Wine Australia for Australian Wine

THE AUSTRALIAN NATIONAL UNIVERSITY

Scale insects and the *Vitis vinifera* cv. Shiraz -reason for high incidence of scales



FINAL REPORT to WINE AUSTRALIA

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1.Abstract

Temperature affects all aspects of the life of animals, especially ectotherms such as scale insects. Development from first instar to third instar for grapevine scales (*Parthenolecanium persicae*) occurs from the beginning of summer through to autumn, although the rate can be changed as temperature increases. Even the development and hatching of eggs is dependent upon the body temperature of the adult females in the spring. Three survey trips and the use of temperature data loggers were used to determine development of scales from third instars through to the appearance of first instars in Langhorne Creek region. No difference in scale presence on vines was observed despite differences in vineyard management.

Fluctuating temperature conditions were transformed into degree-days to compare scale development in vineyards with laboratory conditions. The change from third instar adult appeared to correspond to laboratory expectations, but development from adult to hatching of first instar may be slightly different, although the identification of first instar emergence was limited by rain at the November survey. The pattern of degree-day accumulation may influence the timing of moulting from one scale developmental stage to another between years. Use of degree-days may aid in chemical control procedures for controlling the vulnerable change from third-instar into adult.

Although timing of moulting is important for determining when scale insects may be susceptible to control processes, the size of insects may also play a role in the way that the plant is affected. An increase in size between third instar and adult, along with egg production, may remove nutrients, leading to a reduction in plant development. A second stage that may affect plants is the combined presence of first and second instars, as the most honeydew may be produced then. The high quantity of honeydew may initiate the growth of sooty mould.

Biotic interactions between the scale insects and their host plant may vary depending upon cultivar, as previous work has demonstrated that scale populations differ among grapevine cultivars. A preliminary metabolomics study of grapevine leaves from different varieties indicated that differences exist between cultivars that were more prone to scale infestation and more resistant cultivars. Expansion of this study has the potential to provide scale control methods independent of abiotic conditions.

2. Background

Scale insects have become more noticeable as pests of grapevines in the last 10 years, although Buchanan (2008) suggested that these insects could become pests even before their current outbreak. Two species of scale insects appear to be causing the most damage, the grapevine scale (*Parthenolecanium persicae*) and the frosted scale (*P.* nr. *pruinosum*) as these species seem to be present in all eastern grape-growing regions (Rakimov *et al.* 2013). In the last five years, the level of damage both directly by scale feeding and by subsequent presence of sooty mould has become critical to the industry as both plant and fruit damage is resulting in potentially large financial losses by grapegrowers (Venus 2017).

Scale insects on grapevines in Australia are currently recognised as being univoltine, that is only one generation occurs a year. Females are present in late September-mid October and first instars start appearing in November. The first instars feed on phloem or parenchyma cells (Simbiken 2014) through January-February and then moult into second instars. The second instars of grapevine scales moult to third instars by March and overwinter as third instars. In contrast, the second instars of frosted scales overwinter, with the third instar not developing until August when it is present for only about two weeks before becoming an adult. Frosted scale also moults to adult in late August-early September, so adults of both species are present at the beginning of spring (Simbiken *et al.* 2017). Although the beginning of a second generation may occur in March-April (Venus, 2017), it is unknown whether any of the offspring are able to survive to the following spring, although temperature conditions in some grape-growing regions may permit survival overwinter.

The distribution and abundance of scales can be related to the environment, where the environment for these insects is both abiotic (temperature, wind, general habitat) and biotic (host plant, life history pattern and response to other arthropods) (Andrewartha and Birch 1954; Andrewartha and Birch 1984). While a complete analysis of all the parameters that may be influencing the changes in the presence of scale insects is impossible in a short-term study, some understanding of least some of the important parameters can be examined.

Temperature is the most important abiotic influence on an insect, as the external temperature is the major influence on the body temperature of insects. A theoretical pattern for any scale insect response, or thermal performance norm, is important for understanding how temperature can affect the life and life history of an insect (Figure 1). The lowest temperature for surviving is the critical minimum temperature, and we have determined the lower temperature for scale insect surviving as -13˚C when conditions are dry, and this minimum temperature increases to 0˚C when the insects are wet (Hayes *et al.* 2017). As temperature increases above this minimal temperature, the scale insects

can demonstrate other biological processes, but a minimal temperature for development for *Parthenolecanium* sp. has been suggested to be 10˚C (Camacho *et al.* 2017). The upper temperature for development and the maximal critical temperature are still unknown, and further work is necessary to determine to what extent the instars present in the summer can survive high temperatures and still moult to the next instar.

The most important biotic influence on the scale insect is the plant on which they develop. The plant provides the nutrients necessary for growth and reproduction, with the nitrogenous components mostly being in the form of free amino acids. As sap from the plant is the major food source, a large quantity of fluid must be ingested to meet the requirement for nitrogen that can be used in protein formation within the animal, necessary for both growth and egg development, with part of the nitrogen being produced by symbiont bacteria (Simpson and Douglas 2013). The excess liquid is high in carbohydrates, especially plant sugars, and is excreted from the insect foregut directly into the hindgut without being absorbed in the midgut via the filter chamber (Foldi 1997). This high sugar content fluid, called"honeydew" is found frequently on the plants that scale insects inhabit and is a source of food for ants that tend the scales. However, if too much honeydew is present, then sooty mould can grow on leaves and fruit.

Plants have various defences against pest insects, such as been reported for different grapevine cultivars (Bertin *et al.* 2013; Simbiken *et al.* 2015; Venus 2017). These defences may be secondary chemicals that are present in the leaves of cultivars that repel insects from feeding or even reduce survivorship . Little is known regarding the chemical compounds present in leaves, compared with the vast array of information of compounds in grapes (Teixeira *et al.* 2014; Gismondi *et al.* 2017). However, the compounds present in leaves may help to explain the varying susceptibility of different grapevine cultivars to attack by insects such as scale.

3. Project aims

Within a larger study of the effects of abiotic and biotic influences on scale abundance and distribution, this project examined several specific questions between September and November 2017.

1. What role does temperature have on scale distribution and abundance in the field, specifically within the Langhorne Creek vineyards?
2. How do scale distribution and abundance differ between cv. Shiraz compared with cv. Pinot Noir within and between vineyards?
3. Is there an obvious chemical difference between leaves of different grapevine cultivars which may help to explain differences in susceptibility to scale?
4. Are there sufficient numbers of control insects present in vineyards of Langhorne Creek which would allow biological control of scale?

4. Methods

With the aid of Jenny Venus, three vineyards from Langhorne Creek region were selected that had both Shiraz and Pinot Noir present. The vineyards differed in prior treatment for insects and general vine management practices (Table 1). Six vines for each cultivar from each vineyard were examined monthly for scale quantity and instar distribution, as well as noting what beneficial insects and other potential pest arthropods were present. Approximately 25% of the spurs were examined, with bark being removed to determine whether scales were present under the bark in the September survey. In October, spurs were examined for adult females, while in November, the underside of 20 basal leaves per plant (10 leaves each side of trunk) was examined to determine whether first instars had hatched and moved onto the leaves to feed.

Temperature data loggers (T-TEC 6-1E) were placed in the rows for each cultivar (two data loggers for each vineyard) to continuously measure temperature at the height of the cordon. In September, no shade was present as no leaves were present on the cordons, but shade for the loggers increased as leaf growth occurred. Temperature data was transferred into an Excel file, daily minimum and maximum temperatures determine for each day and then converted into degree-days for each month using equation 1, assuming a simple temperature model.

$$Degree - days = \sum \left(\frac{\max temp + \min temp}{2} \right) - 10℃ \qquad (1)$$

Degree-days in the field were compared with degree-days for development of *P. persicae* measured in the laboratory at 22˚C (Simbiken 2014).

Leaf metabolomics were used to analyse possible chemicals in grapevine leaves involved in scale inhibition. Leaves were collected from different cultivars (late March-early April) in vineyards from ACT and Langhorne Creek regions (latter courtesy of Jenny Venus), placed in labeled paper bags, and bags deposited into a dry LN_2 shipper in which leaves were immediately frozen. Cultivars included Riesling,Sangiovese,Tempranillo,and Shiraz known to be susceptible to scale, and less susceptible cultivars Pinot Noir and Grenache. Leaves were dried using a large freeze drier (Virtis SP Scientific) and ground using a mill (Wiley). The compounds of the powdered leaves were extracted through several steps and extracts run through a single quadropole gas chromatography system connected to a mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). The output from the GC-MS was then run through software (Easy GC, local software) that would align the peaks and then the peak information was imported into R statistical software where a principal component analysis was run. The first two components were plotted on a two dimensional graph to determine how cultivars grouped on the basis of the compounds present in the leaves. No identification of compounds has yet been done, as a library is currently unavailable for leaf compounds in grapevines.

5. Results

Third-instar grapevine scales were predominantly on the spurs in September, although there was also second-instar frosted scales present as well (Table 2). Adults were present in October and November, but only a few first instars in November were observed (Table 2). The latter survey was compromised by heavy rainfall, which may have been washed first instars off the leaves while observations were being carried out.

Temperatures varied daily throughout the two months of the survey, as indicated for the October-September period (Figure 2). During the two months of the survey temperatures varied from a low of -1.9˚C on 17 September to a high of 42.4 on November 13 (Figure 3), although both near 40˚C and below zero temperatures were recorded in both months of study period.

The variation in temperature over the period from the overwintering stage to adults producing eggs to finally the eggs hatching will provide a guide for determining key periods for development in the field. Previous work on scales in North America have used degree-days to determine when spraying might be most effective (Camacho et al. 2017). Degree-days for development were determined using data from previous laboratory studies (Simbiken 2014) and shown in Table 3. The 3rd instar to adult change took about 180 degree-days and the adult-appearance of first instars (egg hatching) required 360 degree-days at a constant 22˚C in the laboratory environment. Degree-days indicate the temperature accumulation necessary for changes in development to occur. The degree-day progression that was determined in the laboratory must be compared with degree-days in the field to understand how the appearance of the various instars in the field may differ as a result of fluctuating temperatures.

Using the daily degree-days calculation for the various cultivar regions in this study shows that from September 14 to October 17, every location had high enough temperatures to meet the 180 degree-day requirement for grapevine scales to moult from 3rd instar to adult (Figure 4). However, that increment of degree-days in the field was longer in days than what was required in the laboratory (15 days, Table 3). The period from 17 October to 15 November does not appear to be adequate to ensure that all adults had laid eggs and the eggs had hatched in the field (Figure 4). This result corresponds to the observation that not many first instars were observed in the field (Table 2), but females that developed early may have young already hatching. Degree-days in the laboratory will underestimate the timing of development, but as shown in Figure 5, a sudden increase in temperature around 9 November, accelerates the degree-day accumulation. A few females that were removed from Shiraz in Vineyard 2 did have first instars present still attached to the ovary (Figure 6a and b).

However, the change in body size with moult, and not just timing of moult, indicates the quantity of energy that is removed from the plant as the insect progresses from one developmental stage to another (Figure 7). The biggest change is from 3rd instar to adult, while the second critical point would be the change from 1st to 2nd instar and the numbers that survive the first instar. The first period would require a large removal of energy from the plant, while the second developmental change would be the stage when most honeydew is produced which is the main source of food for the fungi that produces sooty mould.

Previous work demonstrated that scale infestation varies between grapevine cultivars. Specifically, Pinot Noir and Sauvignon Blanc tend to have lower scale densities than Chardonnay and Riesling (Simbiken 2014, Simbiken *et al.* 2015) and similar variations have been reported in vineyards in South Australia (Venus 2017).

As scale may be influenced by the chemicals in the leaves on which they feed, a comparison of the overall chemical characteristics between leaves of different cultivars may identify differences. Our work on leaf metabolomics is shown in Figure 8 and demonstrates that the scale-susceptible cultivars (Riesling, Chardonnay, Tempranillo, Sangiovese and Shiraz) group in the positive half of the first principal component, separated from the more resistant Pinot Noir and Grenache. Shiraz appears to separate from the rest of the susceptible cultivars along the second principal component.

6. Discussion

Previous work in the NSW/ACT region on the timing of the development of scale insects (Simbiken 2014, Simbiken et al. 2015, Simbiken et al. 2107) was confirmed for the Langhorne Creek region. It must be noted, however, that the degree-day accumulation will vary from year to year as weather changes in any wine region. The timing for controlling the early instars is the key to preventing the accumulation of honeydew that will accompany an increased scale load in December-February. Any spraying regime needs to account for the timing of the insect stages that are most vulnerable (Camacho and Chong 2015), and whatever chemical control processes are used, the developmental timing can be calculated using degree-days. The most vulnerable group would be first and second instars, but they are present when fruit is present on grapevines. The second most vulnerable period is the transformation from third-instar to adult, the development that was predicted by the degree-day technique. Control at that time would reduce adult numbers, and that would lead to fewer eggs produced, and thus a reduced population of first and second instars. Reducing the numbers of scales prior to fruit set will decrease the likelihood that sooty mould will be present in the mid-summer period.

Alternatively, biocontrol can be used to control scale insects, although the likelihood of success is highly dependent upon a high enough incidence of the organisms capable of controlling scales, *e.g.* ladybird beetle adults and larvae (Coccinellidae:Coleoptera) and parasitoid wasps (Hymenoptera). Observations from the Langhorne Creek region suggest that biocontrol has the most potential to occur in Vineyard 1, where many ladybird beetle adults and spiders were present (Table 1). No parasitoids were observed and only one adult lacewing (Neuroptera) was seen. However, Vineyard 1 also had a relatively high incidence of scale (Table 2). In fact, no obvious difference in scale infestation was observed between the vineyards, despite the differences in spraying programs and vine management. A particular vineyard in the ACT/NSW region in which the vines are not sprayed, has been observed to have a high incidence of parasitism on its scale (Table S1 and Figure S1a and b). The grapegrower is using purchased ladybird beetle larvae in an effort to biologically control scales this season.

This work suggested that no first instar scale insects should have emerged (using degree-days) by the survey in November, but the heavy rain at the time of inspection made verification difficult. Examination of some adults that were collected at the time under the microscope showed that first instars were just starting to hatch (Figure 7a and b) and that emergence would then depend upon conditions subsequent to our survey. The increase in accumulation of degree-days starting on 9 November may have been a stimulus for development, and could lead to more eggs hatching between 9 November-1 December, so that first instars would be observed on leaves by 1 December.

The protection of the developing first instars by remaining within the cuticle of the female scale may act to limit the damage that external conditions, such as rain, may have on early instars.

High summer temperatures may further restrict the ability to scales to moult or even survive during the first and second instar stage when they are feeding and producing honeydew on the vines and fruit. However information on high temperature was not gathered in this study. Future work is planned that will permit incorporation of that information into our understanding of scale development, and will allow better degree-day models to be adopted in which higher temperature limits are recognised.

Growth of scales may not typically affect grapevines during the summer months, as scale insects are relatively small (Figure 7). However, the early spring moult to adulthood and the need for acquiring nutrients to develop and mature eggs suggests that growth of grapevines with a heavy infestation of scales may be inhibited. This aspect is not addressed here, but the extraction of nutrients from the plants may be the most stressful period for the vines and any retardation of growth during the spring may impact on grape yield and quality. Previous work suggested that vines are affected by scale with respect to photosynthetic activity (Simbiken *et al*. 2015) but that work only examined plants grown in a greenhouse under short-day conditions.

Previous work in the field and greenhouse demonstrated that scale infestation varies between cultivars (Simbiken 2014, Simbiken *et al*. 2015), and a similar response was reported for vineyards in the Langhorne Creek region (Venus 2017). The leaf metabolomic study shows a clear difference among cultivars using a principal component analysis, with some separation among susceptible and resistant cultivars. Although we have not identified any chemicals that may lead to these differences, a current greenhouse study is underway that will compare the metabolomics of the leaves of Chardonnay and Sauvignon Blanc with and without scales to determine what chemical compounds may be present to explain the separation. This approach may identify volatiles or more stable compounds in leaves which repel or inhibit scale, which could provide a basis for the production of a scale-specific, natural product that can either kill or deter scales on grapevines.

7. Conclusions

- Degree-days can be used to determine developmental pattern in scales to aid in control programs
- Scale control may be influenced by vineyard management practices, including pruning and insecticide use
- High and low temperatures could be influencing scale numbers
- Vines may provide clues to a secondary control system, as chemical differences between cultivars seems to correspond with susceptibility to scale.

8. Future work

Current work that is on-going is following up the information regarding the effect of parasites on scale population in a particular ACT vineyard. The emergence of the adult wasps has been observed during bi-monthly visits, and the extent to which the parasites reduce the effects of scale will be monitored.

High temperature tolerance of first and second instar scales will be examined in January-February under laboratory conditions to determine the upper limit for insect viability.

Chardonnay and Sauvignon Blanc vines, growing in greenhouse conditions, will be used in trials to determine to what extent these two cultivars are resistant/tolerant of scale infestation. Leaves will be removed starting in January to examine what chemical differences may be present using gas chromatography and mass spectrometry (GC-MS) techniques. The GC-MS analysis will lead to potential means of controlling scales using natural products that are already present in some cultivars of grapevines. The experiment will be repeated next growing season using Shiraz and Pinot Noir, as preliminary analysis suggests that red and wine cultivars may differ in their susceptibility to scale attack.

Finally, the effect on grapevines of the development of scales from third instar to adult needs to be assessed to determine to what extent scale insects affect vine growth and how that occurs. It could be that scale feeding on sap removes so much energy that vine development is inhibited, or the insects may affect the plant growth by injection of materials that adjusts plant photosynthetic capacity.

9. Acknowledgements

Thank you to the vineyard owners and managers for allowing me access to their vineyards and showing the interest that is so encouraging to a researcher when I discussed the work with them. I appreciate the help of Jenny Venus in organising the access to the vineyards and Lian Jaensch for supporting research on scale insects in vineyards. The work in the Langhorne Creek vineyards could not have been done without the support of Wine Australia, especially Drs. Sharon Harvey and Liz Waters. Both Jack Egerton and Wes Keys helped with the fieldwork and made valuable suggestions regarding the work. The photographs were taken by Paul Cooper either using a Nikon Coolpix or a Nikon Shuttlepix.

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Table 1. Summary of vineyard management before and during survey work in vineyards in Langhorne Creek, with observations of beneficial insect presence.

Vineyard 1	Vineyard 2	Vineyard 3
Sprayed with oil in August	Sprayed with chloropyrifos in August	Unknown spraying regime.
Minimal pruning	Pruned, with Pinot Noir pruned shorter than Shiraz in September, but then bottom cordon removed from Shiraz after September survey	Extensive pruning, with Pinot Noir pruned shorter than Shiraz
Spiders and webs abundant, several ladybird beetles present in October and November	Pinot Noir with more spider webs present than Shiraz	

Table 2. Results of survey of three vineyards in the Langhorne Creek wine region. Although no first instars were recorded in November, the result may have been influenced by the heavy rain (45 mm) that fell on the day of survey. Light = 1-10 scales, Moderate = 11-50 scales. No adults were observed in September, mostly adults in October and adults again in November.

	Vineyard 1			Vineyard 2			Vineyard 3		
	Sept	Oct	Nov	Sept	Oct	Nov	Sept	Oct	Nov
Shiraz	Moderate	1.5	Moderate	Moderate	Moderate	0	Moderate	>10	0
Proportion plants infested	4/6	4/6	1/6	6/6	6/6	2/6	6/6	4/6	2/5
Pinot Noir	Moderate	5	0	0	0	0	Light	0.85	0
Proportion plants infested	5/6	4/6	3/6	3/6	3/6	0/6	2/6	3/6	0/6

Table 3. Development of grapevine scale in days in the laboratory at 22˚C. Degree-days calculated as in equation 1. Minimum temperature for development taken from Camacho et al. (2017).

Development stage	Time (d)	Temperature (˚C)	Minimum temperature for development (˚C)	Degree-days
Eggs-hatching	20	22	10	240
1st-2nd	30	22	10	360
2nd-3rd	30	22	10	360
3rd-adult	15	22	10	180
Adult-egg	15	22	10	180

Figure 1. Theoretical thermal performance curve for an insect that shows the minimum and maximum critical temperatures, optimal body temperature, range of field body temperatures and thermal performance breadth. In this version, the thermal performance breadth refers to the range of temperatures over which a scale insect will be able to hatch, moult and grow from egg to adult.

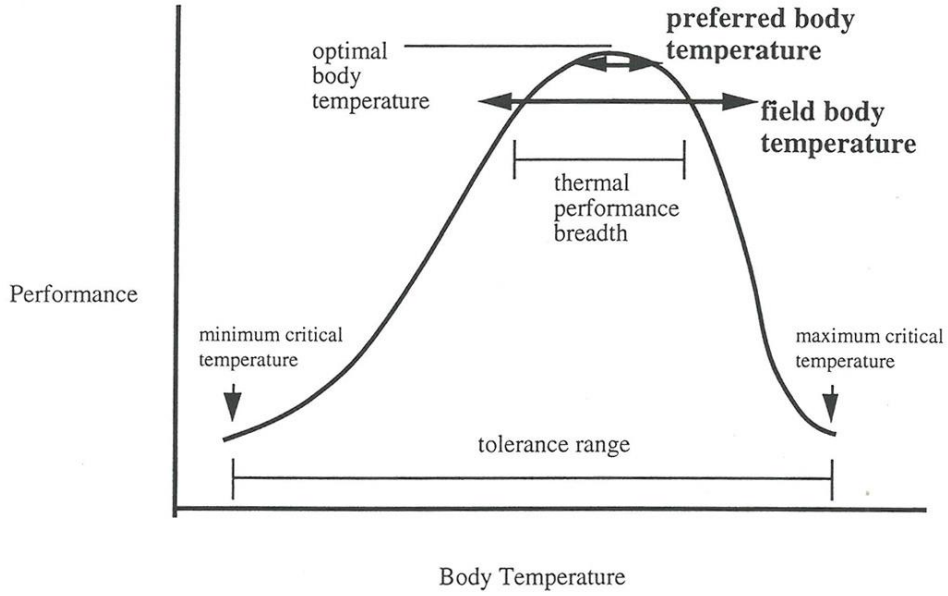


Figure 2. Typical output from data logger in Shiraz showing daily changes in temperature from September to October. All data loggers had similar temperatures in each vineyard, although some slight differences were present before the shading effect of leaf canopy occurred.

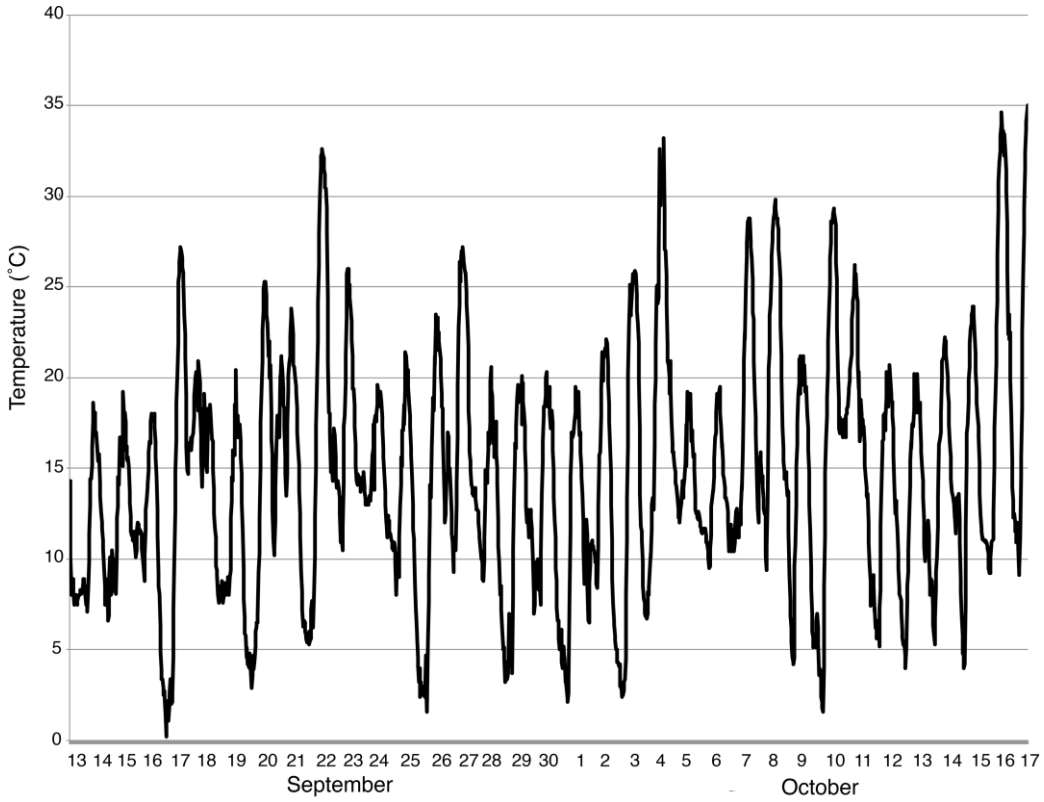


Figure 3. Daily minimum and maximum temperatures recorded during the two-month period. Although the data was similar for each data logger, this represents a data logger that recorded from Pinot Noir on Vineyard 2.

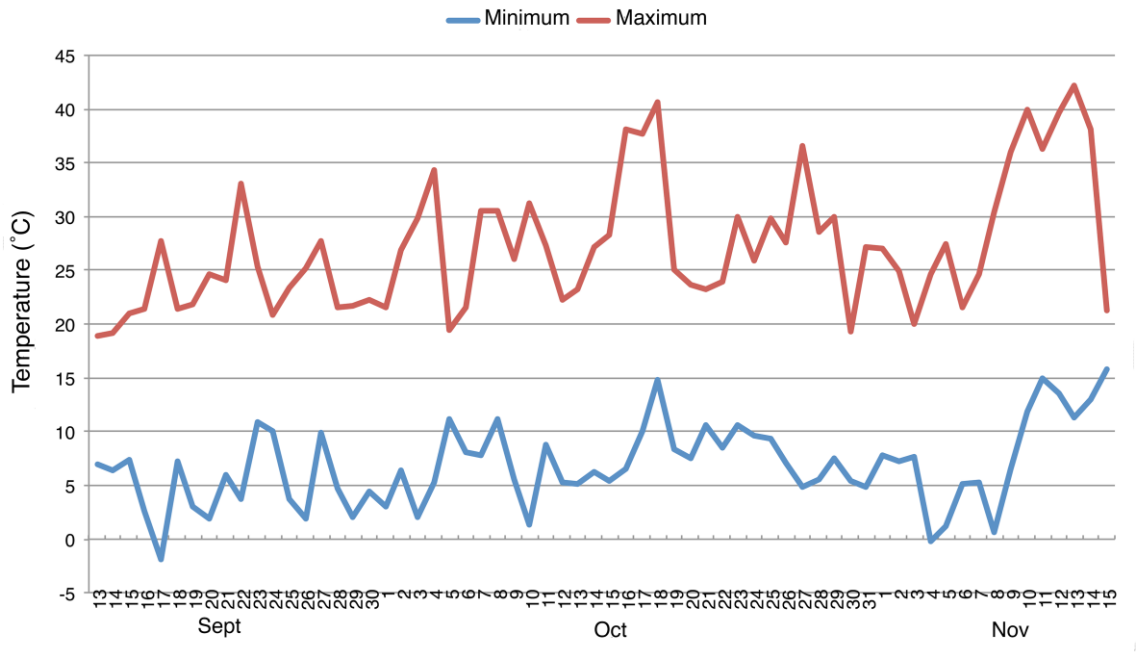


Figure 4. Comparison of degree-days in the vineyards of Langhorne Creek with the calculation of degree-days for development from third instar to adult (Table 3). The degree-days were accumulated over a 30-day period in the field as opposed to only 15 days in the laboratory temperatures.

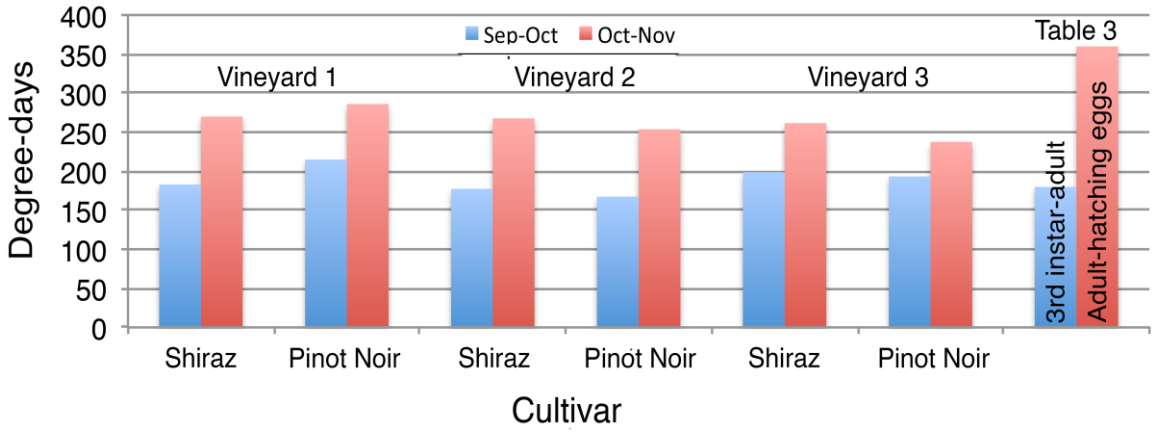


Figure 5. The laboratory measurements (Table 3) had a constant increase in degree-days, but the field situation does not increase in degree-days uniformly as shown by the sudden increase in cumulative degree-days around 9 November.

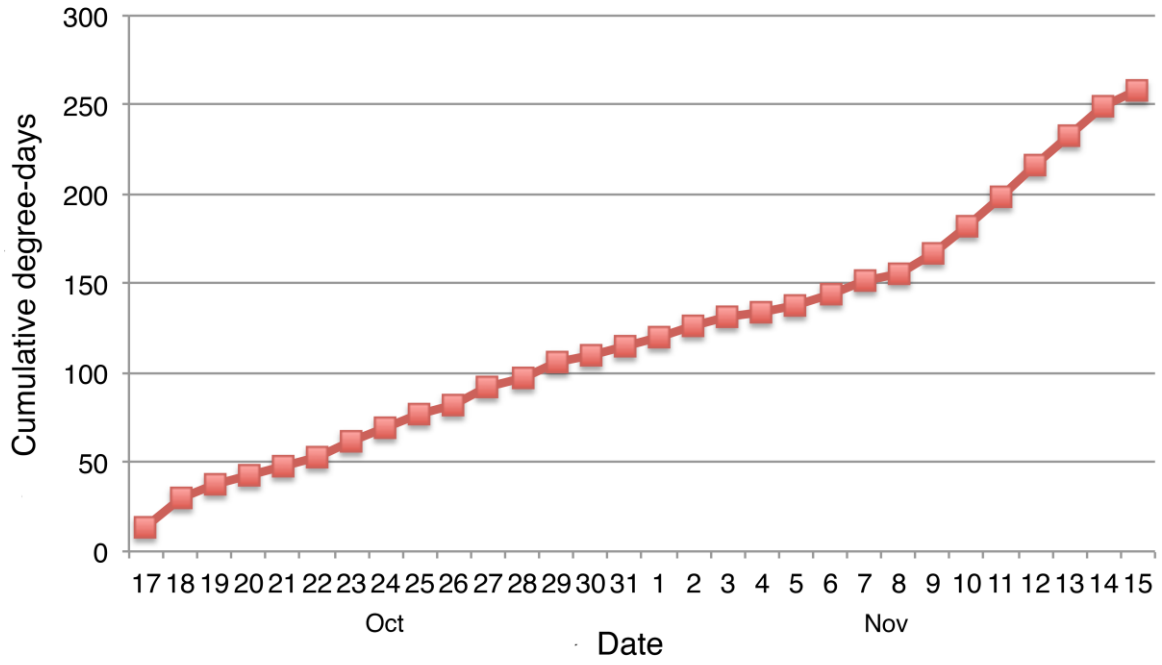


Figure 6. Presence of first instars in ovarian tissue within an adult female removed from Shiraz in Vineyard 2. Although no first instars were observed on the leaves, they are present within the female and still attached to ovarian tissue. The development is overlapping with the presence of eggs.

a) Present within the female in large groups
b) Isolated into a smaller component by removing the ovarian tissue into 70% ethanol.

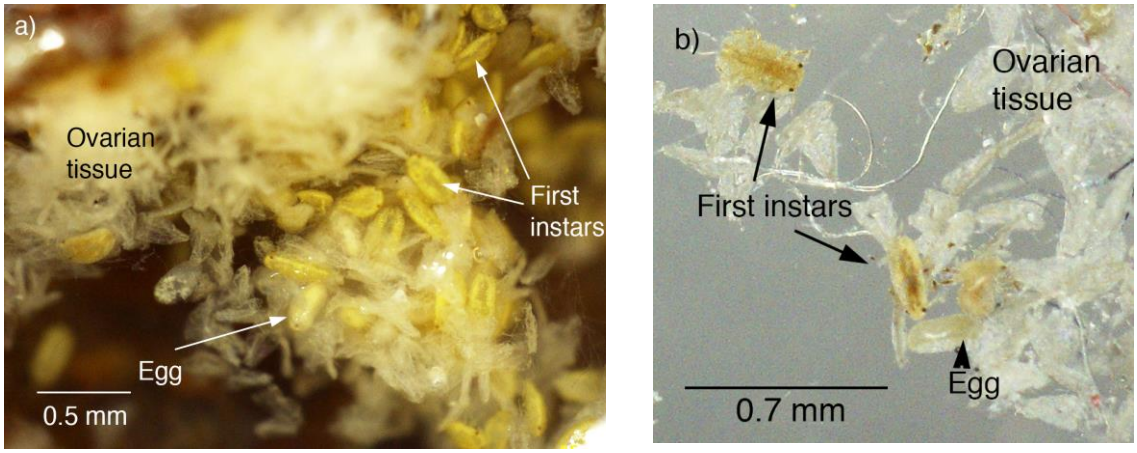


Figure 7. Body length for each instar relative to time since last moult. The time for the first instar is time from egg to hatching into the first instar stage. Mean + 1 S.E.

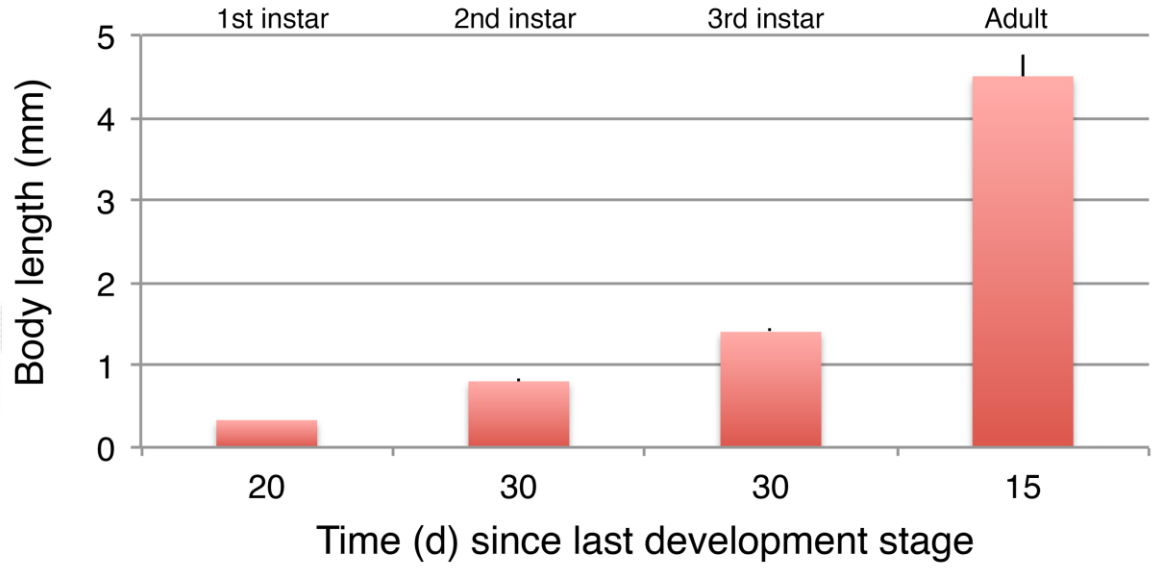


Figure 8. Principal component analysis of output from GC-MS information for chemical compounds in leaves of various cultivars. The separation of Shiraz from Pinot Noir is obvious and the more scale tolerant species tend to separate along the first principal component score, as Shiraz , Riesling, Tempranillo and Sangiovese all have scale, with all but Sangiovese typically having heavy infestation of scales.

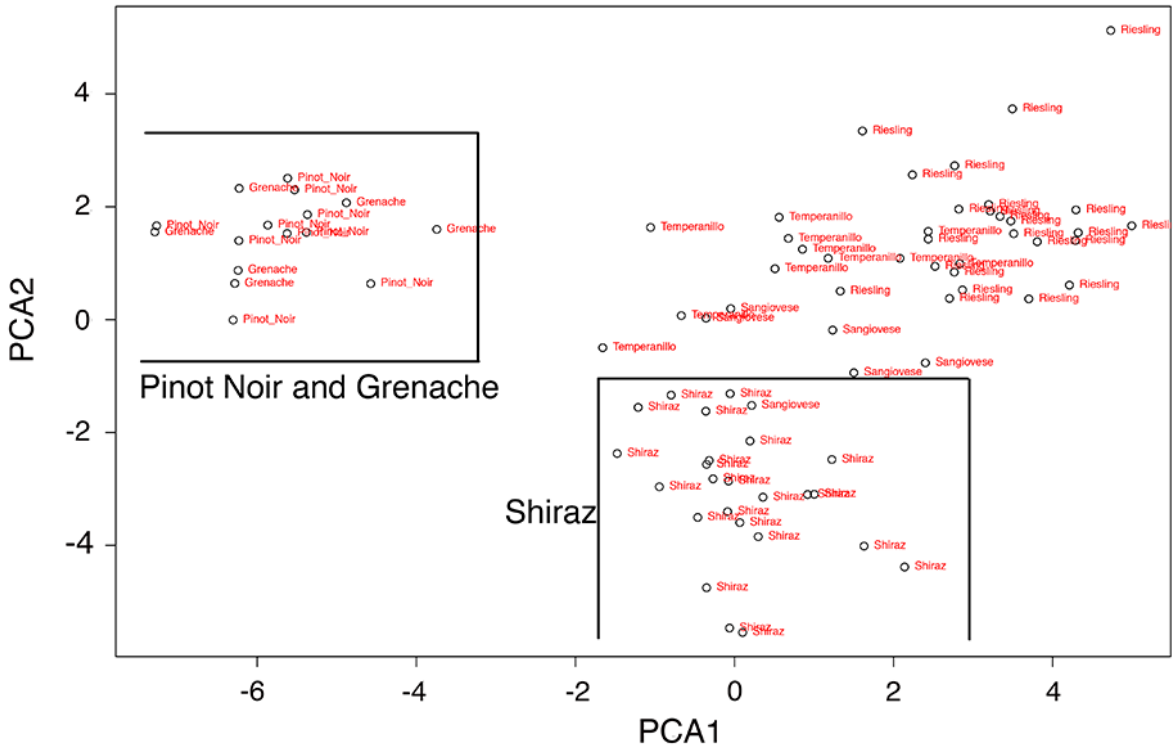


Table S1. Parasitism of grapevine scale in the cool climate wine region of NSW/ACT during 2017-18.

Date	Vineyard	Cultivar	Species	No. of scales	No. with eggs	No. with larval wasps	No. with emerged wasps	% gravid	% parasitised
13 Nov	Mt Majura	Chardonnay	Grapevine	148	85	123	0	57.4	83.1
13 Nov	Mt Majura	Chardonnay	Frosted	64	46	0	0	71.9	0.0
22 Nov	Mt Nanima	Riesling	Grapevine	56	56	0	0	100	0.0
28 Nov	Mt Majura	C. sauvignon	Grapevine	58	54	0	8	93.1	13.8
28 Nov	Mt Majura	Chardonnay	Grapevine	10	4	0	8	40.0	80.0
28 Nov	Mt Majura	Shiraz	Frosted	12	12	0	0	100.0	0.0

Figure S1. Adult with parasitic infections

a) Larvae present within an adult grapevine scale



b) Adult grapevine scale with holes in outer cuticle as a result of parasite emergence from host.

