Impact of frost injury incidence at nodes of Pinot Noir on fruitfulness and growth-stage lag

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Short Running Title: Effect of frost injury on Pinot Noir

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Abstract

Background and Aims~~:~~ ~~An early s~~ Spring frost~~ events~~s can injure primary buds and young shoots and stimulate secondary shoot production in *Vitis* spp. The aim of this study was to develop ~~efficient~~ methods to quantify yield and phenology effects of frost injury during budburst.

Methods and Results: Eight hundred and sixty-nine~~A total of 874~~ ~~869~~ nodes from 92 half-vines of Pinot Noir in eight blocks from four Tasmanian vineyards were sampled; 15–92% of shoots per half-vine were injured after a sub-zero air temperature~~s~~ ≥ -4.5°C. Severity of f~~F~~rost injury ~~severity~~ was spatially variable among vines both with and without frost protection. Generalised linear mixed models revealed that node injury was associated with a mean 27-fold increase in the odds of > 1 shoot per node. Mean December scores for modified Eichorn-Lorenz growth stage were 18.9 and 17.2 for nodes with one shoot and > 1 shoot, respectively. The probability of healthy and injured nodes producing fruit was 0.81 and 0.69, respectively. In a season with poor fruit~~-~~set, the estimated difference in yield per linear m of row between 0 and 100% incidence of injured nodes was 0.2 kg.

Conclusion: Assessment of the incidence of frost injury and fruit ~~weight~~ mass per node was sufficient to estimate the impact of injury on yield at the vine and block-level.

Significance of the Study: Future studies are expected to benefit from application of these efficient sampling, assessment and statistical methods to determine the site-specific impact of early spring frost injury on fruitfulness and growth-stage lag.

Keywords: *damage, grape, mitigation, spatial variation,* Vitis vinifera

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Introduction

Vitis vinifera L. ~~wine and table grapes~~vines in many regions of the world ~~experience injury associated with~~are damaged by frost ~~events~~ during ~~budbreak~~ budburst, when air temperature~~s~~ ~~are~~ is sub-zero but rarely below -5°C. Injury ~~has been associated with~~is due to extra-cellular freezing of tissue water, ~~and~~ the withdrawal of water from the cells and subsequent dehydration of the cytoplasm (Kalma et al. 1992).

The cost of spring frost events across s~~S~~outh-e ~~E~~astern Australia's wine regions in the 2006/07 season was estimated at A$~~UD~~ 140 million (Barlow 2010). Moreover, an ~~extension of the '~~increase in frost ~~season'~~ occurrence has become evident in some locations in Australia with recent changes in climate (Crimp et al. 2016). The extent to which crop yield is reduced will depend on the proportion of ~~N+2 or~~ primary -buds (N+2 buds) injured and the productivity of ~~secondary shoots~~~~N+3,~~-buds (N+3$_n$ buds) -producing secondary shoots (Kasimatis and Kissler 1974, Lavee and May 1997, Friend et al. 2011). ~~There is limited evidence that fruit from primary buds not injured during a spring frost mature earlier than fruit from injured buds (Lider 1965). Injury has been associated with extra-cellular freezing of tissue water, and the withdrawal of water from the cells and subsequent dehydration of the cytoplasm (Kalma et al. 1992).~~

~~The occurrence of f~~Frost injury depends on ~~a~~ complex interactions between plant ~~genetic~~ and environmental factors. Critical temperature is defined as the highest temperature at the surface of a plant organ (e.g. bud) at which injury can be detected after exposure to that temperature for at least 30 min (Young 1966, Johnson and Howell 1981). ~~For p~~Practically ~~implementation of frost protection~~ly, a temperature of -2°C is ~~often~~ usually selected as the critical temperature for injury of non-dormant grapevine tissues during spring (Barlow~~,~~ 2010). In the field, bud surface temperature~~s~~ may vary from the temperature of the adjacent air (e.g. Leuning and Cremer 1988), particularly at low humidity~~ies~~. Other variables affect critical

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temperature, including vine cultivar, surface and soil moisture, the presence and height of cover crops, cultivation, vine root temperature, quantities of ice-nucleating bacteria, and, ~~critically~~most importantly, the stage of vine phenological development (Johnson and Howell 1981, Gardea 1987, Luisetti et al. 1991, Fuller and Telli 1999, Trought et al. 1999, Snyder 2001, Sun et al. 2017).

The region in this study, eastern Tasmania, has a cool-temperate maritime climate with mild winters. Pinot Noir is the most common grape ~~variety~~ cultivar grown in the seven recognised production areas, which differ ~~with~~ significantly ~~variation~~ in climate and soil ~~conditions~~ (Kidd 2014, Webb et al. 2018). Vineyard sites in Tasmania vary from high to low risk for frost injury during spring, with frost risk delineated at a spatial resolution of 80 m (Jones et al. 2010, Webb et al. 2018). Spring temperature~~s~~ as low as -5°C at fruiting wire height have been recorded (Wilson 2001). Overhead irrigation is the most common form of frost protection at high-risk sites where water is available at an acceptable price and where the structure of the cold air inversion limits the effectiveness of frost fans (Snyder 2001).

~~Quantitative r~~Research on the spatial and temporal response of vines ~~within each management unit of~~within vineyards ~~a vineyard~~ to frost injury ~~in spring~~ is necessarily opportunistic; it depends on having accurate local temperature measurements and being able to mobilise ~~field staff quickly~~~~resources~~labour in time to assess ~~the impact once an event happens~~frost damage. Jones et al. (2010) assessed pruning treatments for the recovery of frost-injured Pinot Noir vines in the Coal Valley of Tasmania after multiple frosts in October 2006; however, on-site temperature~~s~~ during each event and subsequent injury prior to pruning treatments were not ~~monitored or assess~~measured. Local records suggest temperature~~s~~ fell below -2°C during these events. A larger dataset on the effect of a frost event on components of grapevine yield is needed to establish the combinations of conditions associated with frost injury~~, and, equally importantly, those conditions when no~~ , or the absence of injury ~~occurs~~.

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Standard methods to assess frost injury in *Vitis* spp. ~~appear to be absent from the refereed literature~~have not been published.

The aim of this study was to develop efficient methods to ~~investigate~~ assess the effects of early spring frost events in eight blocks of Pinot Noir vines at four vineyards in Tasmania. The first objective was to develop and evaluate a method to assess spatial variation in the incidence and severity of injury for frost events during budburst. The second objective was to identify one or more variables able to predict the impact of injury ~~by investigating the relationship between various measures of injury incidence and severity and the following response variables: modified~~ based on: Eichorn-Lorenz (E-L) growth stage (Coombe 1995), number of shoots per node, and components of yield at nodes and on shoots of individual vines. In this context, a node is equivalent to a dormant (latent) compound bud containing a cluster of primordia (Lavee and May 1997). The third objective was to simulate the effect of varying incidences of frost injury at nodes by estimating the resulting average yield (kg) per node, per vine, per linear m of row and per ha. The resulting set of methods integrates a sampling strategy, a method for injury assessment, and a statistical modelling approach.

Materials and methods

Vineyard attributes and sampling method

Four commercial vineyards in three growing regions of Tasmania were sampled during the 2013/14 growing season ~~, with viticultural practices detailed in~~ (Table 1). All vines were cane-~~-~~pruned and drip irrigated. The inter-row vegetation was mown short and the area under the vines was bare ground ~~(post~~after herbicide treatment~~)~~. All viticultural interventions were determined by the grower co-operator, including the timing and method of frost protection.

A block of vines within each vineyard was defined as a ~~discrete~~ contiguous area that received a single, tailored management program (Table 2, Figure 1). Vineyard blocks were

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Alternative wording:
Each vineyard site was managed by the co-operating wine business who applied their standard viticultural practices, including the timing…

Commented [T H13]: Table 2, Figure 1 sampled using a grid pattern, selecting the central vine in every fifth panel of every fifth row, leaving a margin of two rows and panels on all sides of each block to avoid edge effects. In blocks < 0.25 ha, the number of rows between samples was reduced to four, and the number of panels between sample panels was reduced to three or four.

Environmental conditions

Environmental data were collected to show that frosts occurred and to describe some features of the frosts; it was not the objective of this study to describe in detail the physical attributes of the frosts. Air temperature and relative humidity at 1.5 m above the ground on a vineyard headland was recorded every 15 min using a CS215-L3m sensor (Campbell Scientific Australia ~~Pty Ltd~~, Garbutt, Qld, Australia) housed in a mini screen and connected to a Campbell Scientific CR800 datalogger (locations indicated in Figure 1). The positioning of sensors on a vineyard headland removes any influence of structures, such as buildings or vines, and provides some consistency among sites in terms of standardised set up and positioning (Beresford and Spink 1992). Temperature data were also recorded at a second location at vineyard B (Figure 1) using a SHT75 temperature/~~relative humidity~~RH sensor at 1.5 m connected to Libelium Waspmote technology (Libelium Comunicaciones Distribuidas ~~S.L.~~, Zaragoza, Spain), and with a sampling frequency of 1 min. These data were used to derive: (i~~1~~) the minimum air temperature recorded at a given location during each frost event;~~,~~ (ii~~2~~) the time from the first record of a sub-zero temperature to the minimum temperature recorded each night;~~,~~ and (iii~~3~~) the range in ~~relative humidities~~RH during sub-zero temperature events~~s~~.

Sub-zero temperatures= events were recorded in all four vineyards on four consecutive nights in September 2013 (Table 3). ~~T~~A temperature~~s~~ ≤ -2.0°C ~~were~~ was recorded on the first night (12 September ~~12~~) at all vineyards, with 2.75–6.25 h between the first record of a sub-zero temperature and the lowest recorded temperature. The lowest recorded temperature

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during the four-night period was -4.5°C at vineyard A on 13September~~ 13,~~ 2013. During this event, there was at least 9 h between the first record of a sub-zero temperature and the lowest recorded temperature.

The Campbell Scientific sensors at vineyard B were exposed to overhead irrigation during frost protection, indicated by ~~relative humidities~~ a RH of 99.2–100% and a temperature~~s~~ that were higher than those recorded by the Libelium datalogger (Table 3). Relative ~~humidities~~ humidity recorded during sub-zero temperature~~s~~ at other locations were > 84% (Table 3) and the minimum ~~relative humidity~~RH recorded at any site for the period 11–15 September ~~11-15~~ was 43% (Table 3).

Assessment of frost injury, crop phenology and yield components

Commencing approximately 2 weeks after the frost events (Table 2), injury was assessed on all shoots emerging from each node on one cane on the most northerly side of the trunk of each vine sampled. ~~Nodes were identified by number in ascending order from the trunk.~~ For the purpose of this study, a node was classified as a primary-shoot node if a single shoot emerged and a secondary-shoot node if there were two or three shoots. It was assumed that multiple shoots emerging from a secondary node were from $N+3_n$ buds (Lavee and May 1997); however, the precise origin of a shoot from within a compound bud was unknown.

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One thousand seven hundred and ninety-two~~A total of 1792~~ shoots were sampled from 1132 nodes in 9 vineyard blocks 14–36 days after the last frost event. Yield data from one of these blocks ~~was~~ were removed prior to analysis because a physiological disorder was evident on most bunches during the pre-harvest period. This disorder appeared to be unrelated to the frost events. Data from single rows in two other blocks were also removed prior to analyses because grape bunches had been harvested before collection of samples for this study. The final data set comprised ~~1401~~ 1394 shoots from ~~874~~ 869 nodes in 8 vineyard blocks (Table 2).

Each shoot on each node was assigned a modified E-L score (Coombe 1995), and an ordinal score (0–4) for frost injury (Figure 2). The E-L scores were assessed again in December during the pre-flowering or flowering period.

The incidence of frost injury refers to the presence or absence of any injury to plant tissues per shoot or per node, expressed as a ~~percentage~~ proportion of the number of samples and calculated from the severity data. The severity of frost injury per node was expressed as the maximum score for frost injury recorded among all shoots growing from that node. Shoots given a score of 1 for frost injury (trace injury) were included in calculations of frost injury incidence even though the appearance of some of these shoots suggested that shoot growth would proceed similarly to those given a score of 0.

At harvest, the ~~weight~~ mass ~~for~~ of each bunch on each shoot of the sample nodes was measured to one decimal place ~~with the aid of~~using digital scales ~~displaying one decimal place~~ (Soehnle, Supertex Industries ~~Pty Ltd~~, Silverwater, NSW, Australia). These data were used to calculate the total bunch ~~weight~~ mass per node or shoot, and mean bunch ~~weight~~ mass from bunch counts. Nodes were categorised according to the presence or absence of fruit. Nodes were also allocated to categories for maximum frost injury severity (0, 1, 2, 3 or 4~~) for the~~) to calculat~~ione~~ the ~~percentage~~proportion of nodes producing more than one~~>1~~ shoot in each frost severity category. ~~Nodes were also characterised as producing fruit (or not) according to the variable 'total bunch weight per node > 0 g'.~~

More detailed measurements were taken from one fruit-bearing shoot on a primary-shoot node and one fruit-bearing shoot from a secondary-shoot node from each sample vine where both types of node occurred. Bunches on these shoots were weighed and the number of berries per bunch counted to calculate the total number of berries per shoot, mean berry number per bunch (per shoot), and mean berry ~~weight~~ mass per shoot.

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~~Analyses of a~~Associations between frost injury and yield components

Spatial variation in frost injury was visualised using ArcMap 10.1 (ESRI, ~~1999–2012~~Redlands, CA, USA). The position of each half vine was plotted, along with the corresponding values of each of two variables: (i~~a~~) the median value of the maximum frost injury score per node;~~,~~ and (ii~~b~~) the proportion of nodes that produced fruit.

~~Spatial variation in frost injury was visualised by plotting the median for each half-vine of each node's maximum score for frost injury against the proportion of nodes per half-vine that produced fruit, using ArcMap 10.1 (ESRI, 1999–2012).~~

Given that dormant bud number is used by viticulturists to estimate potential yield, the node-level relationships between frost injury and a range of phenology and yield component variables were investigated through a series of generalised linear mixed models (GLMMs) (Pinheiro and Bates 2000, Zuur et al. 2009). The GLMMs were used to accommodate: (i~~1~~) non-Gaussian observations (counts and binary observations);~~,~~ ~~2~~(ii) the nesting of experimental units (nodes within vines within rows within blocks within vineyards);~~,~~ and (iii~~3~~) heteroscedasticity in the dependent and independent variables.

To investigate the relationship between frost injury per node and the production, or not, of more than one shoot per node (primary-shoot or secondary-shoot node status), two Bernoulli ~~generalised linear mixed models (~~GLMMs~~)~~ were generated and a logit link function fitted to the data. The first model accounted for variance among nodes in production of more than one shoot (or not) as a function of the severity of the frost injury, indicated by the maximum frost injury score per node. The second model described co-variation between the incidence of frost injury and the response variable. The models, if significant, were then compared in terms of the amount of variance accounted for in the response variable.

A series of GLMMs were then constructed for the dependent variables per node or per shoot as listed in Table 4. The distribution assumed for each of these GLMMs was dependent

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on the type of outcome: (i~~1~~) continuous variables were fitted with a Gaussian model, unless residual analysis indicated heteroscedasticity; if so, random variation was described using a gamma model;~~,~~ ~~2~~(ii) variables that were counts were fitted with a Poisson model;~~,~~ and (iii~~3~~) binary variables were fitted with a Bernoulli model. Three models were constructed for each dependent variable, each with one of the following independent variables: maximum frost injury score per node, incidence of frost injury, or node status (secondary shoot ~~production~~presence~~,~~ or ~~not~~absence). A binomial GLMM was also used to investigate the effect of frost injury severity on the ~~percentage~~proportion of nodes producing > 1 shoot for each category of frost injury severity. If the overall test was significant, then the different frost injury categories were separated using Tukey's HSD post-hoc test.

In all models, vineyard, block, row, and vine were included as random variables. As above, these sets of models allowed inferences to be made on the relative usefulness of severity or incidence of frost injury in predicting components of yield. Total bunch ~~weight~~ mass and mean bunch ~~weight~~ mass per node were analysed only for nodes or shoots that produced fruit. Similarly, total number of berries, mean berry number per bunch and mean berry ~~weight~~ mass per shoot were analysed only for those shoots that produced fruit.

When a significant association between a binary dependent variable and the independent variable was identified (*P* =<0.05), the association was quantified as a multiplier for the odds of the dependent event. The odds are the ratio of the probability of an event occurring to the probability of it not occurring. The multiplier was obtained by taking the exponent of the GLMM's linear predictor.

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~~Estimation of a~~Average yield loss from frost injury

The results of the GLMMs conducted at the node level were used to estimate changes in Pinot Noir bunch ~~weight~~ mass per node with increasing incidence of frost injury for a hypothetical block of vines. It was assumed that this block had an intra-row spacing of 1.25 m and inter-row spacing of 2.5 m, resulting in 0.8 vines per linear m of row and 2~~,~~800 vines/ ~~per~~ ha. This information was used to convert a per-node yield estimate to a per-vine mean yield estimate, mean yield per linear m of row and mean yield in t/ha. Justification for using the estimate of mean yield per node for nodes producing fruit from this study is presented in the results section, along with probabilities of a healthy or injured node developing fruit, which in turn were used to estimate mean yield per node for 0, 25, 50, 75 and 100% injured nodes. An average price of A$~~UD~~ 2~~,~~672/t for Pinot Noir grapes from Tasmania in 2013~~-~~/14 (Wine Tasmania 2014) was used to calculate revenue ~~(~~A~~U~~$~~D)~~/ha.

Results

The start of the 2013/14 growing season in Tasmanian vineyards was characterised by general observations of sufficient soil moisture, fruitful buds, and weather that promoted good shoot development (Wine Tasmania 2014). Conditions changed during the pre-flowering and flowering period in December when prolonged cool and wet weather presumably affected fruit~~-~~set. The general outcome for the region was smaller than average bunches and a reduced harvest relative to previous growing seasons (Wine Tasmania 2014).

Frost injury

The incidence of injured nodes and shoots per half vine was in the range 17–76% and ~~The incidence of shoots injured per half vine was in the range~~ 15–92%, respectively. ~~.~~ Frost injury severity scores of 0, 1, 2, 3, or 4 were recorded for 52.7, 8.4, 4.9, 24.7 or 9.3% of shoots,

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Commented [KE21R20]: Not measured. I inserted the word 'presumably' respectively. Of those shoots receiving a score of 4 (dead, rotten), 74% were at E-L stage 3 (woolly bud) and 98% were at E-L stage 2–5 (budswell to visible leaf tips); therefore, most shoots were probably at the woolly bud stage (E-L stage 3) during the frost events. A maximum E-L stage of 14 (seven leaves separated) was recorded across all vineyard blocks during the October assessments ~~of frost injury~~.

The severity of frost injury varied within and among vineyards, with 43– 68% of nodes per vineyard having a maximum injury score > 0 (Figure 3). The spatial distribution of the median of maximum node scores for frost injury per half-vine at vineyards A and B was patchy (Figure 4). In contrast, frost injury was ~~uniformly~~ severe and less variable in vineyard D, and less severe ~~though still uniform~~and less variable in vineyard C. The relative proportion of nodes per half-vine that developed fruit also varied spatially within and among vineyards (Figure 4).

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~~Effect on c~~Crop phenology

Shoots from healthy nodes (primary-shoot and secondary-shoot nodes) -had a mean December E-L score of 18.8, whereas shoots developing from injured nodes -(maximum severity score > 0 for frost injury of shoots) had a mean score of 16.9, representing a difference in the means of 1.9 –(*P* < 0.001, Table 4). If frost injury was present, then the severity of injury did not significantly alter the December E-L scores (*P* = 0.44). Primary-shoot nodes ~~(one shoot)~~ had a mean December E-L score of 18.9, whereas secondary-shoot nodes (> 1 shoot) had a mean December E-L score of 17.2, representing a difference in the means of 1.7 (*P* < 0.001, Table 4). These E-L stages precede the first flower caps loosening: the inflorescence is well developed with single flowers separated (E-L score 17) and by E-L score 18 the flower caps are still in place with the colour fading from green.

~~Effect on numbers of s~~Shoots per node

Secondary nodes produced a total of 993 shoots relative to 401 shoots produced by primary nodes. The presence of frost injury per node, regardless of severity, was associated with a 26.7-fold increase in the odds of >1 shoot per node ($P < 0.001$ for node status, Table 4~~, Figure 5~~). There was a significant difference among frost injury severity~~ies~~ for the proportion of nodes producing more than one~~> 1~~ shoot ($P < 0.001$) (Figure 5). The post-hoc analysis indicated that this was due to the injury severity category of '0' (healthy nodes) being different to all others (maximum pairwise $P < 0.001$). In contrast, there ~~were~~was no significant difference~~s~~ among frost injury severity~~ies~~ of 1, 2, 3 and 4 (minimum pairwise $P < 0.001$). The probability of a healthy node producing >1 shoot was 0.28, while the probability of an injured node producing of >1 shoot per node was 0.91.

~~Effect on c~~Components of yield per node

The presence of frost injury affected whether or not fruit was produced from a node (~~total bunch weight per node > 0 g;~~ Table 4). Of the 393 nodes (45%) that had no fruit, 22% were healthy nodes and 78% were injured nodes. The odds of a healthy node producing fruit was 4.3 (probability = 0.81), whereas the odds of an injured node producing fruit was 2.3 (probability = 0.69). Hence, there was a multiplier of 0.53 of the odds of fruit production ~~total bunch weight being > 0 g~~ ($P = 0.002$) when a node was injured. Including the severity of frost injury in the model did not account for additional variance in the dependent variable ($P = 0.10$).

Mean total bunch ~~weights~~mass for primary and secondary nodes ~~were~~was 59.7 g (SD 78.2) and 65.5 g (SD 89.6), respectively, with an overall mean of 62.8 g (SD 84.5). The mean number of bunches per node for all samples was 1.~~13~~09 (SD 1.~~17~~18): primary and secondary nodes averaged 0.998 and 1.17 bunches per node, respectively. Each type of node had similar mean bunch ~~weights~~mass of 30.9 (SD 37.5) and 31.3 g (SD 36.9), respectively. There was a

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significant relationship between the severity of frost injury and the number of bunches per node (*P* = 0.0002) (Table 4). A frost injury score greater than 0 was associated with a 1.09-fold increase in bunch number per node and a frost injury score greater than 1 was associated with a 1.05-fold increase. Further increases in frost injury severity were associated with a decrease in bunch number per node: an injury score greater than 2 or greater than 3 was associated with multipliers of 0.74 and 0.55 for bunch number per node, respectively. Mean bunch ~~weights~~mass and bunch number~~s~~ per node or shoot for each category of frost injury severity score are provided as supplementary data (Table S1).

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For nodes that produced fruit, total bunch ~~weight~~ mass and mean bunch-~~weight~~ mass per node ~~was~~ were not significantly affected by the presence or severity of frost injury (Table 4).

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~~Effect on c~~Components of yield per shoot

Primary-shoot nodes had an average of 0.998 bunches per shoot (SD 1.05) relative to 0.56 bunches per shoot (SD 0.62) for secondary-shoot nodes. The mean total bunch ~~weight~~mass per primary node (one shoot) was 59.7 g (SD 78.2), which was nearly double that per shoot from a secondary node (31.3 g, SD 42.8).

Node status (primary or secondary node), and the incidence and severity of frost injury were associated with the odds of fruit production per shoot (*P* < 0.001) (Table 4). The odds of a healthy shoot producing fruit was 2.05, corresponding to a probability of 0.67 (*n* = 1401 shoots). The odds of fruit production for shoots with frost injury scores > 0, > 1, > 2 or > 3 were 0.14, 0.12, 0.02 or 0.04, respectively. The corresponding probabilities were 0.13, 0.11, 0.02, and 0.04. ~~A total of 117~~One hundred and seventeen shoots (8.4%) had an injury score of 4, and of those, only four shoots (3.4%) produced fruit. The number of bunches per shoot also

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declined as the severity of frost injury increased, from 1.2 bunches per healthy shoot to 0.05 for the most severely damaged shoots (*P* < 0.001).

For fruit-bearing shoots, the presence of multiple shoots (secondary node) was associated with a reduction in total berry numbers per shoot by a factor of 0.95 (*P* < 0.001). The presence of frost injury was associated with a reduction in the total number of berries per shoot by a factor of 0.91 (*P* < 0.0001). There was also a significant association between the severity of frost injury and the total number of berries per shoot (*P* < 0.001); however, the multipliers varied above and below 1.0 with each one-unit increase in the frost injury score. The multipliers were 0.88, 2.23, 0.82 or 1.17 for injury scores >~~-~~0, >~~-~~1, >~~-~~2 or >~~-~~3, respectively. There were no significant associations between each independent variable and mean berry number per bunch (per shoot) or mean berry ~~weight~~ mass per shoot (*P* values 0.07–0.75; Table 4).

A~~Estimation of a~~verage yield loss from frost injury

Given that there was no significant difference between primary-shoot and secondary-shoot nodes in their productivity, a key consequence of the frost events was the reduced likelihood of fruit production when nodes were injured. ~~The proportion of injured nodes per half-vine ranged from 17.3 to 75.9%. Given this result, fruit yield loss at the node and vine levels was estimated by simulating the effect of injured nodes in the range 0–100% incidence. The most biologically relevant difference between nodes with frost injury and those that were healthy was the reduced likelihood of fruit production.~~ As noted above, the probability of a healthy node producing fruit was 0.81 and the probability of an injured node producing fruit was 0.69 – a difference of 12%. These probabilities provided the means to simulate the effect of injured nodes in the range 0–100% incidence. The mean bunch ~~weight~~ mass of a fruitful node in this study was 90.8 g and this ~~weight~~ mass was used to calculate the values presented in Table 5.

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Using a common planting density and node number per vine, a vineyard in which 100% of nodes received some degree of frost injury was estimated to earn, on average, A~~UD~~$1~~,~~630/ha less than a vineyard block with no frost injury (Table 5).

Discussion

Spring frost~~ event~~s are episodic and, thus, have been studied infrequently. An efficient, incidence-based method~~ology~~ was developed to assist future researchers and vineyard workers to ~~mobilise resources quickly after a frost event to~~ assess the likely yield impact of frost injury. In this study, frost injury during budburst in Pinot Noir resulted in a 12% reduction in the likelihood of fruit production per vine node. In 2014, the average purchase price of Pinot Noir grapes from Tasmania and Australia was A$~~UD~~ 2~~,~~672 and A$~~UD~~ 696, respectively (Wine Tasmania 2014, Winemakers' Federation of Australia 2014). Given the relatively high value of Pinot Noir grapes in Tasmania, the reduction in yield (t/ha) from a high proportion of injured nodes translates to a considerable loss of revenue (Table 5). Moreover, the 2013/14 growing season was characterised by poor fruit~~-~~ set and lower than average grape yields (Wine Tasmania 2014) as reflected by the low mean bunch ~~weight~~ mass recorded in this study. A more fruitful season would most likely have resulted in even greater crop and revenue loss.

Spatial variation in frost injury

Within-vineyard spatial variation in frost injury was described, unlike previous studies in which spatial variation was noted but not ~~quantified~~described ~~in this study confirmed previous qualitative observations~~ (Lider 1965, Jones et al. 2010). The study blocks in vineyard A show a distinct increase in elevation from the NE to the SW (Figure 1); however, factors contributing to spatial variation in frost injury in this study remain~~s~~ obscure. It is postulated that the effectiveness of the overhead irrigation system, in relation to the application rates of water,

Commented [KE29]: We would prefer 'viticulturists' rather than 'vineyard workers' The former implies a worker with specific technical skills.

Commented [GD30]: Please provide a reference for this.

Commented [KE31R30]: The evidence is anecdotal – I have cited the 2014 vintage report from Wine Tasmania. If this is insufficient, then I am happy for the following words to be removed: 'poor fruit-set and'

Commented [KE32]: The word 'described' is now used twice in the one sentence. The words 'noted but not described' could be replaced by 'noted only' or 'only noted'?

was near its limit of effectiveness when the air temperature was close to -5°C. A small amount of latent heat is released when water freezes; therefore, a constant supply of water is needed to coat grapevine buds and shoots and to raise the temperature of grapevine tissues. A small variation in system pressure and hence output for individual sprinklers might explain the spatial variability in frost injury recorded. Such variation might also have contributed to spatial variation in frost injury at vineyard B, although blockages of individual sprinklers might have also contributed to their ineffectiveness. Lateral airflow during a frost, while not monitored in this study, might also cause distortion of spray patterns and/or influence spatial variation in environmental conditions.~~.~~

There was no frost protection at vineyard C, where frost injury was less severe than at other vineyards, and frost protection failed at vineyard D where injury was ~~uniformly~~ severe. The lowest recorded temperature on 12 September ~~12~~ at Vineyard C was higher than at vineyard D: -1.9 ~~and~~ versus -3.5°C~~, respectively~~. The difference between bud temperature and the adjacent air temperature during the frost events was probably minimal, given that recorded ~~relative humidities~~RH ~~were~~ was > 84%. Overall, these results are consistent with the findings of Gardea (1987) who sampled one-node cuttings of *V. vinifera* cv. Pinot Noir prior to budburst, subjected them to conditions to promote budburst, and then exposed buds at different stages of development to low temperature~~s~~. The temperature when 50% of buds were damaged at the phenological stages of quiescent, swollen, budburst, first, second and third flat leaf was estimated to be -14, -3, -2.2, -2, -1.7 and 1.1°C, respectively.

This study confirmed that the threshold air temperature of -2°C for potential frost injury during ~~budbreak~~ budburst at these vineyard sites was adequate given that shoots at E-L stages < 5 were injured when exposed to an air temperature~~s~~ < -2°C. Even so, the critical temperature for frost injury is likely to be unknown for any given location because multiple factors have been associated with frost injury (Johnson and Howell 1981, Trought et al. 1999, Snyder 2001, Sun et al. 2017). Even though the full range of potential factors influencing or moderating frost at each site w~~ere~~as not ~~quantifi~~measured~~,~~ the effective application of water was critical ~~as evidenced by the results for vineyard D~~for preventing damage (see vineyard D). In practice, commencement of overhead irrigation before the temperature~~s~~ falls below 0°C would ensure adequate water coverage when injury eventuates at an unknown critical air temperature.

~~Effect on s~~Shoot production and crop phenology

This study not only confirmed that secondary shoots may develop and produce fruit if the primary bud is injured (Kasimatis and Kissler 1974, Friend et al. 2011), it also quantified the difference in the proportion of healthy and injured nodes producing ~~> 1~~more than one shoot (28~~%~~ and 91%, respectively). It is not known why nodes with a frost injury score of 0 produced ~~> 1~~more than one shoot. It is postulated that $N+3_n$ buds were injured non-visibly, which in turn stimulated shoot production. Double primary (latent) buds, in which two N+2 buds are adjacent and separated by an extremely short internode, have occasionally been observed in Tasmania. This phenomenon was not considered a contributing factor in the current study.

The difference in the December E-L stages of shoots from nodes with and without frost injury can be explained by the greater proportion of secondary shoots emerging from injured nodes, presumably after damage to the primary bud. Reports of the consequence of early-season frost injury on crop phenology are rare. Lider (1965) reported lower ~~total soluble solids~~ TSS in bunches from secondary shoots relative to those from primary shoots of Cabernet Sauvignon after a frost event when shoots were 30 to 45 cm long; ~~however, the reproducibility of these results is uncertain because statistical analyses were not applied to the data~~although these reports were not accompanied by statistical anal~~a~~ysis.

Commented [GD33]: By what? Vigour? Number of retained nodes?

Commented [KE34R33]: The suggestion is that injury can occur but may not be visible to the naked eye
The sentence has been reworded accordingly.

Commented [GD35]: Add reference

Commented [KE36R35]: Please see next comment.

Commented [GD37]: Why?

Commented [KE38R37]: We only added this discussion about double primary (latent) buds to address one of the reviewers' comments. The only reference we can provide would be (J. Jones, Tasmanian Institute of Agriculture, personal communication). Jo has seen these double buds but has not counted them to determine their frequency.

The last two sentences of this paragraph could be removed.

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Commented [KE39]: 'this report' (singular?)

Delays in flowering can have unpredictable consequences because there is potential to either escape or increase exposure to episodes of weather promoting poor fruit set. Any subsequent effect on fruit yield and composition at harvest ~~flow-on effect affecting harvest date~~ will depend both on crop load and late-season conditions. In the current study, vine canopies and favourable late-season conditions sustained the ripening of smaller than average bunches. Although not measured, greater variance in the ~~physiological ripeness of bunches~~fruit composition ~~was a potential~~may have been an outcome of the patchiness of frost injury incidence at vineyards A and B (Figure 4).

~~Effect on n~~Node-level components of yield

The most prominent relationship between frost injury and components of yield per node was the reduction in likelihood of fruit production for injured nodes relative to healthy nodes. The apparent lack of association between the severity of frost injury at the node level and the proportion of nodes developing fruit was most likely due to variation in the response of a node to injury in terms of the production of secondary shoots and the fact that secondary shoots had the potential to produce fruit. This variation might also explain the non-linear response of the number of bunches per node with increasing injury severity. Relative to healthy nodes, the mean number of bunches per node was slightly greater among nodes with the least severe injury scores and lower for the highest injury scores.

As noted previously, the assessor did not know whether or not nodes given a maximum injury score of 1 were truly injured, thus raising doubt about inclusion of these nodes in the calculation of injury incidence (nodes injured or not). ~~This~~ ~~However, T~~this inclusion, however, was justified following analyses that revealed the extent of secondary shoot production on nodes with a maximum injury score of 1 relative to healthy nodes (Figure 5).

Commented [GD40]: Please rewrite. All other things being equal, a delay in flowering will strongly tend to expose differentiating buds to warmer weather.

Commented [KE41R40]: This sentence could be contextualized by adding the following words at the start of the sentence:

In the cool, maritime climate of this study, ……

Why?
Long-term average max temperature in November and December are 19.1 C and 20.8 C (Hobart Airport). That is, monthly mean temperatures in Tasmania are relatively 'flat' over the flowering period Nov-Dec. It would be difficult to discern a warming trend from week to week (as early summer progresses) in this cool, maritime climate. It is not uncommon to have two weeks in November that are relatively warm (max temps above 22 C) followed by a week where max temps are 10-15 C.

Commented [KE42]: Remove the word 'the'?

Commented [GD43]: This is not a component. Fewer and smaller bunches?

Commented [KE44R43]: This is about the presence, or not, of fruit per node.
The words "components of yield per node" could be replaced with "a variable relating to yield'

~~Effect on s~~Shoot-level components of yield

Unlike nodes, increasing severity of frost injury for shoots was associated with a reduction in the likelihood of fruit production and the number of bunches per shoot (Table 4). The severity of frost injury was also associated with the total number of berries per shoot although the pattern was difficult to interpret.

~~These r~~Results of this study are consistent albeit not directly comparable with the findings of Friend et al. (2011) who studied the consequences of a 5 h frost event for *V. vinifera* L. Chardonnay 'Mendoza' at bud swell/woolly bud in the ~~Marlborough~~ Canterbury region of New Zealand. These authors reported that total bunch ~~weight~~mass on primary shoots was almost three times greater than that observed for secondary shoots, a difference that was explained by primary shoots having a higher mean number of bunches. It is presumed that the primary and secondary shoots referred to by Friend et al. (2011) were shoots from N+2 and $N+3_n$ buds. ~~Friend et al. (2011) found that the total bunch weight on primary shoots (as defined by the authors) was almost three times greater than that on secondary shoots as consequence a higher mean number of bunches on primary shoots.~~ In the current study, mean total bunch ~~weight~~mass per primary-shoot node (a single shoot) was nearly double that per shoot from a secondary-shoot node and primary-shoot nodes had, on average, more bunches per shoot than those from secondary-shoot nodes. Healthy primary-shoot nodes also had higher mean bunch ~~weights~~mass than injured primary-shoot nodes presumably because $N+3_n$ secondary shoots did not develop post injury. ~~bunch number also declined significantly with increasing severity of frost injury per shoot.~~

~~Unlike Friend et al. (2011), no judgement was made in the current study about whether or not a shoot was from a primary or secondary bud. The difference between the two studies~~

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~~appears to relate to the apparent confidence of Friend et al. (2011) in identifying a primary shoot for the assessment of primary shoot death. More caution was taken in this study by assuming that > 1 shoot per node was an indicator of secondary shoot production and that if a node had a single shoot, then its origin from within a compound bud was not presumed.~~

Friend et al. (2011) found no difference between ~~so-called~~ primary and secondary shoots in average bunch ~~weight~~mass, number of berries per bunch or average berry ~~weight~~mass. Again, results in the current study were similar but not directly comparable to ~~these~~ the findings for Chardonnay. A key difference was the focus in the current study on examining yield components at the node level and the likelihood ~~odds~~ of fruit production. For nodes that produced fruit, the effect of frost injury on bunch ~~weight~~mass, berry count per bunch and mean berry ~~weight~~ mass per node was insignificant. Moreover, the GLMMs were applied across multiple sites and accounted for variance among vineyards, blocks and vines. There appears to have been a significant degree of yield compensation in the current study, attributable in part to the early-season timing of the frost injury when most nodes were at the woolly bud stage, and the subsequent productivity of secondary shoots. ~~Both studies~~Like the study of Friend et al. (2011), this study highlights the importance and contribution of secondary shoots to vine yield after spring frost events, with the response likely to be cultivar dependent (Kasimatis and Kissler 1974). Frioni et al. (2017) also observed yield compensation in the form of abundant, fruitful secondary shoots after frost injury during budburst of the cold hardy *Vitis* interspecific hybrid ~~'~~Marquette~~'~~.

Conclusions

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Recording injury incidence per node, including any visible sign of potential damage, was sufficient to quantify the probability of fruit production at that node. It allowed aggregation of the effects ~~to~~ on vine and block-level fruit production and it was ~~also more time-efficient~~easier than recording severity, which conveyed no additional information for most node-level analyses. Moreover, knowledge of the likely lag in the E-L stage of shoots from injured nodes, especially a delay in the flowering period, can assist forward planning by vineyard managers and heighten ~~alertness~~ awareness ~~to~~ of potential interactions between crop phenology and conditions later in the growing season.

Unlike node-level analyses, ~~the~~ severity scores provided statistically significant information for shoot-level analyses. The descriptive key and scoring scale developed in this study may be applied in future research. Even so, the recording of shoot-level data is unnecessary if the key objective is to understand the effect of frost injury on components of yield, especially spatial analyses to assess the site-specific value of frost protection and/or locations where its application needs to be improved.

Additional studies of frost injury in Pinot Noir across multiple sites and seasons are needed to develop robust, site-specific predictors of the impacts on fruitfulness and yield per node. The ~~methodology~~ methods developed in this study may be applied to generate comparable data sets from standardised assessment of frost injury and associated factors of viticultural importance. Estimati~~o~~ng ~~of~~ likely site-specific effects on yield ~~in relation to the nature of a~~of frost~~ events~~s will allow deeper exploration of vineyard topography~~ of~~ and other factors contributing to spatial variation in the risk of frost injury~~. S~~ ~~uch knowledge~~and, thus, ~~will~~ inform more strategic deployment of frost protection, such as the positioning and timing of overhead irrigation to ~~minimise~~ optimise water use.

Acknowledgements

This study was supported by Sense-T, a partnership between the University of Tasmania, CSIRO and the Tasmanian Government. Sense-T is also funded by the Australian Government. We thank Mr Andrew Terhorst, Mr David Biggins and Mr Chris Sharman from the CSIRO for the sensor data and our vineyard co-operators Mr Matthew Pooley from Pooley Wines, Mr Danny Belbin from Frogmore Creek Wines, Mr Tim Lyne from Spring Vale Wines, and Mr Terry Bennett from Home Hill Wines. We gratefully acknowledge Dr Stephen Wilson and Dr Joanna Jones for reviewing the manuscript.

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Table 1. Location of each vineyard and on-site environmental sensors, attributes of Pinot Noir vines and viticultural practices in four Tasmanian vineyards.

~~Attribute~~	~~Vineyard A~~	~~Vineyard B~~	~~Vineyard C~~	~~Vineyard D~~
		Region of Tasmania		
Attribute~~Region of Tasmania~~	**Vineyard A** **Coal Valley**	**Vineyard B** **Huon Valley**	**Vineyard C** **Coal Valley**	**Vineyard D** **East Coast**
Latitude; longitude; m above sea level (masl) at the location of the Campbell Scientific sensors†	-42.646208; 147.470466; 74 masl	-42.999692; 147.041889; 68 masl	-42.615648; 147.441313; 92 masl	-42.024582; 148.072002; 15 masl
Pinot Noir clone/s	0013; 2051; 8048	114; 115; 2051; 8104	114; 115; 0011; 0013; 2051; 8048	0014; 8048
Vine age (years)	14	7–14	15–16	28
Trellis type	Vertical shoot positioned (VSP)	VSP and Scott Henry	VSP	Modified Lyre
Inter-row x intra-row spacing (m)	2.0 x 1.0	2.7 x 1.5	2.4 x 1.2	2.4 x 0.4
Row orientation	NE–SW	NE–SW and~~&~~ NW–SE	NE–SW	NW–SE
Frost protection	Overhead sprinklers	Overhead sprinklers	None	Overhead sprinklers‡

† Sensors were positioned 1.5 m above the indicated elevation. Libelium sensors at vineyard B were positioned at -43.002222; 147.046667; 43 masl; ‡Assumed to be ineffective due to a pump failure that resulted in little water coverage on 13 September when the lowest recorded temperature was -4.1°C (Table 3).

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Table 2. Sampling details and dates for assessment of frost injury, December E-L score and components of yield. Spring frost events occurred on four consecutive nights (Table 3), with the final event occurring on 15 September ~~15,~~ 2013.

Sampling details or assessment dates	Vineyard A	Vineyard B	Vineyard C	Vineyard D
Total area sampled (ha)	3.5	1.7	1.3	1.8
Number of blocks sampled (see Figure 1 for block location)	2	2	3	1
Total number of half-vines sampled	32	27	21	12
Date/s of assessment of frost injury (days after 15 September~~. 15,~~ 2013)	~~Oct.~~ 7–9 October~~,~~ 2013 (22–24)	~~Oct.~~ 16–21 October~~,~~ 2013 (31–36)	~~Sept.~~ 29 September– ~~Oct.~~ 16 October~~,~~ 2013 (14–31)	~~Oct.~~ 15 October~~,~~ 2013 (30)
Total number of nodes and shoots assessed for frost injury and yield	255 nodes 396 shoots	2826 nodes ~~462~~ 456 shoots	~~187~~ 186 nodes ~~264~~ 263 shoots	146 nodes 279 shoots
Date/s of assessment of December E-L score	~~Dec.~~ 12 December~~,~~ 2013	~~Dec.~~ 17 December~~,~~ 2013	~~Dec.~~ 10–12 December~~,~~ 2013	~~Dec.~~ 11 December~~,~~ 2013
Date/s of assessment of yield components	~~Apr.~~ 14 April~~,~~ 2014	~~Apr.~~ 15–28 April~~,~~ 2014	~~Apr.~~ 3–4 April~~,~~ 2014	~~Mar.~~ 26 March~~,~~ 2014

Table 3. Environmental conditions recorded by on-site Campbell Scientific and Libelium sensors, 11-15 September~~ 11-15,~~ 2013 in four Tasmanian vineyards.

Vineyard	Date when frost event concluded	Lowest recorded temperature during frost event (ºC)	Time (h) from first record of a sub-zero temperature to~~-~~ the lowest temperature	Range in ~~relative~~ RH ~~humidity~~ (%) during sub-zero temperature~~s~~
A	12 Sept~~. 12~~ember	-2.33	6.25	84.2–99.2
	13 Sept~~. 13~~ember	-4.50	9.25	
	~~Sept.~~ 14 September	-2.05	7.75	
	15 Sept~~. 15~~ember	-2.28	6.00	
B - Campbell sensor (irrigated)	Sept. 12	-0.14	1.25	99.2–100
	Sept. 13	-1.27	4.00	
	Sept. 14	-1.36	3.00	
	Sept. 15	-0.81	2.25	
B – Libelium sensor	Sept. 12	-2.30	2.75	N/A
	Sept. 13	-4.07	10.0	
	Sept. 14	-2.23	8.00	
	Sept. 15	-1.91	4.25	
C	Sept. 12	-1.91	4.75	94.1–99.8
	Sept. 13	-2.93	7.45	
	Sept. 14	-1.13	7.00	
	Sept. 15	-0.66	2.50	
D	Sept. 12	-3.50	3.25	85.8–100
	Sept. 13	-4.10	8.00	
	Sept. 14	-1.48	0.75	
	Sept. 15	-2.08	2.25	

N/A = data not available.

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Table 4. Summary of analyses per node and per shoot using generalised linear mixed models (GLMMs).

Level of analysis	Dependent variable	Distribution of dependent variable	Link function used in GLMM	Independent variable		
				Node status†	Incidence‡ of frost injury	Severity‡ of frost injury
Per node	Mean E-L score in December	Gamma	Log	***	***	ns
	Node status†	Normal	Identity	~~NA~~na	***	ns
	Total bunch weight > 0 g§	Poisson	Log	ns	**	ns
	Number of bunches	Gamma	Log	ns	**¶	***
	Total bunch weight†† (g)	Binomial	Logit	ns	ns	ns
	Mean bunch weight†† (g)	Normal	Identity	ns	ns	ns
Per shoot	Total bunch ~~weight~~ mass > 0 g	Binomial	Logit	***	***	***
	Number of bunches	Poisson	Log	***	***	***
	Total number of berries††	Poisson	Log	***	***	***
	Mean berry ~~weight~~mass†† (g)	Gamma	Log	ns	ns	ns
	Mean berry number per bunch††	Gamma	Log	ns	ns	ns

Ns, not significant *P* > 0.1; * *P* ≤ 0.05; ** *P* ≤ 0.01; *** *P* ≤ 0.001. †Primary or secondary node (one shoot or > 1 shoot per node)~~;~~ ‡~~i~~Incidence, ~~=~~ presence (or not) of frost injury; severity, ~~=~~ maximum frost injury score per node. §Fruit present or not. ¶ An injury score > 0; >1; >2; or > 3. ††Only nodes or shoots that produced fruit. na, ~~NA =~~ not applicable; ~~ns = not significant *P* > 0.1; * *P* ≤ 0.05; ** *P* ≤ 0.01; *** *P* ≤ 0.001.~~

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Table 5. Estimated loss of revenue with an increasing ~~percentage~~ proportion of nodes injured by spring frost events at approximately the woolly-bud stage (E-L 3).

Nodes injured by frost events (%)	Mean yield/ ~~per~~ node (kg)	Mean yield/ ~~per~~ vine (kg)	Mean yield/ ~~per~~ linear m of cordon (kg)	Mean yield/ ~~per~~ ha (t)	Revenue/ ~~per~~ ha @ A$~~UD~~ 2~~,~~672/t
0	0.074	1.5	1.2	4.119	11,006
25	0.071	1.4	1.1	3.966	10,597
50	0.068	1.4	1.1	3.814	10,191
75	0.065	1.3	1.0	3.661	9,782
100	0.063	1.3	1.0	3.509	9,376

The probability of a healthy node developing fruit was 0.81 and the probability for an injured node developing fruit was 0.69. It was assumed that there were 20 nodes per vine, 0.8 vines/linear m, 2800 vines/ha and 90.8 g fruit for each node producing fruit. The 2014 vintage in Tasmania was characterised by smaller than average bunches and a reduced harvest relative to previous growing seasons (Wine Tasmania 2014).

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Supplemental data

Table S1. Mean bunch ~~weights~~mass and bunch number~~s~~ per node according to node status (primary or secondary) and mean maximum frost injury score per node for 869 nodes and 1394 shoots.

Nodes status	Total number of nodes (or shoots)	Mean maximum frost injury score/ ~~per~~ node	Mean total bunch ~~weight~~ mass/~~(g) per~~ node (or ~~per~~ shoot) (g)	Mean bunch ~~weight~~ mass ~~(g) per~~ /node(g)	Mean number of bunches/ ~~per~~ node (or ~~per~~ shoot)
Primary	259	0	89.8	46.5	1.47
Secondary	103 (208)†	0	103.8 (51.5)	43.2	1.73 (0.86)
Primary	25	1	15.5	9.84	0.32
Secondary	55 (117)	1	85.1 (38.4)	35.2	1.64 (0.75)
Primary	13	2	20.6	9.13	0.62
Secondary	38 (84)	2	81.8 (39.0)	38.0	1.26 (0.61)
Primary	82	3	0.15	0.15	0.01
Secondary	189 (404)	3	48.3 (22.8)	26.4	0.93 (0.44)
Primary	22	4	1.96	0.98	0.09
Secondary	83 (182)	4	36.6 (17.4)	21.8	0.68 (0.33)

†Values in parentheses represent data for shoots.

Figure legends

Figure 1. Locations in Tasmania of up to three blocks~~-~~ per vineyard in relation to elevation, with contour lines 10 m apart for: (a) vineyard A, Coal Valley; (b) vineyard B, Huon Valley; ~~-~~(c) vineyard C, Coal Valley; and (d) vineyard D, East Coast. Maps, with a grid interval of 100 m, ~~-~~are oriented north-south and within topographic mapping zones GDA zone 94 or MGA zone 55. ~~-and the map grid interval is 100 m (GDA94 MGA55).~~ The base layer images~~-~~were prepared using the Land Information System Tasmania (LIST) service (Tasmanian Government, 2018).~~, Department of Primary Industries, Parks, Water and Environment, Tasmania.~~ ~~The red crosses indicate T~~the location~~s~~ of the Campbell Scientific sensors in the four vineyards (X) and~~.~~ ~~The purple cross at vineyard B was the location~~ of the Libelium sensor~~s~~ at vineyard B (X) is indicated.

Figure 2. Severity scale for frost injury applied to individual shoots of Pinot Noir and commencing 2 weeks after the final of four spring frost events, 12–15 September ~~12–15,~~ 2013. The black arrow indicates the necrotic tissue of an injured bud. The images are from Vineyard C.

Figure 3. ~~Percentage~~ Proportion of nodes at (a) vineyard A, Coal Valley; (b) vineyard B, Huon Valley; (c) vineyard C, Coal Valley; and (d) vineyard D, East Coast ~~each vineyard~~ in each category representing the maximum severity of frost injury. Injury was assessed between ~~-~~29 September ~~29~~ and ~~-~~21 October ~~21,~~ 2013. The ~~total~~ number~~s~~ of nodes sampled in vineyards A, B, C and D ~~were~~ was 255, ~~286~~282, ~~187~~ 186 and 146, respectively.

Figure 4. Map of block-level patterns of frost injury with each circle representing a half-vine in (a) vineyard A, Coal Valley; (b) vineyard B, Huon Valley;~~-~~ (c) vineyard C, Coal Valley; and (d) vineyard D, East Coast. Frost injury per half-vine (intensity of shading in each circle) was calculated as the

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Commented [KE48R47]: I have edited the text. These are references to the corresponding map regions for two different map projections. GDA is the acronym for Geocentric Datum of Australia and UTM is the acronym for Universal Transverse Mercator. The number refer to the geographical zone. 55, for example, covers Tasmania and parts of eastern Australia.

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median of the maximum frost injury scores for all nodes assessed. The size of the circle indicates the relative proportion of nodes per half-vine that developed fruit.

Figure 5. The ~~percentage~~ proportion of nodes (*n* = 869~~74~~) across ~~all~~ the four vineyard sites in the Coal Valley, Huon Valley and East Coast of Tasmania producing >~~-~~1 shoot by category of maximum score for the severity of frost injury assessed between 29 September ~~29~~ and 21 October~~ 21,~~ 2013. There ~~were~~was no significant difference~~s~~ among the severity of frost injury ~~severities~~ of 1, 2, 3 and 4 (minimum pairwise *P* < 0.001), whereas healthy nodes were significantly different from all others.

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