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Protection of plants from frost using hydrophobic particle film and acrylic polymer

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Summary

Frost damage to potatoes, grapevine and citrus plants was assessed following treatment with either an acrylic polymer (Antistress^{TM}) or with a hydrophobic particle film (CM-96-018). In large freezing tests, the application of the hydrophobic particle film consistently led to less damage whilst the acrylic polymer led to the same amount or more damage when compared to control plants. Detailed examination of the freezing of leaves of all three species using infrared thermal imaging revealed that the hydrophobic particle film delayed the entry of ice from a frozen water droplet containing ice nucleating active bacteria and in some cases for the complete duration of the frost test. In contrast, the acrylic polymer was only able to influence the time of ice nucleation of the leaves of citrus plants. It was concluded that the hydrophobic particle film shows considerable promise as a frost protectant applied to susceptible crops just prior to a freezing event.

Key words: Frost protection, Antistress^{TM}, hydrophobic particle film, citrus, potatoes, grapevine

Introduction

Freezing damage to sensitive crops can only occur after the formation of ice in the plant tissues. Whilst the universal melting point of frozen plants is close to 0°C, studies on various crops have demonstrated that the freezing point of plants varies and the degree to which this occurs is termed the supercooling ability (Chen *et al.,* 1995). The amount of supercooling in turn depends on the presence and activity of ice nucleators which can be extrinsic, such as the ice nucleating bacterium *Pseudomonas syringae* (Lindow, 1995), or intrinsic compounds of plant origin (Ashworth *et al.*, 1985). Despite a large body of published information on extrinsic ice nucleators, there is still some doubt as to the exact nature and importance of these in natural freezing events. There are several reports of the importance of surface water leading to early ice nucleation of plants (Fuller & Le Grice, 1998; Fuller & Wisniewski, 1998) suggesting that free water facilitates the activity of ice nucleators present in the phylloplane. Le Grice (1993) maintained that the surface water was the most important aspect with regards early freezing and subsequent freeze injury in early potatoes. In most field situations, dew fall precedes freezing ensuring that leaf surfaces are nearly always wet prior to freezing (Pescod, 1965).

Protection of sensitive crop plants from freeze damage has always been a major challenge to agriculture. Most damage occurs during radiative freezing conditions in spring when physiological cold hardiness has been lost (Fuller & Le Grice, 1998; Fuller & Telli, 1999). Various field techniques are employed in valuable crops and generally involve interfering with the freezing environment by the use of turbines to mix cold and warm layers of air or by smoke blankets to prevent the crop losing long wave radiation (Kalma *et al.*, 1992). In some cases crops are sprayed with a continuous fine mist of water which freezes on the plant and raises the leaf temperature to zero by the release of the latent heat of freezing of the water (Hamer, 1986, 1989). In this strategy, care must be taken to keep the mist continuous so that the leaf surface continues to remain at zero and, although it is encased in ice, the leaf does not actually freeze. A risk of this technique is that the ice on the plant can build up so extensively that it causes physical damage to the plant such as branches snapping on fruit trees.

The application of field applied compounds for frost protection has been an elusive dream of many researchers and agri-chemical companies. Many compounds have been screened and some have been reported in the literature for flowering fruit bushes (Wilson & Jones*,* 1980; 1983*a,b*). Exogenously applied cryoprotectants, such as sorbitol or polyethylene glycol, however, appear to be either

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phytotoxic at active concentrations or only marginally effective in controlling ice nucleation in vegetative field crops (A S Robinson, personal communication). More recently some acrylic compounds have been marketed on the basis of giving frost protection by covering the leaf surfaces with an inert layer (Anon., 2002). Le Grice (1993) examined the use of kaolin dusts as an absorbant of free water but found little frost protective effect whilst Glenn *et al.* (1998) reported hydrophobic and hydrophilic formulations of kaolin dust with biologically active properties. Recently, Wisniewksi *et al.* (2002) reported the use of a hydrophobic kaolin as a protectant of freezing in tomato.

This paper examines the effect of two compounds in the frost protection of three frost sensitive species, potatoes, grapevine and citrus, and examines their mode of action at the ice nucleation level using the technique of infrared thermal imaging.

Materials and Methods

Plant materials

Three plant species were tested, potatoes (*Solanum tuberosum* cvs Maris Bard and Premiere), grapevine (*Vitis vinifera* cvs Madeline Angevine and Siegrebbe), lemon (*Citrus limon* cv. unknown). The potatoes were raised from certified seed tubers in 10 cm diameter pots and were 25 cm tall and at the 7 to 9 leaf stage when tested. The grapevines were 35 cm tall, 2-yr old, direct rooted cuttings. The lemons were 25 cm tall, 2-yr old plants raised from seed collected directly from mature commercial fruit in Greece. Potato and lemon plants were grown in peat-based compost and maintained in the glasshouse with a minimum temperature of 10°C. The grapevines were maintained outside.

Frost protection compounds

Two frost protection materials were assessed, AntiStress™ (supplied by Agrihandlers Ltd) an acrylic polymer capable of forming an elastic coating on the leaves of plants, and an hydrophobic kaolin particle film, CM-96-018 (Engelhard Inc., Islin, NJ, USA). Both materials were applied in water by hand-held sprayer until run-off. The AntiStress was applied at a rate of 1:50 (vol:vol) and the hydrophobic dust at a rate of 30 g litre^{-1}. The latter was first mixed to a paste in methanol and this added to water at a rate equivalent to 10% methanol. Both spray mixtures were agitated periodically during spraying. Control plants were sprayed with distilled water. All materials were allowed to dry on the leaves of the plants for 2 to 4 h prior to frost testing.

Frost testing

Prior to frost testing, plants were held at 10°C in a Phytotron with 16 h daylength at a photon flux density of 120 μM m^{-2} s^{-1} for 7 days. Two frost tests were conducted, firstly on whole plants in a walk-in convective freezing chamber and secondly on isolated leaves in a radiation frost chamber (Fuller & Le Grice, 1998).

Since the species under test were all regarded as frost sensitive, the object of the convective frost test was to apply a mild freezing representative of a spring frost. During the frost tests, whole plants in pots were placed in a completely randomised arrangement in the freezing chamber, sprayed with distilled water and then subjected to the following regime: 3°C for 30 min, 0°C for 60 min, -3.0°C for 2 h, 3°C for 16 h then returned to 10°C for recovery and damage expression. Each stem or branch on each plant was scored for damage after 5 days recovery on a 0 to 4 scale (where 0 = no damage, 1 = slight, 2 = moderate, 3 = severe, 4 = complete kill). Each species was frost tested independently with seven to 10 plants tested per spray treatment in each. Air temperatures recorded at four locations in each test showed that the testing environment was uniform and consistent from test to test. Score data were analysed in pooled Kruskal-Wallis non-parametric ANOVA.

For radiation freezing tests, leaves were removed from treated plants, their petioles sealed with silicon grease and arranged on an observation platform beneath an Inframetrics Model 760W Infrared Camera in a radiation frost chamber (Fuller & Le Grice, 1998). Images were collected in real time on videotape (Wisniewski *et al.* 1997; Fuller & Wisniewski, 1998) and videotape was then analysed using custom software (Thermagram Plot™) and data transferred to Excel™ for further analysis. Leaves were each inoculated with a 10 μm droplet of ice nucleating active (INA) bacteria (*Pseudomonas syringae* pv. Cit7) with an ice nucleation point of -2.3°C to -2.5°C. Freezing was slow (less than 1°C h^{-1}), declining exponentially as temperature fell, as is typical in a natural radiation frost (Fuller & Le Grice, 1998) and continued for 2 h after the freezing of the INA bacterial droplet. Thermocouple recorded temperatures of the observation platform confirmed that all frost tests were virtually identical. Leaves were then thawed slowly, 2 h at 4°C, and covered with a moist tissue and incubated overnight at 10°C. Frost damage was assessed visually by the degree of water-soaking of the leaf. Each species was tested independently with three replicate tests carried out for each species and two leaves used per spray treatment in each test.

Results

Convective frost tests

The freezing regime used was sufficiently mild to enable the expression of a range of frost damage symptoms in all the species tested. Damage score data revealed a clear bi-modality with a preponderance of scores at 0 and 4, i.e. either no damage or complete damage, and both symptoms could occur on separate stems/branches on the same plant. Such damage symptoms were similar to those observed in the field following transient freezing (M P Fuller unpublished) and are interpreted as shoots which supercooled and escaped freezing (score of 0) and those in which ice nucleation had occurred leading to complete freezing damage (score of 4). Intermediate scores were interpreted as stems that were still in the process of freezing at the termination of the frost test. Analysis of the score data showed that the hydrophobic particle film (average rank 188.9) performed significantly better ($P < 0.001$; Kruskal-Wallis analysis) than either the control (average rank 234.1) or the acrylic polymer (average rank 239.6) and there was no significant difference between the control and the acrylic polymer. The proportion of stems/branches per plant undamaged by freezing most clearly illustrated the effects of the treatments (Table 1) (data were transformed by Logit transformation prior to analysis of variance; $\mathrm{Ln}\{(x + 0.5)/(n - x + 0.5)\}$ where n = number of branches/stems per plant, x = number of undamaged branches/stems per plant, 0.5 = arbitrary correction factor).

Radiation frost tests

The droplets of INA bacteria applied to the leaves in all of the radiation frost tests froze at the predicted temperature (-2.3°C to -2.5°C) and the droplet took approximately 2 min to freeze out completely. There was then a delay before the leaves froze ranging from a few seconds to over 2 h (i.e. beyond the duration of the test) and leaf freezing temperatures were in the range -2.5°C to -4°C. In all species, frost damage took the form of complete water-soaking, in those leaves which were observed to freeze, or leaves were completely undamaged in those which did not freeze.

The leaves of potato froze most readily with control leaves freezing very soon after the INA bacterial droplet had completely frozen out. Grapevine leaves showed a short delay of several minutes whilst the citrus had a considerable delay sometimes not freezing at all despite the presence of a frozen INA droplet. Observations of the videotape indicated that all leaf ice nucleation commenced in the vicinity of the INA droplet and rapidly radiated outwards to encompass the whole leaf in a matter of a few seconds.

Of the leaves that did freeze, it was always the control leaves that froze first. Analysis of the mean time delay in leaf freezing after the freezing of the INA droplet showed that the presence of the hydrophobic particle film led to a significant delay in leaf freezing in all species (Fig. 1). The acrylic polymer, however, only significantly delayed leaf freezing in lemon.

Discussion

The results of this investigation demonstrated that

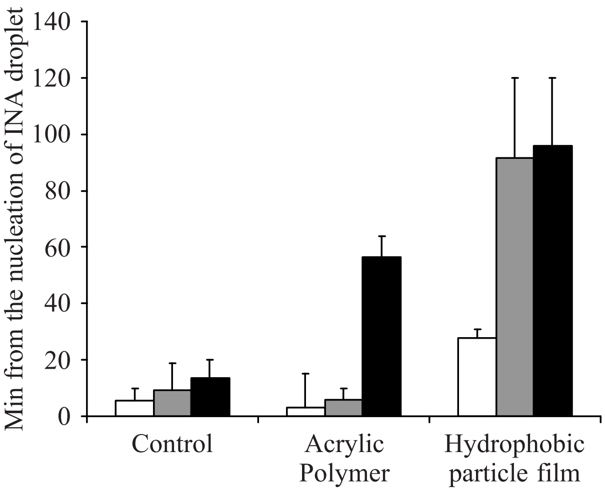

Fig. 1. Comparison of the mean delay in ice nucleation of leaves of potato (mean of cvs Premiere and Maris Bard) (unshaded bars), grapevine (mean of cvs Madeline Angevine and Siegrebbe) (grey bars) and lemon plants (solid bars) treated with frost protection materials (bars = SE).

Table 1. *Performance of two treatments (acrylic polymer and hydrophobic particle film) against frost (% shoots/branches undamaged per plant followed by Logit transformation) on three frost sensitive species (grapevine, potatoes and lemon)*

		Grapevine cv. Madeline Angevine	Grapevine cv. Siegrebbe	Potato cv. Maris Bard	Potato cv. Premiere	Lemon cv. unkown	Mean
Control	%	56.67	53.33	44.00	28.89	50.32	48.39
	(logit)	(0.330)	(0.093)	(-0.220)	(-0.903)	(-0.132)	
Acrylic	%	63.00	65.00	50.70	35.10	30.00	47.74
Polymer	(logit)	(0.398)	(0.439)	(0.057)	(-0.410)	(-0.699)	
Hydrophobic	%	66.67	85.00	62.58	43.57	80.00	70.14
Particle film	(logit)	(0.694)	(1.286)	(0.999)	(-0.182)	(1.153)	
Pooled SE	(logit)	(0.2823)	(0.2436)	(0.2487)	(0.3497)	(0.2168)	

a hydrophobic particle film was capable of giving some protection against freezing in a range of frost susceptible species. The method of protection was shown to be by prevention of the ice nucleation of leaves by delaying the penetration of ice from a frozen droplet on the leaf surface. The efficiency of this protection was such that ice penetration was delayed on average by up to 2 h and in a short freezing test this was sufficient to enable a higher proportion of plants to supercool and avoid freezing and thus remain undamaged. Such short durations of freezing have been shown to be commonplace during radiative spring frosts (Fuller & Le Grice, 1998) and thus a delay in freezing could have significant beneficial effects in the field. The findings also support the work of Wisniewski *et al.* (2002) who evaluated the same product on tomatoes and found that it enabled treated plants to supercool to -6°C compared to untreated plants which froze at -1.5°C to -2.5°C. The product evaluated here (CM-96-018) was a pilot formulation of this material and there may be scope for improved formulation to further enhance its efficacy now that its mode of action has been established.

In contrast to the hydrophobic particle film, the acrylic polymer (Antistress™) did not clearly demonstrate a consistent frost protection effect and was not significantly different from the control treatments. It was unable to delay the penetration of ice in potatoes and grapevine leaves although there was some evidence of a delay in lemon. Other results in our laboratory suggest improvement will not simply be a function of the concentration of the product applied (L Keene, personal communication) and we conclude that it is not an effective frost protection formulation as presently presented. Wisniewski *et al.* (2002) evaluated a similar product (Moisturin™) and concluded that it too was ineffective in consistently preventing ice nucleation in tomatoes.

It was clear from the observations using the Infrared Thermal Imaging that the ice nucleation of the leaf was caused by the frozen droplet on the leaf surface and that differences in leaf morphology influenced the penetration of the ice. Such observations support the findings of Wisniewski & Fuller (1999) who showed that leaf ice nucleation could be prevented by the presence of an impermeable silicone grease barrier and was severely delayed in species with leaf morphologies which were very waxy or had thick cuticles. The precise nature as to why potato leaves are so susceptible to rapid ice nucleation from external ice is unknown but it may be related to the abundant presence of trichomes or abaxial stomata. In contrast, the thick waxy cuticle of the lemon leaves was apparently responsible for the delay in leaf freezing in this species.

This investigation brought together two methods of examining freezing effects on plants applied to the screening of two commercial products for the protection of plants during stress (hydrophobic particle film and acrylic polymer). The two methods (convective and radiative freezing) yielded similar conclusions in terms of the effects of the products tested and confirmed that both approaches could be used to assess frost protection. The infrared thermal imaging, however, enabled a mode of action to be investigated and quantified and illustrates the usefulness of this technique in ice nucleation and frost protection studies.

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