018 hydrophobic kaolin particle film, or coated with a hydrophilic kaolin. All treatments except the dry leaflets had a single droplet of Ice$^+$ bacteria placed on the upper surface of each leaflet. Treatment effects were significant at $P \leq 0.001$ by ANOVA. Treatment means ± SD with different letters are significantly different at $P = 0.05$ (n = 12).

Fig. 6. (**A–C**) Infrared thermography and (**D**) a photograph of noncoated and M96-018 coated tomato plants subjected to freezing temperatures. Each plant was sprayed with water containing Ice$^+$ bacteria using a hand-operated aerosol sprayer. The coated plant is on the left in (**A–C**) and on the right in (**D**). Large drops of water can be seen as black areas on the noncoated plant in **A**. The black areas are a result of these areas being colder than the set temperature range of the camera (–0.2 to 1.7 °C). The lower temperature of these areas is due to evaporative cooling. In **B**, the noncoated plant is in the process of freezing, as evidenced by the warmer temperature resulting from the exothermic reaction of ice formation, while the coated plant is unfrozen at about –2.5 °C. In **C**, the noncoated plant has almost completely frozen, except along the stem, petiole, and midvein, and is approaching temperature equilibrium with air temperature. The coated plant in **C** is still unfrozen at about –6.0 °C. In **D**, noncoated (**left**) and coated (**right**) plants can be seen after exposure to –6.0 °C, removal from the chamber, and thawing. The noncoated plant is completely killed while the coated plant appears uninjured.

temperature of –9.0 °C. Variability in the freezing response was again greatest in the leaflets treated with the hydrophobic particle film. In preliminary experiments, the freezing response of hydrophilic kaolin-coated and hydrophobic kaolin-coated leaves, without the application of moisture, was examined. There was no difference between these treatments in the absence of applied moisture and both froze at temperatures similar to dry, noncoated leaves (data not presented).

Freezing whole plants. The effect of the hydrophobic material was twofold. It prevented droplets of water applied with the aerosol sprayer from adhering to the leaf surface, thereby lowering the number of potential extrinsic sites for ice nucleation, and secondly, it prevented extrinsic nucleation of the tomato plants to temperatures of at least –5.0 to –6.0 °C. Accumulation of water on the nontreated plants can be seen as large, black areas (Fig. 6A; black representing a temperature cooler than the set range of the camera), a result of evaporative cooling which led to drops of moisture present on the surface of the plant, and hence the subtending leaf surface itself, to be significantly cooler by 1.5 to 2.5 °C. In Fig. 6B, the nontreated plant (right) had frozen whereas the treated plant (left) was unfrozen. This is indicated by the in the natural frost experiment, however, were not significant, most likely due to the small sample size.

Discussion

Common methods for frost protection include use of water sprays (both standard and microsprinklers), wind machines, and burning of organic matter (Perry, 1998). More recently, use of aqueous foams has been proposed (Choi et al., 1999; Choi and Giacomelli, 1999). All these methods are based on providing a mechanism by which the temperature of the plant tissue is kept above its freezing point.

Another approach to frost protection that has been studied extensively is lowering the temperature at which a plant will freeze by controlling epiphytic ice nucleation induced by ice-nucleation-active bacteria, such as *Pseudomonas syringae* (see review by Lindow, 1995). This approach is based on the premise that plants can supercool to some extent below 0 °C, and that the extent of supercooling is determined by the presence of ice-nucleating agents of plant or bacterial origin (see review by Ashworth and Kieft, 1995). These agents may also be external (extrinsic) or internal (intrinsic) in origin and activity. Factors involved in determining the temperature at which plant tissue freezes include the size (mass and surface area) of the specimen, cooling rate, presence of moisture on the tissue surface, and the population density of INA bacteria present (Ashworth and Kieft, 1995).

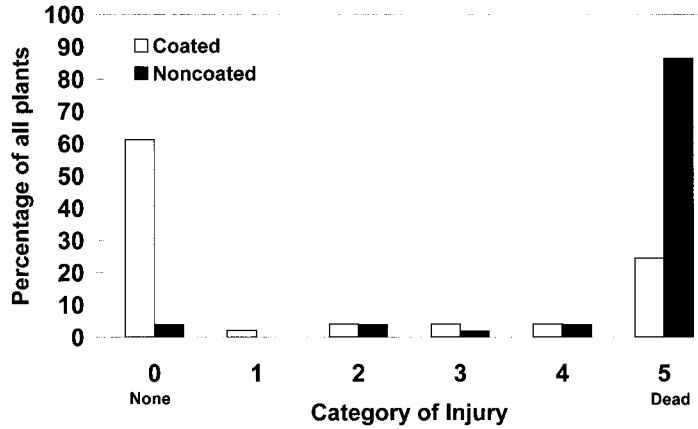

Fig. 7. Percentage of M96-018-coated and noncoated tomato plants in different injury classes after exposure to –3.0 °C. Freezing injury was rated on a scale of 0 to 5 where 0 = no injury, 1 = slight damage to leaf margins, 2 = injury to leaf margins and a few leaves killed, 3 = a moderate number of leaves killed, 4 = only a few leaves uninjured, and 5 = the plant completely killed. Each plant was sprayed with water containing Ice$^+$ bacteria before being placed in the environmental chamber. The injury levels, combined for all classes, was analyzed by an paired *t* test for coated vs. noncoated. The probability of a difference based on the *t* test is $P \leq 0.001$ (n = 51 for noncoated plants and n = 49 for coated plants).

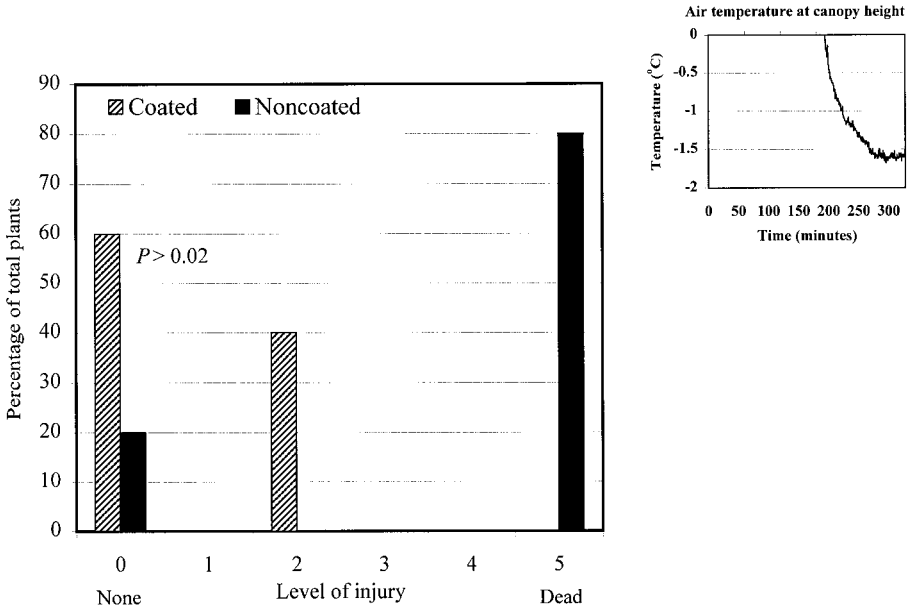

Fig. 8. Percentage of M96-018-coated and noncoated tomato plants in different injury classes after exposure to –1.6 °C for 1 h in a radiative frost chamber. All plants were sprayed with water containing Ice+ bacteria before being placed in the frost chamber. The graph in the upper right represents temperature data obtained during the course of the experiment by placing a thermocouple at the canopy height of the tomato plants. A paired *t* test indicated that there was a significant difference in the level of injury between coated and noncoated plants ($P \leq 0.02$, n = 6).

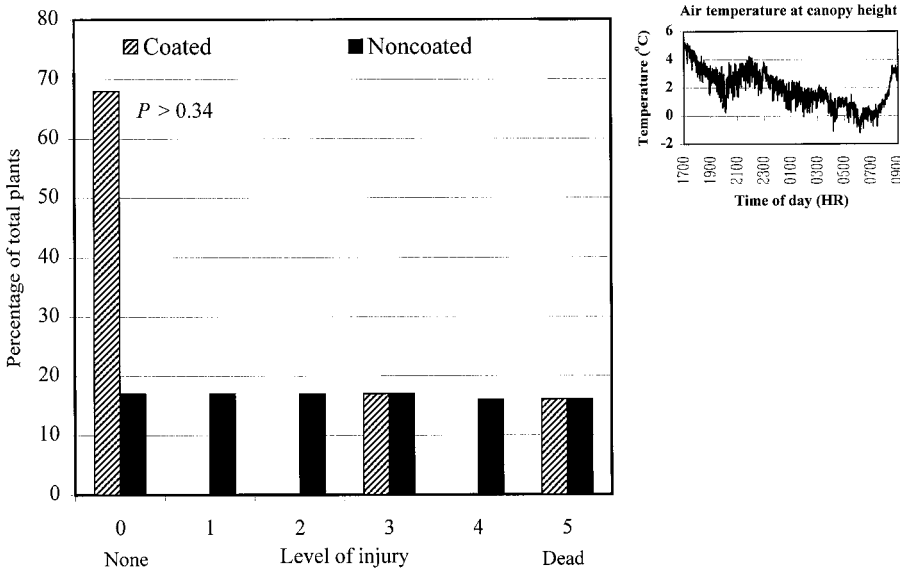

Fig. 9. Percentage of M96-018-coated and noncoated tomato plants in different injury classes after exposure to a natural frost. Plants were placed outdoors in the evening and then retrieved the next morning and placed in a growth chamber at 18 °C. Levels of injury were recorded after 10 h. The graph in the upper right represents temperature data obtained during the course of the experiment by placing a thermocouple at the canopy height of the tomato plants. No water or Ice+ bacteria were administered to the plants before placing them outdoors. The lowest recorded air temperature was about –1.2 °C. More of the noncoated plants were uninjured (class 0) compared to the M96-018-coated plants, however, the differences were non significant (paired *t* test, $P \leq 0.34$), most likely due to small sample size (n = 6).

Recently, infrared thermography has been used to monitor ice nucleation and propagation in plants and has provided new insights into the freezing process (Carter et al., 2001; Fuller and Wisniewski, 1998; Pearce and Fuller, 2001; Wisniewski et al., 1997; Wisniewski and Fuller, 1999; Workmaster et al., 1999). These studies have confirmed the presence of intrinsic nucleators in woody plants that are active at warm, subzero temperatures, and that ice nucleation temperature can be a function of plant size or mass. The studies have also confirmed the presence of barriers to ice propagation and the ability of some herbaceous plants to supercool to temperatures well below 0 °C . Collectively, data from these studies have indicated that in herbaceous plants, the presence of external surface moisture and epiphytic, ice-nucleating agents are the main factors that limit the ability of many plant species to supercool to temperatures of –6.0 °C or lower. These observations led to the present study where the ability of hydrophobic particle films to block extrinsic ice nucleation was examined.

The material used in the present investigation consisted of a proprietary formulation of kaolin particles that have been coated to impart hydrophobicity. It is our hypothesis that if moisture can be prevented from forming on the plant surface and activating epiphytic ice nucleators, or itself freezing and acting as a source of nucleation activity, then there would be a higher probability of the plant expressing its intrinsic ability to supercool. Results herein support this hypothesis on both individual leaves and whole plants of tomato.

In the present study it appeared that the particle film, due to its hydrophobicity, prevented moisture from collecting on the plant surface (in many cases water would simply roll off) and lowered the amount of contact that any individual droplet had with the plant surface, effectively raising the droplet above the leaf surface (Fig. 1). Additionally, it is assumed that the moisture barrier prevented the wetting of epiphytic ice nucleators present on the leaf surface and hence their activation. Collectively, this would have reduced the number of potential extrinsic nucleation events, and diminished the ability of ice crystals that did form from propagating into the internal portion of the leaf and inducing the leaf to freeze. Access of ice crystals to the internal portion of the leaf is believed to occur through stomates, cracks in the cuticle, broken epidermal hairs, etc. (Wisniewski and Fuller, 1999).

While the degree of supercooling in M96-018-coated tomato leaves or plants with moisture present on the surface was quite significant (–8.0 to –12 °C), the variability observed in the average freezing temperature was greater than in the other treatments (Figs. 3 and 5). This may be attributed to both a failure in achieving complete coverage of the plant material with the hydrophobic particle film and/or the ice crystal present on the leaf surface overcoming the barrier presented by the hydrophobic kaolin (Fig.

1). This was also reflected in experiments with whole plants exposed to –3 °C (Fig. 7), where it appeared that generally the hydrophobic particle film either worked or did not, i.e., most of the plants either had no injury (class 0) or were killed (class 5). In our initial studies, we did not use temperatures below –3 °C for several reasons. We wanted to use a slow cooling rate and were apprehensive about the wet soil freezing in the pots and causing the plants to freeze, a scenario that does not occur during natural freezing events because of the reservoir of heat present in the soil. We did not have the space or facility of providing an external heat source for the pots, although they were insulated with plastic bubble wrap. We were also aware of the difficulty in providing complete coverage of the plant with the present formulation of the hydrophobic particle film and the complexity of protecting whole plants from hundreds if not thousands of sources of nucleation. This is reflected in the fact that plants will freeze at much warmer temperatures under field conditions (Fig. 9) than they will in environmental chambers (Ashworth and Kieft, 1995).

Application of the hydrophobic particle film provided protection to tomato plants when experiments were conducted in a radiative frost chamber (Fig. 8) or under natural frost conditions (Fig. 9). The frost conditions in both of these experiments would enhance radiative heat loss from the plants and so even though it appears that the frost was relatively mild (–1.2 °C) and plants froze at a warmer temperature, the plants may have been considerably colder than the air temperature. Thermocouples were not placed on or in the plants to ensure that the thermocouples did not induce a freezing event. Induction at the site of thermocouple placement has been observed in previous studies (Fuller and Wisniewski, 1998; Wisniewski et al., 1997). Lack of statistical significance between the amount of injury in treated and nontreated plants in the natural frost episode was likely a product of the small sample size (n = 6). Nevertheless, it appeared that freezing had been delayed in the plants treated with the hydrophobic particle film as the majority of them were completely uninjured while a broad range of damage was observed in the nontreated controls. The particle film has been observed to delay ice crystal growth from a frozen droplet present on leaf surfaces for an average of one hour and in some cases for the whole duration of a frost test (data not presented). This time delay is significant in that it is representative of the duration of transient radiation frosts under field conditions (Fuller and LeGrice, 1998).

In summary, previous research using infrared thermography has indicated that the major factors limiting supercooling in herbaceous plants are the presence of moisture and epiphytic (extrinsic) ice nucleating agents on the plant surface. Use of a hydrophobic material to delay or block the effect of moisture or ice-nucleating agents appears to be a valid approach. Results herein indicate that coating plants with a hydrophobic particle film delayed or prevented the ability of external ice crystals from growing into the plant and inducing it to freeze, thus providing a degree of frost protection. Large scale studies under field conditions will be needed, however, to determine if the hydrophobic particle film (or a similar type of compound) can be used to provide frost protection under the complex freezing conditions that are present during natural frost episodes.

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