Frost Management in Cool Climate Vineyards

FINAL REPORT to
GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **UT 99/1**

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Executive Summary

This project, developed as a result of devastating frosts across southern vineyards in the late 1990's, was designed to indicate the need, and possible future directions, for research on two aspects of frost protection causing concern among vignerons. In cooler areas, damage to vines by spring frosts and subsequent losses in yield has become a critical issue determining profitability in production of high quality, cool climate wines. Frost protection options include various types of irrigation, heaters, wind machines and some commercial spray-on materials promoted as frost protectants. Heaters have become too expensive to operate, and irrigation (although of proven effectiveness) places heavy demands on water supplies and drainage systems. In many areas, wind machines are preferred in spite of possible noise pollution restrictions, but installation guidelines generally do not cover sloping sites. Anecdotal evidence is that on some sites machines have been less effective than expected, even increasing frost damage in parts of vineyards.

Although attractive to growers, the concept of a spray-on protectant has found little support in the scientific literature. Occasional reports of success have not generally translated into commercial use and products claiming to provide protection do so with little scientific support. Worldwide there seems to be only one example of an industry, which has been using a spray-on protectant routinely and successfully for a decade or more.

The present project was divided into two separate studies, an examination of wind machine effects on temperature on sloping sites, and an evaluation of spray-on protectants aimed mainly at confirming current views. The objective in both cases was to assess the need, and possible directions, for further research.

Wind machines

A series of measurements of temperature at fruiting height in two vineyards planted on moderate slopes were used to assess the effect of a wind machine on ambient temperatures during two early spring frosts. In one case, the change in temperature due to the machine was too small to be of practical value. Investigation of inversion conditions suggested that faulty operation of the machine was the cause of lower than expected gains in temperature. On the second site, there was an initial rise in temperature of about 1.3°C averaged over the area influenced by the machine, and the increase remained relatively constant throughout the two frosts studied. Over the area of machine influence there was a strong multiple linear regression relating both distance from the machine and elevation to the temperature gain at each measurement position. A decline in effect with increasing distance confirmed previously published reports and the increase in effect down - slope from the machine appeared similar to reports of downwind distortion of protected area.

Estimated temperature down - slope from the machine in undisturbed air decreased with increasing depth in the inversion negating the increase in ambient temperature due to the machine. The model also indicated potential reduction in ambient temperature up - slope or at extended distances from the machine, confirming published reports and anecdotal evidence of increased damage up - slope or on the margins of protected areas. It is concluded that, in general, the area protected by a machine on a sloping site will be less than for a near flat site under otherwise comparable conditions. Down - slope distortion of the protected area did occur, but did not extend beyond the expected range of the machine under still air conditions on a flat site. Although the up - slope influence of the machine was reduced with increasing elevation, increased ambient temperature at higher elevation compensated and there was no evidence of increased damage. Overall the pattern of damage in a mild frost reflected the model of temperature modification.

It was concluded that present design guidelines regarding inversion strengths and wind speeds appear to be valid on sloping sites, but selection and positioning of the machine needs to take changes in both machine

effectiveness and ambient temperature with elevation into account. Reduced effectiveness at elevations above the machine and a down - slope decrease in ambient temperature may substantially reduce the area protected and hence economic return on the capital cost of the machine on some sites. The results suggest that the system is amenable to more complex mathematical modelling, perhaps providing a computer based site analysis. Such a development should attract commercial funding.

Spray on protectants

A review of the scientific literature revealed few examples of effective, spray on frost protection. There have however been several reports of field applied sprays which have resulted in depressed freezing point for excised leaf, flower and shoot tissue measured under laboratory conditions. Generally these results have failed to translate into commercially viable frost protection, but anecdotal reports of successful field treatments continue to circulate. A series of spray materials, which have been reported as providing a level of frost protection in the scientific literature or in commercial product labels, were tested. Phytotoxicity and effect on leaf water potential of mature vines were tested before influences on shoot tissue freezing behaviour were measured in laboratory, frost simulation chamber and field trials. Materials tested were, glycine betaine, potassium dextro-lac (KDL), Teric 12A23B, Seasol (a seaweed extract), sodium alginate and two commercial film forming materials, Antistress and Envy.

Potassium dextro-lac and higher concentrations of betaine and 12A23B were phytotoxic when applied to actively growing tissue. Lower concentrations of the latter two did not induce phytotoxicity symptoms. Seasol, alginate and betaine all caused a decrease in osmotic potential (Teric at low concentration was not tested), but the change took more than 3 days to develop. Betaine, alginate and Seasol all decreased freezing temperature in laboratory studies, but in simulation trials on potted plants there was no effect of spray treatments at frost temperatures of -6 and -4 °C.

In line with several previously published reports, the present laboratory results were not duplicated with whole plants in a frost simulation chamber. Temperatures tested may have been below the limit of protection and further work is required. Overall the results give some cause for optimism and perhaps suggest that at least some of the negative reports on these materials may result from insufficient time being allowed for a protective effect to develop before a damaging frost. It is recommended that further work should be considered and should concentrate on the mechanism(s) and development time of the protective effect.

Technical Summary

Two studies on frost protection are described. In both cases the objective was to clarify concerns about efficacy or economic value, which have been raised by industry. It was anticipated that the investigations would establish whether further work should be considered and provide a basis for any recommended ongoing research.

Wind Machines

Wind machines or frost fans are an accepted and widely used method of frost protection for vines and tree fruit subject to relatively mild (ca. –4.0 ^{o}C) inversion frosts. On flat or near flat terrain, in still air conditions, machines have been shown to increase ambient temperature 30 – 50% of the 1.5 to 15 m. temperature profile in the inversion, over a radius of about 100 m (greater for larger machines). The literature offers little, if any, comment on machine effectiveness on sloping or undulating sites. On flat land effectiveness has generally been measured as the immediate change in temperature when the machine was started or the increase in temperature compared with records taken out of range of the machine. This type of analysis, or comparison, assumes little spatial variation in temperature over the area influenced by the machine and the surrounding area. Topographic influences on both surface and inversion temperatures during an inversion frost are well documented, and data on effects of a wind machine on ambient temperature on a flat site are not necessarily applicable on a sloping site as this spatial variation is not taken into account. Consequently a correlative technique was used to estimate values for ambient temperatures (ie temperatures had the machine not been operating) during machine operation.

Regressions of temperature logged at various positions in a vineyard, without the machine running, were plotted against equivalent temperatures recorded at a nearby “reference” site, out of the expected range of the machine. With the machine running these regressions were then used with corresponding temperatures at the reference site to estimate ambient temperature at each position for comparison with corresponding recorded temperatures. The differences between estimated ambient and measured temperatures were taken as an estimate of machine effectiveness at each logger position.

The effect of the machine estimated at various elevations and at varying distances from the machine was highly correlated with both elevation and distance, with effectiveness decreasing with increasing distance and elevation. The spatial variation in effect on temperature was discussed in relation to decreasing ambient temperature at lower elevation.

Chemical Protection

Although there are a number of scientific publications demonstrating no useful protective effect from various spray – on materials, there are persistent reports in trade journals (and elsewhere) of success with both osmolytes and film forming sprays. There are a few scientific papers demonstrating increased freeze tolerance in – vitro, but laboratory studies have not been developed into proven commercial product.

A range of non phytotoxic osmolytes including natural seaweed extracts and glycine betaine, were found to lower both leaf osmotic potential and freezing point of excised shoots. Tissue freezing point was determined in a glass, temperature controlled chamber using either exotherm temperature or a rapid change in electrical impedance to mark the start of intercellular ice formation. A characteristic increase in electrical impedance was found to closely correspond to the first low temperature exotherm.

Actively growing cuttings in pots were treated with the osmolytes and two commercial polymer film (antitranspirant) materials and subjected to frosts of –4 and –6°C in a frost simulation chamber. All treatments suffered severe damage at both temperatures.

Observations during these experiments, published scientific data and anecdotal evidence from industry, suggest that both time and rate of application may determine whether spray materials are effective and account for the lack of success in field trials. In view of this readily identifiable deficiency in trials seeking to evaluate sprays, further work is recommended. Emphasis should be placed on mode of action of applied sprays with particular attention paid to the time taken for tolerance to develop and the advantages or disadvantages of repeat application.

Introduction

In cooler regions ideal for growth and production of grapes for high quality wines, damage caused by spring frosts has become a significant factor limiting returns on many established vineyards. Site selection favours a gently sloping land, usually on sheltered valley floors, to maximize exposure to sunlight and allow for a long growing season. Such sites are frequently prone to frost and temperatures as low as -5 ^{o}C at fruiting wire height have commonly been recorded. Where frosts are severe and frequent, growers have been forced to install capital expensive physical protection methods including wind machines and purpose designed irrigation. Where frosts are less frequent growers have tried various “spray - on” treatments as a cheaper and less reliable alternative frost protection.

The present project was developed in response to industry concerns regarding the efficacy of “spray – on” type frost protectants and some inconsistent results with wind machines. The two issues were examined separately in a series of experiments with an overall objective to provide enough preliminary information to decide on the need for more detailed research and, if necessary, to provide a basis for planning future work.

Frost development and structure

As defined by Kalma et al. (1992), a “frost” occurs when the air temperature near the earth’s surface drops below 0^{o}C. Generally it results in a deposit of interlocking ice crystals formed by freezing of dew or direct sublimation of atmospheric water vapour (Reiger, 1989). Under suitable conditions, plant temperature drops quickly reaching a temperature lower than that of the surrounding air. If air relative humidity is high, dew is formed before air temperature reaches 0 ^{o}C. When surface temperature falls below 0 ^{o}C a ground frost occurs and continued de-humidification of the air results in ice crystal growth and the formation of a white frost.

The atmospheric conditions leading to frost formation are closely linked to the local earth/atmosphere energy exchange as described by Oke (1978). During the day, solar short-wave radiation emitted from the sun and transmitted through the atmosphere to the surface of the earth. Absorption of this short-wave radiation causes the surface temperature to rise and long-wave radiation is emitted back to the atmosphere and to space. The temperature of the surface and its relative radiating power or emissivity control the amount of radiation emitted by the earth. The surface also receives radiation back from the atmosphere. The amount of atmospheric counter-radiation is related to the atmospheric temperature and emissivity. Unlike the earth’s surface emissivity, the emissivity of the atmosphere is highly variable spatially and temporally and is strongly dependent on atmospheric water vapour and (longer term) the carbon dioxide content. Under certain conditions clouds can counter radiation loss by the surface and hence reduce radiative heat losses from the surface (Kalma et al., 1992). Conversely, radiation loss will be at its greatest when there is no cloud cover. At night there is no incoming solar radiation, and surface temperature is related to residual energy stored from incident radiation during the day and radiative heat loss.

During the day air above the surface is heated by radiation from the heated surface, which results in a “lapse” profile in which temperature increases with height. At night the absence of incoming radiation from the sun, and radiative losses from the surface may allow surface temperature to fall low enough for heat to transfer from the adjacent atmosphere to the surface. Therefore, during clear calm nights the lowest air temperature is observed near the surface (Kalma et al., 1992) producing a ground-based energy and temperature inversion, i.e. temperature increases with height. This creates an air temperature and buoyancy gradient, directed towards the surface, so that any air motion results in a downward sensible heat flux. This process mixes warmer air to the surface from the warmer layers above it. Heat is thus transferred to the cooling surface and consequently the air near the surface cools, leading to progressive thickening of the surface inversion layer (Gopalakrishnan et al., 1997). The time of formation of the nocturnal inversion is dependent upon the atmospheric conditions and the physical characteristics of the earth's surface (Godowitch et al., 1985). The nocturnal inversion layer is usually strongly stable and there is no mixing of this layer near the surface. Above the inversion a neutral or weakly stable layer exists as a remnant of the previous day's mixed layer.

On windy, clear nights with strong atmospheric mixing, strong vertical temperature gradients (a large temperature differences between the earth's surface and the top of the inversion profile) are rare because efficient turbulent exchange rapidly replaces any losses from the surface with heat from the lower layers of the atmosphere. When there is a lack of efficient atmospheric mixing strong inversion profiles can develop and both the surface and adjacent layers cool to a greater extent because of poor convective heat transfer (Kalma et al., 1992).

Thus radiation frosts, which commonly occur in southern Australia are characterised by still, cloudless nights and strong surface inversions, developing in stable atmospheres. Most damaging frosts are brought about by radiative processes and local scale advection of dense cold air, leading to pooling of cold air into low - lying areas.

The height of the nocturnal inversion layer represents the vertical extent of cooling due to turbulent and radiative heat fluxes and will be effected by the processes of katabatic drainage and ventilation/stagnation (Kalma et al., 1992). Godowitch et al. (1985) noted that unless there is a change in synoptic conditions, the height of the inversion continues to increase throughout the night but the increase is most rapid early in the evening, rising to two-thirds of its final height at sunrise within 3-4 hours after formation. The conditions conducive to this rapid cooling of the ground by long-wave radiation are clear, dry and light wind conditions often leading to initial formation of a surface-based inversion by sunset**.**

Frost development becomes more complex in more complex terrain. It is possible to divide topographic effects into those due to the varying input of solar radiation, and those related to the generation, or modification, of airflow (Oke, 1978).

The short - wave radiation input, is dependent upon the angle at which it strikes the receiving surface. At a given time and location, the incident radiation is unlikely to vary much spatially (depending upon atmospheric conditions) and hence variations in the slope and aspect angles presented by topography determine radiant heat loading across a landscape. The slope that most directly faces the sun, i.e., incident radiation striking the surface at 90° will receive the most radiation, whereas if the incident angle is near tangential to the earth's surface, minimal radiation is received. The surface angle at which incident radiation is greatest depends on the latitude the site, and the time of year. Both factors change the effective height of the sun in the sky and thus the incident angle of the direct-beam radiation. In general, the effect of moving from high to low latitude in the southern hemisphere is to increase the illumination of the north-facing slopes at the expense of the south-facing slopes (Oke, 1978).

Topographically-induced radiation variations lead to energy balance differences across the landscape, and as a result in an area of non-uniform topography a range of microclimates can develop. Because nocturnal climate under still cloudless conditions is closely related to soil heat, inversion strength of radiation frosts will vary markedly with topography.
To further complicate the issue, hill - slopes and valleys, especially those in mountainous regions, produce their own wind systems as a result of topographically induced thermal gradients. At night the valley surfaces cool by the emission of long-wave radiation and sensible heat flux divergence. Colder, higher density, air moves down - slope until it is immersed in a layer of air of the same or greater density (Kalma et al., 1992). While these thermally induced winds can reach very high speeds under alpine or polar conditions, under

most circumstances, a less spectacular gentle flow (<1m/s) is common. Elevation differences of less than one meter may allow cold air to drain to the lowest lying portions of the landscape thus the coldest (densest) air settles in the lowest areas increasing the depth and strength (thermal gradient) of the inversion.

Frost Damage in plant tissue

Opinion remains divided on the processes of internal freezing which result in tissue damage in plants. Allard et al. (1998) reported that during freezing stress, extracellular ice formation creates a water vapour deficit that increases water migration from interior to exterior of cells, resulting in the dehydration of the protoplasm. Under these circumstances, the plasma membrane functions as a block against ice seeding of the cell contents. However, Stout (1987) reported that freezing injury raises electrical impedance of tissue owing to membrane damage and there is a persistent but largely unfounded view that ice crystal growth causes direct physical damage to cell walls or to membranes. For example, in discussing frost damage on grapevines, Magarey et al. (1994) suggested that when water freezes it expands vine tissues and damages cells. The current view is that direct physical damage is rare under natural frost conditions and that dehydration injury is the main cause of damage. The nature of dehydration damage remains a matter of debate.

Opinions on critical temperatures for freezing injury also vary, but there are clear differences between species, tissues and stages of growth. Jackson et al. (1987) noted that at any time of the year, a frost of –4°C or less, 30cm above the ground will effect the leaves of grape vines. Exposed flower cluster and young shoots are more sensitive, with damage occurring as temperature falls below –2°C. Vines, unlike some fruit trees, bear a number of dormant buds, which can become fruitful if the growing buds are destroyed. These secondary and tertiary buds are only 50 to 70 percent as productive as primary buds and remain prone to frost damage if further frosts occur. Thus, depending on timing and frequency, frosts in late spring can partially or totally eliminate the year's crop (Seyedbagheri and Fallahi, 1994).

Wind machines for frost protection

Introduction

Wind machines sometimes called "frost fans" are designed to provide atmospheric mixing in a near horizontal plane and commercial and scientific publications generally refer to the atmosphere above a flat or near flat terrain when reporting machine effectiveness. Cool climate viticulture has moved increasingly into hill country in search of lower summer temperatures. Wind machines are successfully employed for frost protection in these areas, but installation is often on a trial and error basis with little information available on the effect of slope on the total area protected or the likely gain in temperature. Growers face the difficult task of deciding whether gains in temperature will be sufficient to reduce frost damage over a large enough area to make the installation cost effective.

Reese and Gerber (1969) and Rieger (1989) described the design and operating principles of commercially available wind machines (Plate 1), in detail. A large fan, typically about 5 m in diameter, mounted on top of a 10 - 15 m tower, produces an air flow inclined slightly downward drawing air from above the crop and mixing it into the canopy and fruiting zone. As a temperature inversion usually accompanies damaging frosts, warmer air from aloft is mixed with cooler air near the surface and net warming occurs at crop height. Mixing of the inversion is partly due to the incursion of air from above and behind the fan and partly due to warmer air entrained by the jet. At distance from the fan, the jet slows and cools and the volume of entrained air also decreases. The fan also slowly rotates about a vertical axis resulting in periodic mixing in a near circular pattern. Under ideal conditions there is sufficient mixing to give a near isothermal temperature profile within the natural inversion, throughout the effective radius.

Doesken et al. (1989) reported that a wind machine could raise temperatures within the plant canopy more than 50% of the inversion temperature gradient between 1.5 and 15m above soil level. Other authors, McGann and McNaughton (1987) Reese and Gerber (1969) and Carran (1981), have suggested lower gains in the range 20 to 33%. This simple relationship between ambient temperatures and machine effectiveness appears to be an oversimplification and Bates (1970) and more recently Nanos et al. (1997) have both demonstrated decreased machine effectiveness with increasing distance from the tower. Nanos et al. also reported a substantial rise in air temperature at each pass during rotation but temperature dropped towards ambient between passes.

Estimates of temperature increases and protected areas vary. Nanos et al. (1997) reported that a wind machine effectively increased the lowest temperature at vine height 1 to 2°C for a 2.5 hectare farm with open-field lowest temperature at 1m high of –4°C. The further away from the machine the lower the temperature measured, and bud mortality was actually increased at the edges of the protected area. Bates (1970) recorded an isothermal profile throughout a radius of 97m, concluding that the protected area was about 3 hectares. The size of the protected area will vary with size and type of wind machine used and recently released machines with 15 m towers claim a significantly greater protected area. Nanos et al. (1997) observed that the rotation time is important for effective warming. They reported that an increase in air temperature occurred at each point of rotation but the temperature dropped substantially until more warm air was introduced. Clearly in commercial use, the time in which the wind machine can rotate without damage occurring will depend on the thermal inertia of the particular plant tissue in question.

Plate1 Wind machine at Meadowbank. Study area was to left and right of tower.
View is approximately NNE.

replaced by warmer air from aloft. However there is anecdotal evidence that further up the slope, the cold air may 'bank up', and thus wind machine effectiveness may be reduced.

Wind machine effectiveness is ultimately measured as ability to hold temperature at above damaging levels, but commercial and some scientific literature generally refers to an increase above an unstated ambient. Doesken et al. (1988) suggested that a minimum inversion strength of 2°C from 1.5 to 5 m was required for a wind machine to have any useful effect on a frost sensitive crop. This, however, may be slightly conservative based on the experience of other researchers who found that a 1.5°C inversion strength was enough for warming to be provided by wind machines (Bates and Lombard, 1978, cited in Doesken et al, 1988). The effectiveness will clearly depend on the absolute temperatures and critical temperature for damage as well as the inversion gradient. Throughout the literature it seems to be generally accepted that machines will raise temperatures by 30 - 50% of the 1.5 - 15m difference in temperature (inversion strength) as noted above. This "rule of thumb" needs to be modified if there is a surface wind and the effective gain may decrease with distance from the machine.

Various methods have been used to estimate wind machine effectiveness. Several workers, including Reese and Gerber (1969), used arrays of sensors within the protected zone to record spatial variation in temperature during machine operation. Doesken et al. (1988) adopted a more sophisticated approach using hourly temperature data at 1.5m, to calculate cumulative degree-hours below arbitrary threshold or base temperatures. Machine operation was observed to decrease the accumulated degree-hours by 42% for a 0°C base temperature. This increased to a 51% reduction for a –1°C base, a 60% reduction for a –2°C base, and a 79% reduction for a –3°C base. At the –4° and –5°C bases, wind machine operation was observed to have eradicated all occurrences of temperatures below these levels. Most published studies have been on flat sites with protected area temperatures simply compared with temperatures taken from outside the range of the machine.

Studies designed to show how wind machines change air temperature compared with ambient temperature in undisturbed air have generally been restricted by difficulties in measuring or estimating temperatures had the machine not been operating. Figures quoted for influence of wind machines have been based on observed temperature rise when the machine was started, or ambient temperature recorded outside the radius of influence of the machine. Both methods are adequate for a stable inversion on a flat site, with little spatial variation in temperature in undisturbed air. However on a sloping site, in similar conditions, in-canopy temperature would be expected to vary with elevation, resulting in greater spatial variability.

Materials and Methods

Lyons (1977) working with wind data criticised the use of single point data to describe temporal and spatial effects and used correlations between sites of interest and a common reference point to develop a simple correlative model of wind flow in a region. The present study adopted a similar approach and utilised a reference temperature record taken outside the influence of the wind machine for comparison with measured in - vineyard temperatures to track the effect of the machine during frosts. For more detailed estimation of spatial differences in machine effects, regressions between in-vineyard temperatures and the reference without the machine operating were plotted. During machine operation these equations were then used to estimate in - vineyard temperatures in undisturbed air from the reference position records. Wind machine effectiveness at fixed positions within the vineyard was then taken as the difference between the measured and estimated temperatures.

Two methods were used to obtain regressions for estimation of ambient temperatures in undisturbed air. At both sites, all sub zero temperatures recorded during four winter frosts were plotted against corresponding temperatures at the reference position. Results are not shown, but for individual frost events, correlation coefficients were generally greater than 0.9, but when data was combined across frosts separated by several weeks variability increased markedly, reflecting a variation in inversion structure between frost events. Consequently, results shown below use regressions based on temperatures recorded on the same frost night, before the machine was started, to obtain estimates for ambient temperatures during machine operation.

Measurements

For night time air temperature measurements, radiation screening is not required and unscreened "TinyTag Ultra" dataloggers were suspended from the fruiting wire, approximately 0.7m above ground. For measurement of the inversion profile at Springvale, a logger with an external sensor (time constant less than 2 seconds) was lifted with a tethered helium balloon.

The loggers used for in – vineyard and reference positions had a specified time constant of 5 minutes to 90% accuracy. Records were taken at 20 minute intervals throughout frost nights and at the conclusion of measurement, loggers were calibrated by operating together in a controlled environment chamber. Readings across all units were within 0.1°C. Times and temperatures for all in-vineyard positions and the reference position were recorded throughout the nights (sunset to sunrise) on which the wind machines were operated.

In early December after detailed measurements had ceased, there was a late season frost during which the wind machines operated on both sites. Shoot damage was quantified as number of leaves per vine showing frost induced necrosis on every third vine in every second row.

Description of sites

Spring Vale

"Spring Vale Vineyard" is situated near Cranbrook on the East Coast of Tasmania in a broad valley, close to the Swan River which runs in a southerly direction past the vineyard. The area is noted for production of high quality Pinot Noirs, but all vineyards in the area are plagued by spring frosts. The experimental site had a uniform slope of 2-3 degrees facing south east, with the northern corner having the greater elevation. Shelterbelts were present along all sides except for the lower southwest, with the wind machine situated near the centre of the vineyard. The electric powered machine had proven underpowered in the local electrical supply system and the fan diameter was reduced from 10 to 9 m.

Due to the relatively small area, central position of the wind machine and even slope, temperatures were recorded in a grid pattern, with loggers near equidistant from the machine. A reference logger was placed outside the range of the machine approximately 100 m from the northern boundary. Only one mild frost occurred during the study period and the wind machine was turned on for 2 hours on July 4 when the ambient temperature dropped to –1°C at the base of the machine tower. During this frost, inversion strength, i.e. temperature difference between the ground surface and the top of the inversion layer, was measured at the TL position.

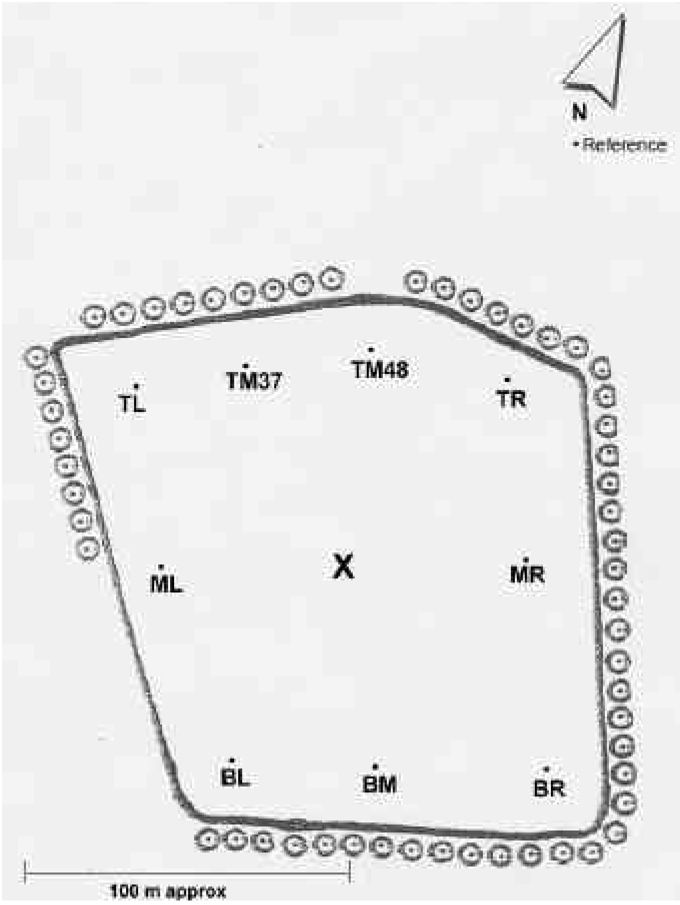

Fig 1 Sketch map of Springvale, showing general

layout, wind shelter and datalogger positions

Meadowbank

“Meadowbank Vineyard” is one of the oldest commercial vineyards in the relatively new cool climate viticulture area of southern Tasmania. The contour map (Fig 2) shows the general layout and topography of the site. The contour interval is 5 m and all elevations quoted use the base of the wind machine tower as a datum. Vine row direction is north - east to south - west. Lower elevation rows towards the north - west boundary had a history of severe frost damage, frequently failing to produce a useful crop. Vines on the higher, south eastern side rarely suffered frost damage. Down - slope air movement and expected distortion of the pattern of protection were the main factors taken into consideration when a wind machine was installed approximately 10 years after initial planting. The machine was placed near the centre of the vineyard, to protect the vulnerable north - western side. Although the machine reduced frost damage, moderate to severe crop losses have continued to occur in the areas where damage was most severe prior to installation of the machine.

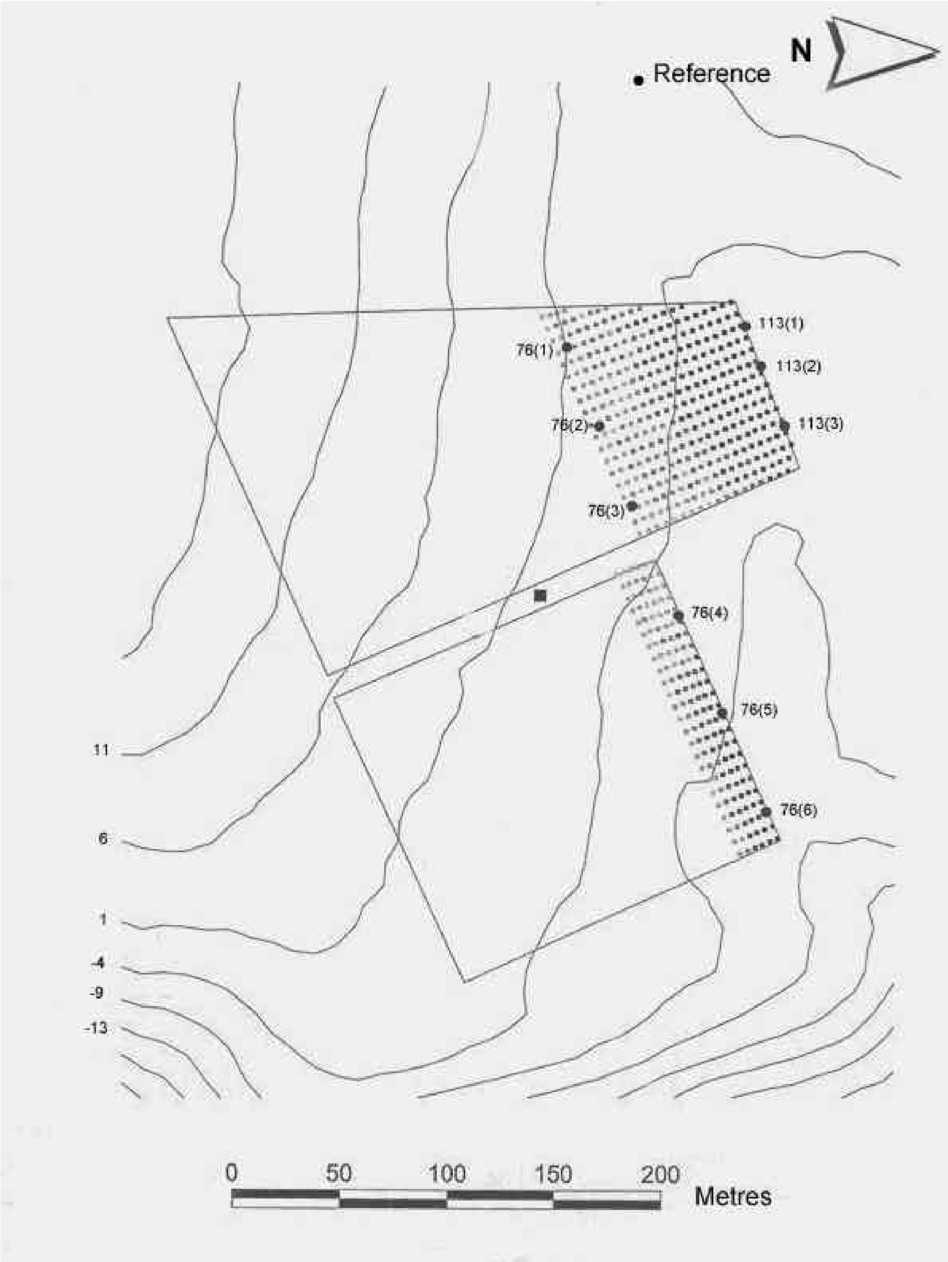

Fig.2. Contour map of Meadowbank, showing layout, logger and wind machine (square symbol), contours and contour interval relative to base of machine tower. Shading on left of map shows frost damage on a continuous greyscale from just visible to most severe.

During two frosts, just before bud burst, temperature was recorded in two fixed transects through the damage prone area between the wind machine and the north western boundary (Fig 2). A reference logger was placed outside the range of the wind machine, up - slope from the most distant logger, approximately 300 m from the wind machine (Fig 2).

The machine was operated for 7 hours on consecutive nights (September 19 and 20), when the ambient temperature dropped to 1°C at the base of the machine. Inversion strength was not measured at Meadowbank, but in vineyard means were plotted against elevation to give an estimate of inversion structure over the site.

Data Analysis

Using ambient temperatures estimated as noted above, machine effectiveness for each logger position was calculated as the difference between estimated ambient temperature (using the regressions with the reference position when the machine was not running) and measured temperature. Data were then combined and means across times for positions, or across positions for times were used to plot spatial and temporal effects on machine effectiveness. Regressions were calculated using the general linear models package in Fastat (Systat Corp). For the Meadowbank data, temperature gains were plotted against elevation and distance from the machine. Data for Meadowbank was averaged across the two frost events for analysis.

Results

Spring Vale

Correlations between the reference position and in vineyard positions (Fig 1) before the machine was started ranged from quite strong ($r^2 = 0.88$) to relatively weak ($r^2 = 0.39$) as shown in Table 1.

Position	Equation	Regression	Mean Effect (°C)
TL	y = 0.723x - 0.238	$r^2 = 0.80$	0.29
TM37	y = 0.522x - 0.969	$r^2 = 0.66$	0.20
TM48	y = 0.745x - 0.450	$r^2 = 0.82$	0.21
TR	y = 0.722x - 0.136	$r^2 = 0.80$	0.21
MR	y = 0.741x+ 2.650	$r^2 = 0.52$	-0.62
BR	y = 0.561x - 0.977	$r^2 = 0.73$	0.33
BM	y = 0.898x+ 0.279	$r^2 = 0.88$	0.06
BL	y = 0.517x - 0.245	$r^2 = 0.39$	0.13
ML	y = 0.614x - 0.596	$r^2 = 0.78$	0.09

Table 1. Regression equations (y = estimated ambient temperature, and x = measured reference temperature) and the mean temperature change during machine operation for each in-vineyard position at Springvale. Codes for positions refer to Fig. 1

Every position in the vineyard, except MR, showed a small mean temperature increase of around 0.2 °C during wind machine operation. However, the greatest temperature modification was in the MR position, where the machine apparently decreased the temperature by an average of 0.62°C. Inversion structure, measured at the TL position, was a uniform gradient of 0.22 °C/m from –1°C at ground level to +3°C at a height of 18 metres (Fig 3).

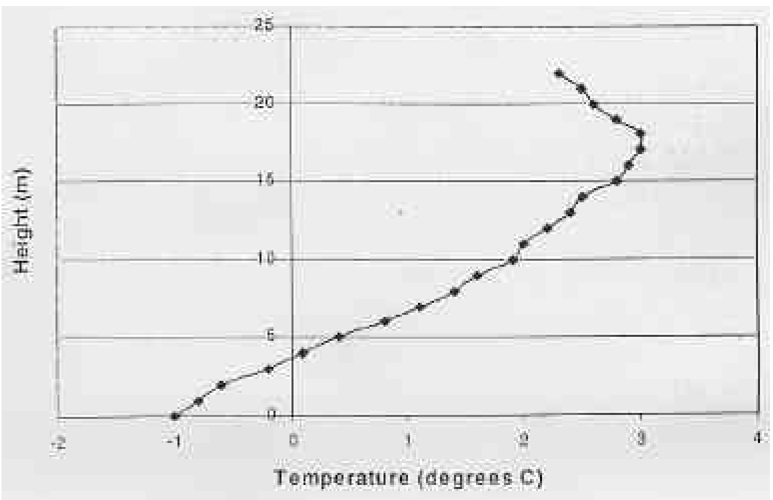

Fig.3 Inversion temperature profile for TL position at Springvale

After the December frost, during which minimum temperature at the tower base fell to -1°C, there was no discernible tissue damage anywhere in the vineyard.

Meadowbank

In-vineyard temperatures were recorded for 11 hours on each of two frost nights. On both nights, the wind machine was turned on when temperature at the base of the tower reached -1°C, and off when the temperature reached +1°C at the same position. Machine run times were 7 h on both nights. In-vineyard temperatures for the 4 h of recording time when the machine was not running were then combined and plotted against equivalent temperatures logged at the reference position. Linear regressions were calculated as described. Regressions and estimated mean change in temperature at each position, during machine operation are shown in Table 2

Position	Equation	Regression	Effect
76/1	$y = 1.079x - 0.417$	$r^2 = 0.94$	-0.52
76/2	$y = 1.231x - 1.013$	$r^2 = 0.95$	0.30
76/3	$y = 0.991x - 1.176$	$r^2 = 0.87$	2.12
76/4	$y = 0.867x - 2.137$	$r^2 = 0.84$	2.14
76/5	$y = 0.848x - 1.904$	$r^2 = 0.86$	2.87
76/6	$y = 0.797x - 2.344$	$r^2 = 0.79$	2.47
113/1	$y = 0.803x - 1.749$	$r^2 = 0.91$	0.69
113/2	$y = 0.934x - 1.894$	$r^2 = 0.93$	0.67
113/3	$y = 0.801x - 1.931$	$r^2 = 0.92$	1.21

Table 2. Regression equations (y = estimated ambient temperature, and x = measured reference temperature) and the mean temperature change during machine operation for each in-vineyard position at Meadowbank. Codes for positions refer to Fig. 2

The decline in temperature during the two frost nights at the reference position and the mean for all positions in the vineyard is shown in Fig 4. Prior to the machine being started, temperatures at the more elevated reference site were higher than the vineyard mean. When the machine was started, there was an immediate increase in temperature of 0.8°C on the first night and 1.6°C on the second. After this initial rise, mean temperatures in the vineyard then continued to fall remaining close to the reference position temperatures throughout the period of machine operation.

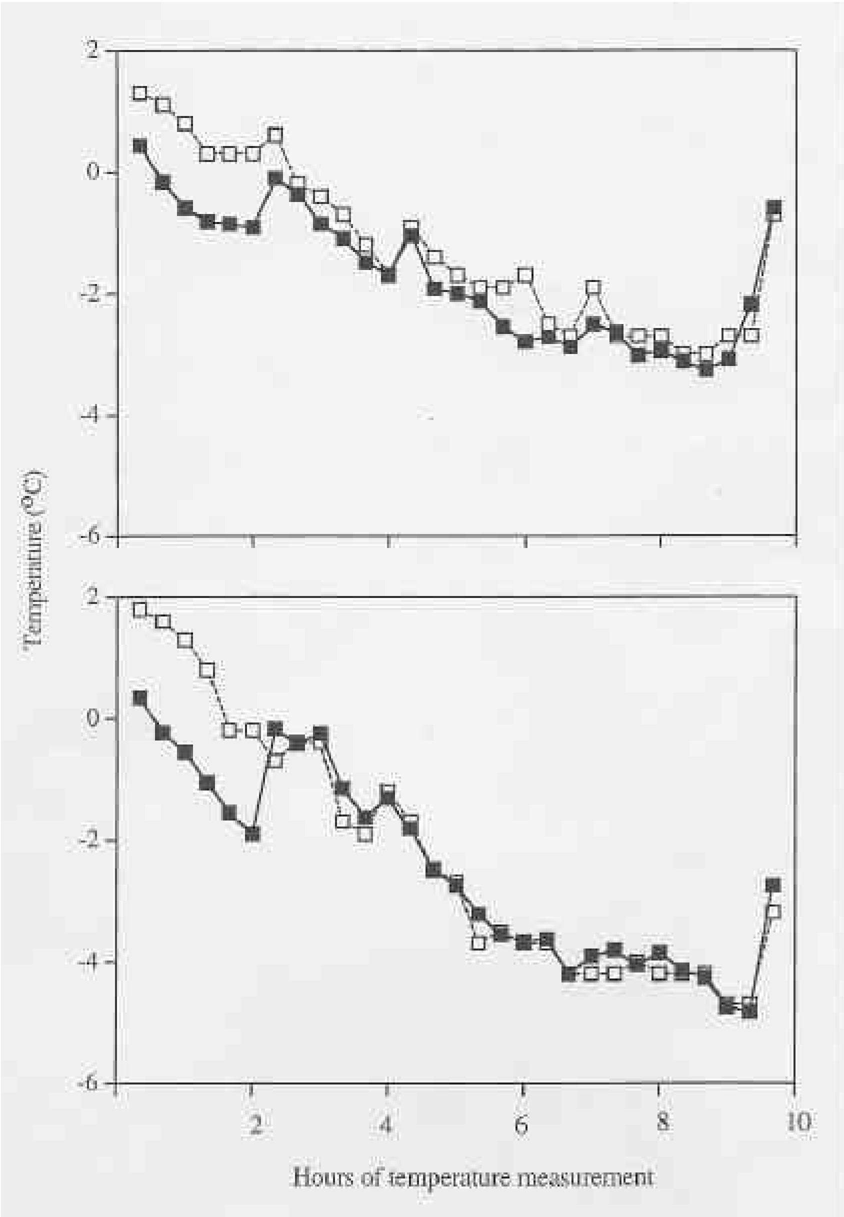

Fig 4 Temperatures at reference position (open symbols) and means for all

in-vineyard positions during two frosts at Meadowbank. Machine started at 2 h 20 min.

Distance from the machine and elevation were related to machine effectiveness in a highly significant (P<0.001) simple multiple regression model with an adjusted r^2 value of 0.90 (Fig 5). Sixty metres from the tower the measured mean temperature increase above estimated ambient was 2.87°C. The machine was least effective at the position one metre above the level of the tower base and 115 m distant, where there was a decrease in the estimated mean ambient temperature of 0.52°C.

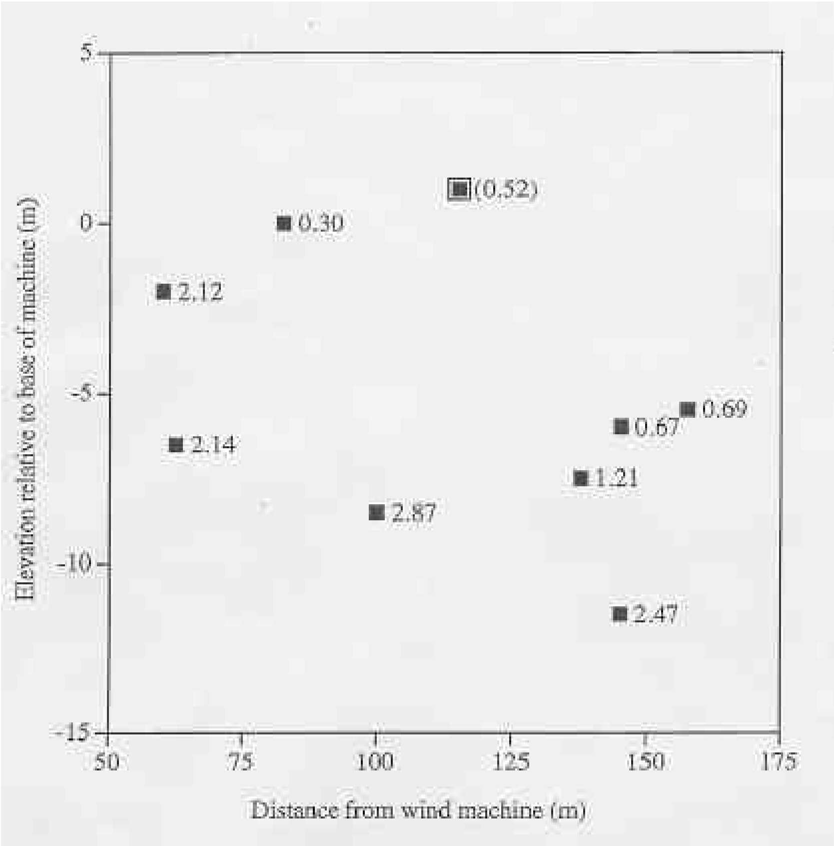

Fig. 5 Plot of data logger positions (distance from, and elevation relative to, base of machine tower), with position labels showing mean changes in temperature in response to machine operation.

Minimum temperature recorded at the base of the tower during the late spring frost was 0.7°C (with the machine running). Damage was confined to the lower slopes, as shown in Fig 2. At all elevations, vines within 100 m of the tower suffered only minimal damage. Outside the 100 m arc, vines in the north-west and northeast corners, suffered heavy damage to flowers and shoots.

Discussion

Springvale

With a nominal inversion strength of about 3.3 °C in the lower 15 m, a change of 30 to 50% of this figure in response to machine operation should have resulted in a rise in temperature of about 1 - 1.6 °C. In fact changes were markedly less over all positions, at around 0.2°C. Mid - slope on the north eastern side (MR), there was a decline in estimated temperature during machine operation. While this figure is cause for concern it is based on a relatively weak regression to obtain the base (machine not working) estimated figure, and may overestimate the negative effect of the machine. The grower reported that, after machine operation under regular frost conditions, this area of the vineyard appears to suffer most severe damage. Apart from this outlying figure, the response to the machine was contained within a very narrow range and from approximately 60 to 110 m from the machine there was no change in effect. Similarly there was no pattern of change with elevation or ambient air movement.

Overall, these small increases in temperature show that the machine was of little value in spite of the measured inversion strength which indicated sufficient accessible warm air to make the site suitable for wind machine operation. Ambient wind speed during the frost was not measured, but growers records indicate speeds about 2 m s^{-1} are common in frost conditions. This is well below the upper limit recommended by manufacturers and would not have been expected to almost totally negate the potential effect of the machine. Consequently, it appears that reduced machine efficiency associated with the modifications to the machine have created an installation which is of little value to the grower.

Because of the lack of overall machine effectiveness and of any spatial pattern in the small temperature increases observed detailed analysis of the effect of topography on machine operation were not carried out on this site.

Meadowbank

The main practical considerations in frost management using wind machines are the capacity of the machine to maintain temperatures above tissue damage levels, the area over which this can be achieved, and the distortion of the expected circular pattern by ambient airflow and topography. The present results indicate that on this sloping site, the effect of the machine was generally in accordance with previously published results and commercial experience. Average gains in temperature were within the range expected for the apparent strength of the inversion. Attenuation of the mixing effect was approximately linear with distance from the machine and useful gains in temperature were limited to about 100 m, the generally accepted radius of protection. There was distortion of the pattern down - slope as would perhaps be expected from published reports of wind effects on protected areas, assuming that the study area experiences some down - slope air movement under inversion conditions.

In spite of the accord between these results and previously published material, when the positive effects of the machine on in-vineyard temperature are considered in relation to the topographically related estimated ambient temperatures in undisturbed air a different picture emerges.

The gain in temperature above ambient is critically dependent on the inversion temperature profile as noted by McGann and McNaughton (1987) and others. On both frost nights (Fig 2) there was an initial rise in temperature when the machine was started and the mean in-vineyard temperature then closely followed the temperature recorded at the reference site. A weak regression between machine effectiveness and ambient temperature confirmed that there was no change in machine effectiveness with declining temperature. It follows that the slope of the inversion profiles also remained almost constant throughout the duration of the two frosts. There was however, an apparent difference between the two nights with the machine more effective in the second (colder) frost.

Using mean temperatures while the machine was not operating, at all elevations during the two frosts, there was an estimated temperature gradient in the lower inversion of 0.27°C / m. This gives an estimated mean difference in temperature between fruiting level and 15 m elevation of 3.89 °C. Thus, the gain in mean temperature of 1.33 °C with the machine operating was 34%, comparing favourably with reported gains of 20 to 50% of the temperature change in the lower 15 m of the inversion (McGann and McNaughton, 1987). These results highlight the distinction between the temperature gain due to mixing in the lower inversion and the actual in - canopy temperature. A relatively constant gain of 1.33 °C means that frost damage will occur when ambient declines below -3.33 °C regardless of machine operation.

The combined influence of elevation and distance from the tower base on the capacity of the machine to raise ambient temperature is given in Fig 5. The machine was most effective near the tower, with increasing distance resulting in a decreased effect, whilst effectiveness increased at lower elevations relative to the tower base. The distance component of the relationship aligns closely with Nanos et al. (1997) as the model suggested a maximum effective range of 100 m at the same elevation as the tower base. Also according to the model, at distances greater than 100 m or elevation more than 8 m above the tower base the machine may decrease ambient temperature. Clearly attenuation of the mixing effect is unlikely to follow the linear model at extreme distance. However the measured reduction in estimated ambient temperature at the most elevated position, 115 m from the tower, and increased frost damage at extreme range reported by Nanos et al. (1997), suggest that the predicted decrease in temperature is at least partially correct. Growers have also occasionally reported an apparent increase in frost damage at the margins of protected areas.

Although the model was based mostly on observations down - slope from the machine the predicted decrease in effectiveness up - slope could be anticipated. As the machine rotates about the vertical axis it will shift air from the same level and hence the same temperature in the inversion. Introduction of this air into relatively warmer air at higher elevations would result in net cooling. Conversely at successively lower elevations, air introduced from higher in the inversion, should provide larger increases in temperature than would result from mixing in the same plane. In contrast there is no obvious explanation for a decrease in temperature beyond 105 m from the tower. Reese and Gerber (1969) did note a pool of cold air in advance of the rotating jet. With reduced mixing at the greater distances in the present study, sufficient displaced or entrained cold air may have accumulated to result in a net reduction in air temperature.

From the regression model, effectiveness of the machine increased by 0.28°C / m down - slope from the base of the tower. As estimated ambient temperature decreased 0.26°C / m, with decreasing elevation, the corresponding increase in effectiveness of the machine at lower elevation was negated by decreasing ambient temperature. Thus although there was a down - slope increase in the machine effect on ambient temperature, the increase did not represent a down - slope distortion of the area of protection. Up - slope, according to the model, the machine provided no gain in temperature when elevation above the tower base exceeded about 8 m. Considering both the distance and elevation components, the up - slope radius of positive effect was substantially less than down - slope. As ambient temperature increased with elevation, this reduction in effect would not necessarily have been reflected in increased frost damage.

Overall, the results show that wind machine effectiveness expressed as gain in ambient temperature on a sloping site was distorted down - slope in a manner similar to what would have been expected if there were a down - slope wind. Unlike near level sites, ambient temperature on the slope changes with elevation and down - slope distortion of influence of the machine was not an accurate reflection of temperature during machine operation. The positive influence of the machine on temperatures down - slope was counterbalanced by lower ambient temperature at lower elevation and the resultant practical protective effect was simply related to the effective range of around 100 m. This was evident in the damage from the late spring frost, in which the up - slope limit to damage followed the -4 m contour, except where it was within 100 m of the wind machine. In this area, where vines at an elevation less than -4 m were within the effective range of the machine, damage was substantially reduced. On the eastern boundary, reduced damage outside the 100 m circle and below the -9 m contour appears to be due to a localised air drainage effect and not related to machine operation.

Inversion structure over a sloping site is difficult to measure and describe, involving multiple balloon soundings throughout the vineyard and adjacent area and a detailed analysis of the inversion was beyond the scope of this study. (The single sounding at Springvale was taken to confirm the presence of an inversion after the machine was found to have such a small effect on temperature.) The literature indicates that the achievable gain in temperature by the machine is directly related to the temperature differential in the inversion. Thus, a relatively constant gain in temperature throughout the hours of wind machine operation at all positions suggests that the rate of temperature increase with height did not alter significantly as the frosts developed overnight. This is consistent with the observation of Gopalkrishnan et al. (1997) that once established, the inversion layer continues to thicken during the night but the temperature gradient (strength) changes little. Whilst minimum temperature and inversion structure may change with soil heat and synoptic conditions the lack of change in structure overnight means that within any one frost event, effectiveness of the machine is unlikely change with declining temperature.

The specific results and relationships obtained here will clearly not be directly applicable at other sloping sites or with other machines, but the general principles should apply. On this sloping site there was a clear distinction between the effect of the machine on ambient temperature and an ability to maintain temperature above a critical level for tissue damage. The latter is the sum of the effect of the machine and ambient air temperature. On a flat site, machine effectiveness varies with inversion profile and distance from the machine whilst ambient temperature in undisturbed air is relatively constant. Machine effect on temperature is therefore a suitable guide to effectiveness for frost protection. In contrast, on a sloping site, the distance effect remains but both ambient temperature and machine effectiveness are influenced by elevation. The resultant capacity to prevent frost damage is therefore influenced by the same variables as normally operate on a flat site plus a complex slope or topography factor. Consequently guidelines for machine installation on flat land may lead to a substantial overestimation of the area under effective protection.

Conclusion

The above analysis and interpretation of the Meadowbank data was submitted for publication in the Australian Journal of Grape and Wine Research. Referees commented that more information was required on the spatial distribution of frost damage and more detail on the effect of the machine on temperatures particularly up - slope from the machine before the concepts put forward should be published in a scientific journal. One referee did however suggest immediate publication in a commercial journal and the editor has invited resubmission with further detail included. Clearly more work is required to confirm the picture for this site and perhaps check the application (in a qualitative sense at least) of the model to other sites particularly where frosts may be characterised by strong down - slope flows. The additional work required for on site confirmation is minimal and dataloggers will be placed in the vineyard in the coming (2001/2) season. If there is a frost it is anticipated that sufficient additional data to satisfy the referees suggestions can be obtained and publication early in 2002 is expected.

With regard to the objectives of the project, the results indicate that growers concerns with wind machines on sloping sites are valid. Of critical importance is the fact that the achievable increase in temperature must be considered along with the actual temperatures at various elevations in the vineyard. Specifically, the following points should be noted.

- Machines are limited to a temperature gain of 30 to 50% of the 1.5 – 15 m inversion gradient.
- Temperature gain is related to inversion gradient, not the actual temperatures in the inversion
- Effectiveness is attenuated with distance from the machine - maximum distance for a useful temperature increase is about 100m but probably greater with larger machines.
- Temperature modification by the machine is influenced by the slope, with greatest temperature increase down-slope and a possible decrease up-slope.
- Lower temperatures at lower elevation require greater machine effectiveness to obtain useful protection.
- The effect of slope (elevation) may mean that the area over which a useful change in temperature can be achieved is substantially less than it would be on a flat or near flat site.

Detailed on-site measurements giving the distribution of surface temperatures with elevation should allow growers to use existing wind machine specifications and installation guidelines to determine whether a machine would be effective and to decide on position and estimate effective protected area. It is anticipated that the simple model describing temperature responses to machine operation in this study will be published in 2002. With further work, it could be developed into a more complex analysis, applicable across a range of sites.

Spray-On frost protection

Introduction

Lack of consistent results with commercially available frost protectants and failure to commercialise published results showing other products to be effective in particular situations has made scientists justifiably sceptical about chemical or spray on protection and there has been little effort to clarify mechanisms. The general scientific view has been that spray - on materials either mimic natural tolerance mechanisms or simply establish a physical barrier preventing ice crystals from proliferating from the surface into sensitive tissue. The literature on acclimation to low temperatures is extensive, but much of the work has been on tolerance of dormant tissue to the low winter temperatures of continental Europe and North Amarica. The links between this body of work and damage to actively growing tissues under relatively mild frost conditions remain unclear.

Working with leaves of broadleaf evergreen species Rajashekar & Lafta (1996) associated cold acclimation with a number of cellular level changes, including cell wall structure. Cold acclimation was found to increase cell wall rigidity leading to greater turgor pressure and higher total water potential during extracellular freezing. Loss of turgor in leaves during freezing was attributed to cavitation within the cell wall structure. They also noted that in cultured cells of grapes and apple, cell wall pore sizes were reduced and cell wall breaking pressures increased in response to cold acclimation.

Sakai and Larcher (1987) suggested that increased intracellular sugar concentration would decrease osmotic potential and decrease dehydration by extracellular freezing. Changes in other soluble cell constituents have been noted with various authors reporting changes in amino acids and the frequently reported association between proline levels and various aspects of water stress. Once extracellular ice is formed the progressing ice/water interface produces a potential gradient between the ice and the cell contents. Water potential of the cell contents is primarily determined by the osmotic potential. As a result of the potential gradient, intra-cellular water diffuses through the semi-permeable plasma membrane to the extracellular ice at lower vapor pressure. The rate of diffusion of water to ice loci outside the cells is related to the water potential gradient and the permeability of the membrane. Thus, extracellular ice formation results in a process of freeze dehydration of cell protoplasm, with water from cells accumulating in intracellular spaces giving a flaccid water soaked appearance upon thawing.

Behaviour of the plasma membrane has also been reported to change with cold acclimation. Li and Palta (1979) discussed membrane consistency, and control of membrane transport systems. The plasma-membrane protein PM-ATPase was reported by Repo et al. (1997) as the first target of frost injury. This protein is responsible for maintaining intracellular ion concentration and functional change results in leakage of ions into extracellular spaces after exposure to freezing temperatures. The PM-ATPase has been found to recover, to an extent, from frost damage and during recovery it pumps the leaked ions from the apoplasm back into the cytoplasm.

Jones et al. (1999) pointed out that most studies on frost or freezing tolerance assume that a single component limits freezing injury and ignore potential interactions among metabolites, osmolytes and membrane effects in protecting the plant from freezing temperatures. Further, many of the reported changes in osmotic potential resulting from exogenous applications of osmolytes (glycine betaine and others) are much greater than would be expected from the amount or concentration of applied material. Successful demonstrations of spray - on frost protection using polymers and compatible solutes, almost certainly reflect changes in osmotic potential as suggested in previous work by the present author (Wilson and Jones, 1983). However this is probably an oversimplification and it has been suggested that some compatible solutes may also enhance membrane stability and Coughlan and Heber (1982) suggested that osmotic and membrane protection are both involved in induced protection mechanisms. Nuccio et al. (1998) expanded the view of naturally occurring osmoprotectants describing the small, nontoxic, molecules as lowering the osmotic potential of the cytoplasm without disrupting metabolism and with the additional functions of stabilising protein and membrane structures.

The major naturally occurring osmolytes found in micro - organisms and higher plants are non-reducing sugars (sucrose and trehalose), polyols (glycerol, sorbitol and mannitol), amino acids (glutamate and proline) quaternary ammonium compounds (e.g. glycine betaine) and tertiary sulfonium compounds. Several commercial products such as growth regulators, anti-transpirants, dormant oils and other materials of apparent cryoprotectant activity have been used to increase cold hardiness and/or delay bud break of horticultural crops. Although these chemicals have been promoted commercially, their effectiveness has been inconsistent, often having little or no effect in field trials. Dami et al. (1996) commented that the use of chemicals is an attractive method of frost protection because of the low cost and ease of application, but noted they may also pose a threat to the environment. Frost or freeze protection has been reported for sensitive plant tissue following exogenous application of all of the following materials:

Glycine Betaine

Coughlan and Heber (1982) found that glycine betaine ("Betaine") accumulates during water stress in a range of plant species. The exact mechanism for increased water stress tolerance remains unclear but betaine appears to play at least some role in osmoregulation. As the extracellular ice formation in freezing stress causes cell dehydration closely analogous to water stress, betaine may also have a cryoprotective role. Allard et al. (1998) found betaine accumulation was correlated with the development of freezing tolerance in wheat, with a three fold increase during winter acclimation. Zhao et al. (1992) suggested several physiological functions for this accumulated solute including membrane protection, enzyme protection and osmoregulation. Exogenous betaine application also resulted in an increase in total osmolality apparently mostly due to betaine accumulation in the cytoplasm.

Zhao et al. (1992) also reported that betaine was effective in protecting cabbage leaves from freezing injury at -25°C and that betaine was superior to sucrose as a cryoprotectant for spinach thylakoids. It was also found that when alfalfa plants were frozen at -6 °C, previously applied betaine reduced leakage of ions from shoot tissues. In a discussion of how betaine or proline might postpone damage or reduce injury from dehydration Kramer (1983) noted that concentrations are usually too low to contribute significantly to lowering osmotic potential and hence freezing point of vascular sap during osmotic adjustment. However, Allard et al. (1998) described betaine accumulation in different cellular compartments to adjust osmotic balance and increase stability of membrane and protein structure.

Glycine betaine is osmotically less active than sucrose when compared on a molal basis. Coughlan and Heber (1982) gave a possible explanation for the enhancement of thylakoid protection by betaine at low concentrations, as a weak interaction between the positive quaternary ammonium compound and the anionic carboxyl residues of exposed surface proteins. This caused an increase in stability either by direct membrane protection and/or by stabilization of the water layer surrounding the thylakoid. High betaine concentrations do not provide complete membrane protection because of the low cellular membrane permeability to betaine. It was suggested that if betaine does not penetrate into thylakoid vesicles, the inner thylakoid surface is not fully protected during the freeze thaw regime, leading to leakage of some components. If betaine influences membrane structure and function it may induce changes in symplasmic cation concentrations (probably K^+) which could account for larger osmotic potential changes than would be expected from the molal concentration of applied betaine.

High molecular weight surfactants.

A range of high molecular surfactant materials has been shown to reduce freeze injury in micro-organisms, tissue cultures of higher plants and whole plants. Himelrick et al. (1991) studied cryoprotectant effects of high molecular weight surfactant materials including DEPEG (Surfactant WK), Teric 12A23B and ethylene glycol on *Vitis labrusca*. It was found that all three chemicals reduced the low temperature exotherm between 3.5 and 5.5 °C. DEPEG and Teric 12A23B were shown to be superior to other products. All spray applications were to dormant vines, with pre-budburst (early spring) applications giving potentially useful reductions in freezing point in leaf tissue after budburst. Results were based on laboratory tests on actively growing tissue excised from field treated vines and were not confirmed with natural or simulated frosts.

Wilson and Jones (1980) found that DEPEG applied to flowering blackcurrants (*Ribes nigrum*), produced minimal phytotoxic symptoms, leaf water potential was decreased and abscission of flowers after exposure to freezing conditions was reduced. Similarly, Wilson and Jones (1983) reported that summer application of Teric 12A23B in low concentrations on blackcurrants lowered leaf water potential and a single early spring application gave protection of flowers down to -6 °C in the field. This reduction in freezing point was however greater than would have been expected from the observed change in water potential in spite of the fact that freezing point depression and leaf water potential measurements were made at different times of the year and on different tissue types. Sutcliffe (1977) calculated that a change in osmotic potential of -1.2MPa should result in a freezing point depression of -1 °C. Himelrick et al. (1991) also reported that, in dormant grapevine buds, freezing resistance of young leaves was improved by 4.1 °C with application of Teric 12A23B. Again this change appears to be in excess of what would be expected if the effect were based only on osmotic potential changes.

A commercial frost protectant "Frost Gard", containing a block polethoxy polymer, was shown by Anderson and Whitworth (1993) to delay freezing when infiltrated into strawberry leaves but there was no effect on intact plants. Several papers by Perry and co-workers, (eg. Perry et al., 1992) showed that this product (and several others) gave no useful field frost protection on a range of vegetable crops.

Seaweed Extract

Burchett et al. (1988) reported that a seaweed extract (Maxicrop) sprayed on winter barley increased frost tolerance. Wilson (unpublished data) also found small changes in leaf osmotic potential when *Vicia faba* leaves were treated with various seaweed extracts including food grade sodium alginate, but there was no discernable increase in frost tolerance in laboratory freezing tests.

Anti transpirants

Reiger (1989) reported that anti-transpirants including commercially available products Antistress and Envy, reduce desiccation related injury. However they had no effect on freeze survival or cold hardiness of developing peach fruits and young citrus trees. Anti-transpirants also increased mortality of almond and plum flowers after exposure to -4.4 °C. Commercially, Potassium Dextro-Lac (KDL) is recommended to complement antitranspirants, especially Envy (Anon 1999a and b), but no mode of action has been suggested and evidence supporting its use appears to be anecdotal. A suggested mode of action for antitranspirant films as frost protectants is prevention of surface ice acting as a nucleating agent for inter cellular water (Anderson and Whitworth, 1993), but the work by Perry and others referred to above failed to show any useful effect.

Control of Bacterial Ice Nucleation.

Apart from the much publicised trials on genetically engineered non-ice nucleating *Pseudomonas syringae* in California, there have been few reported successful trials involving modification of surface ice formation (Anon 1988 and 1990). Increased frost damage has been recorded following application of *Ps. syringae* by Arny et al. (1976). Unpublished work on citrus in Arizona (Caple pers. com. 1984) suggested that one of the effects of Teric 12A23B may be to modify ice nucleating behaviour by surface bacteria. There is also some anecdotal evidence that control of surface bacteria using antibiotics or copper fungicides might offer some frost protection but results have not been confirmed experimentally.

Materials and Methods

Plant material

Spray trials were carried out on Chardonnay, Pinot Noir, and Cabernet Sauvignon vines in the small research vineyard located at the Horticultural Research Centre at the University of Tasmania in Hobart and Bishops Rock Vineyard at Cranbrook on the east coast of Tasmania. For frost chamber studies, rooted Pinot Noir cuttings in 10 cm diameter pots were treated and transferred to a frost simulation chamber for tolerance assessment. Each of the treated plants had one vigorous, actively growing shoot, at the time of treatment.

Phytotoxicity

During spring, treatments were applied to individual shoots and leaves of similar size and phenological age by spraying to runoff using a small hand held domestic sprayer. Visual observations and sampling of leaves and/or stems was carried out three to seven days after spraying. Phytotoxicity of sprays and frost damage were assessed visually using an arbitrary scale from 0 (no damage) to 5 (all normally green tissue turned brown).

Measurement of critical temperatures

An electrical impedance method of measuring freeze injury was detailed by Grey and Isaacs (1991). It is based on the observation that the electrical resistance of a cell system is made up of apoplasmic and symplasmic components in parallel. Because membrane resistance is very high, a direct or low frequency alternating current through the tissue will flow mainly through the apoplasmic solution (Stout 1987). As frequency is increased the resistance of the membrane decreases and current flows both through apoplasmic and symplasmic tissue. At very high frequency, impedance of the membrane is effectively zero and the equivalent circuit reduces to apoplasmic and symplasmic resistance in parallel. Hayden (1969) noted that temperature damage to cell membranes is associated with a drop in impedance (complex resistance) at a critical temperature. In particular, low frequency resistance of living tissues is high whereas the resistance of dead or damaged tissue is low. Zhang and Willison (1992) and Stout (1987) attributed the reduced impedance of tissue after freezing to membrane damage resulting in electrolyte leakage during the freezing processes. Stout (1987) suggested the decrease is larger at lower frequencies, and the effect of freezing injury is typically evaluated using low frequency or by temperature related changes the ratio of low to high frequency impedance measured on the same tissue.

During freezing, impedance increases as a result of intercellular ice formation. While electrolyte leakage may occur at ice formation, Zhang and Willison (1992) suggested it is undetectable in the frozen tissue because the increase with ice formation overrides any decrease associated with the increase in apoplastic ion concentration. In the present study this increase in impedance with ice formation was used to determine the start of intracellular freezing as tissue was cooled.

Leaf and shoot samples were gathered before 8.00 a.m., sealed immediately into self sealing plastic bags and held at 2 °C until required. For determination of freezing point, shoots were defoliated and inserted into a jacketed glass cylindrical sample chamber, 10 mm in diameter by 60 mm long. Coolant was pumped through the jacket to control temperature and actual temperature inside the chamber was monitored with an electronic (thermistor) thermometer. The chamber was then cooled to –0.2 °C and allowed 5 minutes to equilibrate before ice crystals were introduced to act as a nucleation source and the cooling treatment commenced.

A non-polarizing Ag/AgCl electrode in liquid (water) contact with plant tissue was used for impedance measurement. The electrode consisted of 40 mm of silver wire with most of the length electrolytically coated with KCI, then inserted into plastic capillary tubing containing agar and wrapped with Parafilm at the electrical connection end. Electrodes were inserted into sample cups containing water at each end of the excised shoot and current was measured at frequencies of 100, 1,000 10,000 and 100,000 Hz. Preliminary experiments showed that electrode resistance remained stable and junction potential errors were minimal. Zhang and Willison (1991) reported that total measured impedance was linearly related to inter-electrode spacing and, the excised defoliated shoots were cut to 100 mm length and inter-electrode spacing kept constant.

Electrodes were connected to a low frequency, sine and square wave signal generator and current measured at 70 and 7000Hz. Temperature was reduced at a rate of 1 °C every five minutes and the impedance of the shoot and sample chamber temperature recorded every minute.

Osmotic potentials

Osmotic potentials were measured on thawed leaf tissue after the psychrometric method described by Turner (1981). Immature leaves were excised and frozen at -18 °C in sealed containers. Tissue was then transferred to the sample chambers of a Decagon Devices model SC-1OA Thermocouple Psychrometer and allowed to thaw and equilibrate for 2 h before water potentials were read using a Decagon Devices NT-3 nanovoltmeter calibrated against an NaCl dilution series. As noted by Turner this method overestimates osmotic potential due to mixing of symplast and apoplast solutions.

Simulated frosts

A frost simulation chamber, owned by the Tasmanian Department of Primary Industries, Water and Environment was used to test effects of treatments on potted vines. The chamber was built in a standard 3 x 3 m coolroom. An upper chamber operating at a constant -20 °C is separated from the lower (working) chamber by a blackened ceiling containing a heating mat. The ceiling provides a temperature controlled "sky" absorbing radiation from the contents and floor of the lower chamber. Frost temperature is controlled by a variable level thermistor in the lower chamber, and rate of cooling, minimum temperature levels and duration and rewarming rates can be programmed and controlled for a selected level. The chamber establishes a true inversion frost with temperature gradient controlled by the "sky" ceiling temperature.

Experimental details

Expt 1 Phytotoxicity. All experiments included an assessment of phytotoxicity but available spray materials were initially screened and those showing significant phytotoxicity were deleted from further experiments. Concentrations used throughout were based on commercial recommendation and the range of concentrations quoted in scientific publications. Immature shoots were selected from Chardonnay and Pinot Noir vines and sprayed to runoff with one of the following treatments.

Control - Unsprayed
Teric 12A23B 0.25%(W/V)
Betaine 4 and 25 μM
Anti-stress 2%(V/V)
Envy 5%(V/V)
Antistress 2% plus 3.2% Potassium Dextro Lac (KDL)
Envy 5% plus 3.2% Potassium Dextro Lac (KDL)
Seasol 0.4% (V/V)

Sodium alginate 0.05% (W/V)
KDL 3.2% (W/V)
Visual assessments were made every 2 days following spraying, and any symptoms of phytotoxicity noted. Two weeks after spraying, damage was visually graded as noted above. The design was completely random using three replicates (single shoot plots) of each treatment, on each variety.

Expt 2 Effects of non phytotoxic spray treatments on osmotic potential. Sprays understood to be acting as osmolytes and which produced no phytotoxic symptoms in experiment 1 were applied to immature shoots of Cabernet Sauvignon. Additional higher concentrations of Seasol and betaine were included.

Control — Unsprayed
Seasol 0.4%
Seasol 0.8%
Sodium alginate 0.05%
Betaine 4 μM
Betaine 6 μM

The design was completely random with three replicates of single shoot plots. Five, 9 and 12 days after spraying, the last fully expanded leaf on each treated shoot was removed for measurement of osmotic potential as described.

Expt 3 Tissue critical temperatures. The treatments listed in Expt 2 plus Antistress and Envy at recommended rates (Expt 1) were applied to individual shoots of Cabernet Sauvignon. Shoots were excised four days after spraying and leaves removed before critical temperature determination using the method already described.

Expt 4 Field evaluation of protectants. Seasol, Antistress, Envy, Betaine and a water sprayed control were applied at the rates noted in Expts 1&2 on one year old vines in Bishops Rock Vineyard the night before a mild frost in early December. Minimum temperature at 0.7 m in the trial area was -2 ^{o}C. Damage was assessed 10 days later using the scale described above for phytotoxicity.

Expts 5&6 Effects of sprays under simulated frosts. Pot grown vines were sprayed with the following treatments: Envy and Antistress at commercially recommended rates, Teric 12A23B at 0.02 and 0.05% (w/v), Betaine at 4 μM, a mixture of Betaine at 4 μM and 12A23B at 0.02%. Teric 12A23B was reintroduced into trials at this stage after confirmation of ongoing commercial use on *R. nigrum* in New Zealand, but at a lower concentration (0.02%) than that which caused severe phytotoxicity in the initial experiment. This concentration is the lowest to be found effective on blackcurrants as reported by Wilson and Jones (1983)

Four days after treatment in Expt 5, vines were transferred to the frost simulation chamber and the temperature allowed to fall to -6°C at the top of the vines at a rate of approximately 1°C/h, before rewarming to above freezing at the same rate. In Expt 6, vines were also transferred to the frost simulation chamber on day 4. The temperature was allowed to fall to -4° C and return to 0° C at the same rate as in Expt 4. Damage was assessed and ranked as described above. Vines were also assessed for return growth after damage measured as number and length of new shoots 6 weeks after frost treatment.

Both experiments were the same design being five replicates of randomised complete blocks, blocked according to position in the frost simulation chamber.

Results

Expt 1. Results were similar across all replicates and both varieties and no statistical analysis was necessary. The higher concentration of betaine, Teric 12A23B, and all sprays containing KDL produced moderate to severe phytotoxicity but there was no apparent damage with the low concentration of betaine, seaweed extract, sodium alginate or either of the surface film commercial treatments.

Spray	Phytotoxicity
Seasol	0
Antistress	0
Envy	0
Alginate	0
Betaine (0.05 g/l)	0
Betaine (2.92 g/l)	3.5
Teric	2.5
Envy/KDL	2
Antistress/KDL	3
KDL	3
Control	0

Expt 2 Three days after treatment there was no change in osmotic potential in response to treatment and results are not shown. However after 9 days there was a significant treatment effect and the following table shows means for measurements taken at 9 and 12 days. There was a significant ($P<0.01$) effect of treatment on osmotic potential with betaine (3μM) and sodium alginate both lower than the control.

Spray	Osmotic potential (MPa)
Seasol (0.4%)	-1.46
Seasol (0.8%)	-1.57
Alginate (0.05%	-1.60
Betaine 3 µM	-1.79
Betaine 6 µM	-1.47
Control	-1.51
LSD (P<0.05)	0.07

Expt 3 Electrical impedance of stem tissue remained near constant as temperature declined before increasing markedly at freezing point in some samples whilst there was only a small and indistinct change in others. Exotherms were not evident in all cooling runs possibly due to variable contact between thermistor and tissue, but exotherms temperatures invariably co-incided with an increase in electrical impedance. With freezing point measured as exotherm temperature and/or the change in impedance, there was a significant effect of treatment on critical temperatures. All of the osmolyte treatments produced significant reductions in freezing points compared with the control. Antistress and Envy had no effect on critical temperature.

Expt 4 Under the relatively mild frost conditions in this field trial, there was little apparent damage on the unsprayed controls and no treatment effects were evident, as shown below. There were no significant differences between treatments ($P>0.05$) and results are not shown.

Spray	Freezing Point (°C)
Betaine (0.3μM)	-5.60
Alginate	-5.50
Seasol	-6.10
Envy	-3.00
Antistress	-3.43
Control	-3.67
LSD (P<0.05)	0.33

Expts 5 and 6 At minimum temperatures of both -4 and -6°C damage was severe (rank 5) across all treatments and replicates in both trials and there were no significant treatment or block effects on either assessed damage or return growth (results are not shown).

Discussion

Overall the results confirm existing uncertainty with spray-on type protectants and align closely with the common finding that laboratory results do not translate to field or simulated frosts. In simulated frosts in the present study, spray treatments afforded no apparent protection, but temperatures may have been below the practical limit for whole plant protection. This result is in spite of significant changes in both water potential and freezing temperature of excised tissue. Marked changes in mean freezing temperatures of excised tissue in response to exogenous osmolytes in the present study were consistent with previously published work by Himelrick (1991). The synthetic surfactant, 12A23B, found to be most effective by Himelrick (ibid) on grapes and on *Ribes nigrum* by Wilson and Jones (1983b), was phytotoxic when applied to actively growing tissue. Himelrick (1991) avoided the phytotoxicity problem by spraying whilst vines were still dormant and obtained a useful gain in frost tolerance measured *in-vitro* after bud burst and during early growth. Reduced solution concentration in the present study succeded in avoiding phytoxicity but had no effect on freezing behaviour in whole plants.

The application of high molecular weight surfactants to dormant tissue has resulted in a small protective effect in other species. Working in Oregon in the late 1970's, D.O. Ketchie applied DEPEG (see Wilson and Jones, 1980) to dormant apple trees and obtained a useful protective effect in field trials, but the response was not persued commercially.

Failure of the present treatments in the higher temperature (-4°C) simulated frost need not indicate lack of field effect. The reduction in freezing point was around 2°C in response to osmolyte treatment. In the trial reported, -4 °C was chosen as the temperature most likely to discriminate between osmolyte and untreated or film protectant treated vines. In earlier studies, Wilson and Jones (1983a and b) and Himelrick (1991) both found that untreated tissue supercooled in laboratory studies giving freeze initiation (exotherm) temperatures several degrees below intact tissue. If however in this study, damage to controls occurred at the nominal damage temperature of -2 °C (Kalma, 1992), the gain due to treatment may have been just below the 2 °C required to avoid damage at -4 °C. The temperature in the field trial on young vines did not fall low enough to initiate damage on untreated vines. Consequently it appears that if there was a commercially useful effect of the applied osmolytes, the trials reported may have included temperatures just outside the effective range.

At a more theoretical level the results confirm that reported gains in freeze tolerance cannot be explained by changes in osmotic potential alone. Using the general formula suggested by Sutcliffe (1977), the measured decrease in osmotic potential for betaine treated vines shifts the freezing point from -1.2 °C to -1.5 °C. Indeed the observed change of around 2 °C in excised tissue requires a change in osmotic potential of approximately 2.4 MPa. This is clearly excessive. The relatively small changes in osmotic potential observed both here and by Wilson and Jones (1983a) and the tendency for excised tissue to supercool observed here and by Himmelrick (1991) suggest a more complex mechanism than originally suggested by the former authors. In the present study there was not a strong relationship between osmotic potential and freezing point, but results particularly for osmotic potential, were inexplicably variable and variability in both osmotic potential and freezing temperature may have masked any relationship.

The delay of several days from spray application to the change in osmotic potential also corresponds with observations by Wilson and Jones (1983 b and unpublished data), that both the adjustment in osmotic potential and development of frost tolerance in *Ribes nigrum* took several days to develop and stabilise. These workers also noted that high concentrations or repeat applications appeared to diminish the protective effect. This suggests an acclimation process and may account for failure of some of these materials to give positive results in field trials. That is, freezing treatments may have been applied too soon after spray treatment before tolerance had developed. A feature of most of the published studies (cited above) showing that exogenous osmolytes are not effective, particularly in field studies, is that materials have been applied within 24 h of a damaging frost and treatments have often been repeated in the same trials. These time and concentration effects appear to be factors previously unrecognised in studies evaluating these products and may account for some of the conflicting and confusing results. Concerns about lack of attention to this aspect of chemical frost management were also raised in a recent communication on "Vaporgard" (a film type protectant) claiming that this material increased physiological freeze tolerance in addition to a nucleation barrier effect (Sansermino pers. Com. 2001). With rapidly growing grape vines, the difficulty of balancing time for protection to develop against dilution as tissues expand may however mean that successful protection using materials of this type is not achievable.

The lack of any effect of Antistress and Envy in the laboratory study is as expected, as neither of the two film-forming products were likely to decrease freezing point in the excised stem tissue. In the simulation study, failure to protect below -4 °C could simply indicate that the limit of their effectiveness was higher than the temperature used. Effectiveness, or otherwise, of these two materials remains to be demonstrated.

Conclusion

The results underline the confusion in the literature on the practical value of spray on frost protectants. The present results provide independent confirmation of the observations by Himelrick (1991) that (for vines) applied osmolytes will alter freezing characteristics of excised tissue. The results also confirm that the response cannot be explained only by an osmotic effect and that a more complex mechanism is indicated. However the difficulty or unreliability in extending laboratory results to a practical field situation continues to frustrate. If there is a practical (field) gain in freezing temperature, the present results suggest that it will be less than 2 °C. While this is small it compares favourably with the gain estimated for wind machines in this and other studies, and protection would be at a lower cost and more uniform across sites.

Further work is clearly needed to relate laboratory results on excised tissue to whole plants in simulated or field frosts. To extend this to a useful field treatment will then require further work to determine the delay from initial application to the development of tolerance and the persistence of treatment. Aspects of the present results and published work suggest that reasons for lack of consistent field effects include a failure to consider that tolerance may take time to develop and that multiple applications may exceed a concentration threshold for effective protection. This then diminishes the commercial value, as application of spray in response to a frost forecast may not be an option. That is, growers in marginal frost areas opting for spray on protection may need to spray on a routine rather than an "as required" basis.

As indicated in the grant application, this study was speculative, but the literature review and experimental results do indicate that with further research, spray on frost protection may be a useful alternative to engineering solutions to frost incidence. Because of the low cost and potential for large scale protection, further work is recommended, with the following issues taken into account:

- Tissue or cellular level changes in response to osmolyte application require clarification. While osmotic potential changes appear to partly explain the increase in freezing tolerance, other factors such as membrane structure, function or stability should be considered in more detail.

- Both field/simulation and tissue/cellular level studies should include the time for adaptive changes to take place and the upper and lower concentration limits over which treatment is effective.

- A gain in frost tolerance is probably less than 2^{o}C, thus field and simulation studies need to concentrate on field frost temperatures between -2 and -4 ^{o}C.

Technology has changed in recent years and MIFE and other membrane physiology tools may allow a better understanding of the influence of osmolytes like betaine, alginate and the synthetic surfactants on cell membranes. If such influences, speculated on in the past, can be confirmed and related to frost tolerance, a better physiological basis may be established for a more objective evaluation of frost protectants. Alternatively, field trials need to recognise that spray concentrations may be critical, gains will probably be limited to less than 2^{o}C and that the tolerance to frost may take some time to develop after spray treatment.

Disclaimer

Results contained herein should not be used as recommendation for any of the products mentioned. The study was designed as a preliminary investigation and should be treated as such.

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