

**BIOLOGY AND ECOLOGY OF GRAPEVINE SCALE**  
***PARTHENOLECANIUM PERSICAE* (FABRICIUS)**  
**AND FROSTED SCALE *PARTHENOLECANIUM***  
***PRUINOSUM* (COCQUILLET) (HEMIPTERA:**  
**COCCIDAE) ON GRAPEVINES *VITIS VINIFERA* L.**

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# DEDICATION

**To God be the Glory**

This thesis is dedicated with love to my wife Balba Simbiken, two daughters Shelomith Lapiwan Simbiken, Abigail Kwalitakua Simbiken and two sons Jedidiah Kamsause Simbiken and Jeremiah Warasangun Simbiken

Special gratitude to my Mother Catherine Mairakin Simbiken  
and Father Simbiken Kunukhwi Yanbangi

In memory of Boaz Simbiken

11/06/1996 – 17/01/2003

# DECLARATION

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree in any tertiary institution, and to the best of my knowledge and belief, contains no material previously published or written by another person, except when due reference is made in the text of this thesis.

Chapters two, three, four, five and six have been submitted for publication. For all the Journal submissions I am the principle author and contributor to the work.

.....

Nelson Awanim Simbiken

27<sup>th</sup> July 2014

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# THESIS ABSTRACT

Grapevine scale (*Parthenolecanium persicae* Fabricius) and frosted scale (*Parthenolecanium pruinosum* Cocquillet) (Hemiptera: Coccidae) are significant economic pests of several deciduous fruit trees and ornamentals worldwide, including grapevines in Australia. Management using biological control and pesticides can control these scale insects however sporadic outbreaks can still occur across wine zones and districts. This thesis addressed aspects of the biology and ecology of these scales and examined possible responses of *Vitis vinifera* L. varieties to scale feeding activity.

A non-hierarchical cluster analysis of morphological characteristics identified three cluster groups that corresponded to first instars, second instars and adults in frosted scale. Four cluster groups were identified in grapevine scale; first instars, second instars, third instars and adults. Only female scales were present in the populations, and the second and third instars of frosted and grapevine scales respectively were the overwintering stages. Increased growth of appendages did not match total body development, so that the legs, antennae and stylets were relatively shorter as scales matured. Stylet lengths for frosted scale suggested that feeding site did not vary, although location of feeding changed from leaf for first instars to woody branches for later life stages.

Females of grapevine scale had a similarly high fecundity on both Chardonnay and Riesling grapevine varieties. Females of frosted scale had reduced fecundity when present on *V. vinifera* Sauvignon Blanc compared with individuals present on *V. vinifera* Chardonnay

and Riesling varieties. The differences in fecundity of frosted scale were not a result of reduced body size, although smaller individuals were present on Sauvignon Blanc and Riesling. Fertility was at least 94% for both species and was not affected by grape variety.

The pest status of grapevine and frosted scales across the wine zones does not appear to be associated with warm and cold climates. Vineyard size and monoculture and/or mixed cropping of grapevine varieties differed with locale in the pest status of scales, as more small vineyards in the Hunter Valley had infestations, but mixed cropping reduced the incidence of scale in northeastern Victoria. More scales were apparent on white wine grape varieties compared to red wine grape varieties across the regions.

Field and glasshouse studies identified that grapevine and frosted scales exhibit different responses on grapevine varieties. In field studies, population growth of grapevine and frosted scales were much higher on established vines of Riesling and Chardonnay compared to vines of Pinot Noir and Sauvignon Blanc. In greenhouse studies on the response of rootlings of Pinot Noir, Riesling and Sauvignon Blanc, the presence of frosted scale insects reduced chlorophyll content and internode, but only the number of internodes per branch was further reduced as the number of scale insects increased.

This thesis provided several findings that can be used in future studies to address further questions on pest persistence, outbreaks, monitoring and management. Future research on grapevine and frosted scales pest population response and grapevine damage may need to consider the interaction between grapevine nutrition and secondary metabolites, and the differences that are present among grapevine varieties.



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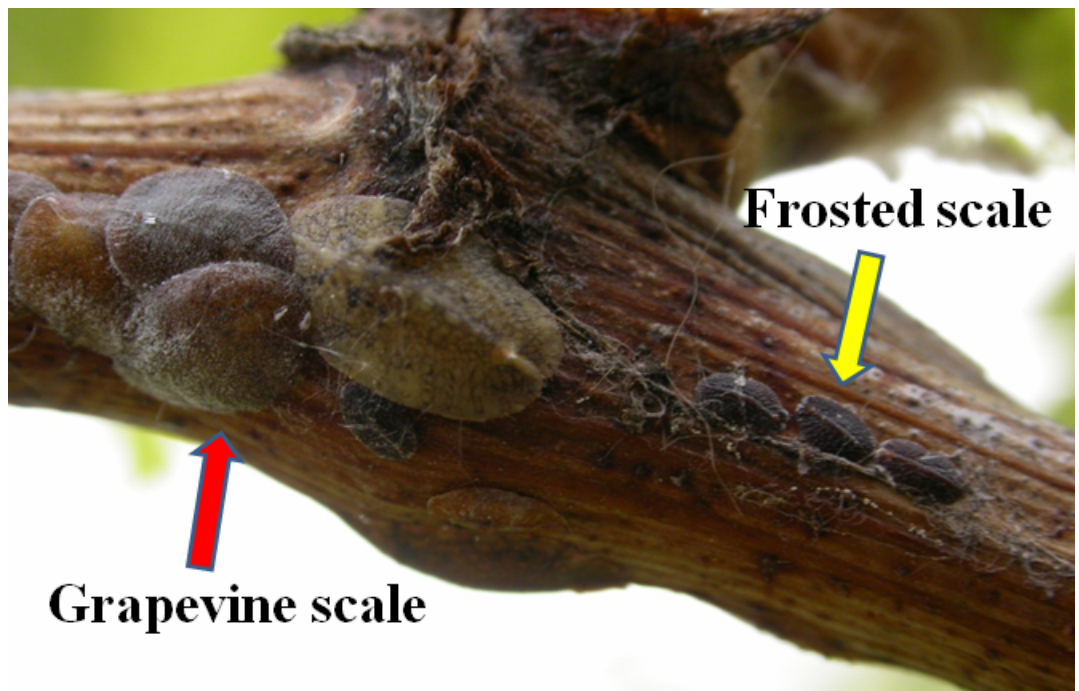
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# CHAPTER 1

## General Introduction



## 1. 0 Historical Background

Wine grape production is an economically important product in Australia. However, as with any agricultural crop, insect pests can be a problem for maintaining production, and the future of the role of pests becomes difficult to predict with any potential change in environment (Risch 1987, Tschardt et al. 2002, Tschardt et al. 2005, Bianchi et al. 2006, Médiène et al. 2011, Jonsson et al. 2012, Segoli and Rosenheim 2012, Tschardt et al. 2012, Scopel et al. 2013).

Currently, there are several species of insects within the soft scales (Coccidae) that are known to feed on grapevines in Australia: *Parthenolecanium persicae* (Fabricius), *Parthenolecanium pruinosum* (Cocquillet), *Coccus hesperidum* (Linnaeus), *Coccus longulus* (Douglas), *Parasaissetia nigra* (Nietner) and *Saissetia* species (Rakimov 2010, Rakimov et al. 2013). Grapevine scale (*Parthenolecanium persicae*) and frosted scale (*Parthenolecanium pruinosum*) are reported to be the dominant and most widespread species in vineyards. Both insects can be found either as single species or in mixed infestations (Rakimov 2010). Feeding activity of these sap feeders results in the production of copious amounts of sugary faeces for sooty mould fungus growth. Sooty mould fungus produces unsightly effects on grapevines and tainting of grape fruits resulting in reduced photosynthesis and market quality of fruits (Miller and Kosztarab 1979, Gill and Kosztarab 1997), as well as potentially affecting wine quality (Bordeu *et al.*, 2012). Plant viruses may be vectored by scales (Cretazzo et al. 2010, Tsai et al. 2010), and direct effects of feeding within the plant vascular tissues leads to plant tissue damage and depletion of plant nutrients.

Grapevine and Frosted scales are polyphagous and can cause significant damage on a wide range of economic crops and ornamentals in temperate and sub-tropical climate worldwide (Hely et al. 1982, Ben-Dov and Hodgson 1997, Pellizzari 1997, Pfeiffer 1997, Swirski et al. 1997) including grapes in Europe and Australia (Hely et al. 1982, Nash et al. 2010, Rakimov 2010, Rakimov et al. 2013). Infestations of both scales can be found in most grape growing zones of Australia. But, the degree of field infestation and damage may vary between geographic zones, individual vineyards and between the grapevine varieties (*Vitis vinifera* L) (Rakimov 2010). In vineyards with high prevalence of scale, infested vines may gradually lose vigour and yield (Figure 1). Keeping the scales below the minimum injury level (MIL) is necessary for maintaining grape production and is part of agricultural pest management (Tang and Cheke 2008).

## **1.1 Ecology**

Grapevine and frosted scales are cosmopolitan scale species, distributed mainly in temperate and sub-tropical climatic zones. Grapevine scale was first reported in Australia in 1901 in vineyards in Western Australia, whereas *P. pruinosum* was first found on plum in NSW in 1928 (Hely et al. 1982, Rakimov 2010). Recent surveys showed that grapevine scale infestation is extended to hosts in sub-tropical and temperate regions of Australia, while *P. pruinosum* is restricted to plants growing in the temperate regions (Rakimov 2010).

Grapevine and frosted scales have univoltine life cycles in most habitats around the world



**Figure 1. Presence of grapevine scale and frosted scale on grapevine leads to retarded growth (red Arrow).**

(Pfeiffer 1997). Young and mature adults of frosted scale produce frosty wax secretion over the sclerotised dorsal surface (Gill 1988, Rakimov 2010), but frosty wax production is absent in grapevine scale. Grapevine scale was reported to be larger in body size than frosted scale on grapevines (Figures 2 and 3) (Rakimov 2010), but body size in scale insects can be influenced by many parameters, including environment and nutrition (Speight 1994, Wardhaugh and Didham 2005). The overwintering stage of grapevine and frosted scales may differ in body size and appearance (Figures 4 and 5), but as the life cycle of *P. pruinosum* is relatively unknown, the stage at which overwintering occurs may differ between the two species. Although *P. persicae* is known to have three nymphal instars and the adult form (Brittin 1940, Rakimov 2010), the number of life stages of *P. pruinosum* has not been examined in southern hemisphere populations. Further work is necessary to clarify both the number of juvenile instars and the instar that overwinters in this species.

## **1.2 General Biology of *P. persicae* and *P. pruinosum***

General aspects of reproduction have been reported for several species of soft scales (Brittin 1940, Michelbacher and Hitchcock 1956, Boratynski 1970, Hely et al. 1982, Gill 1988, Pfeiffer 1997). Rakimov (2010) reports on the number of eggs and fecundity of *P. persicae* and *P. pruinosum* in Australian vineyards. There is considerable variation in the fecundity of *P. persicae* and *P. pruinosum*, presumably as the fecundity depends on climate, host plants, female feeding site and presence of egg parasites (Rakimov, 2010). On Australian grapevines, *P. persicae* were reported to produce 2352 nymphs per female (range 110 – 4343) (Rakimov 2010). *P. pruinosum* produced 1529 nymphs per female

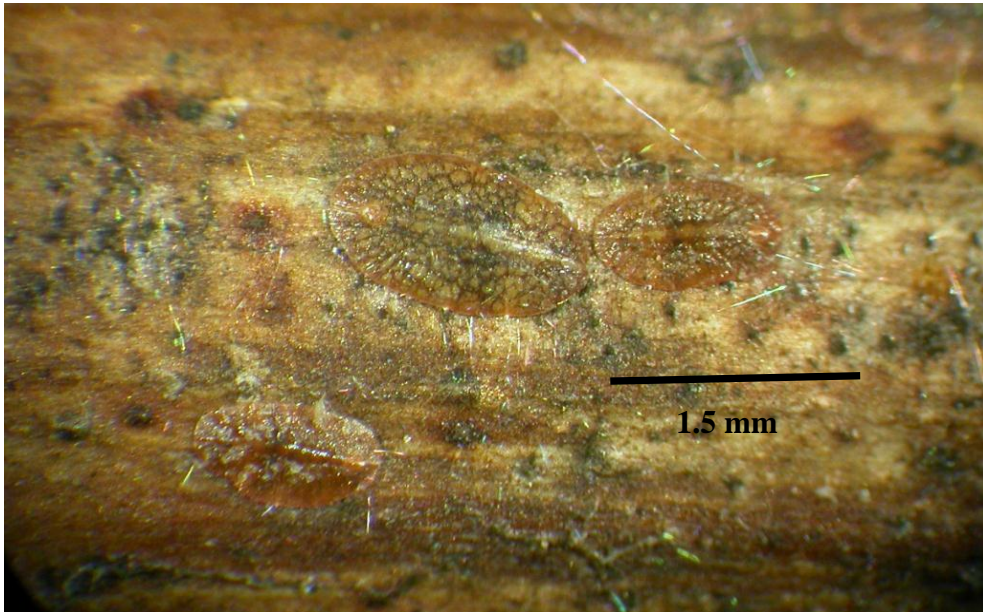


**Figure 2. Mature Female Adult of grapevine scale *P. persicae*.**



**Figure 3. Mature Female Adult of frosted scale *P. prunosum*. The dorsal cuticle is secreted with frosty wax.**





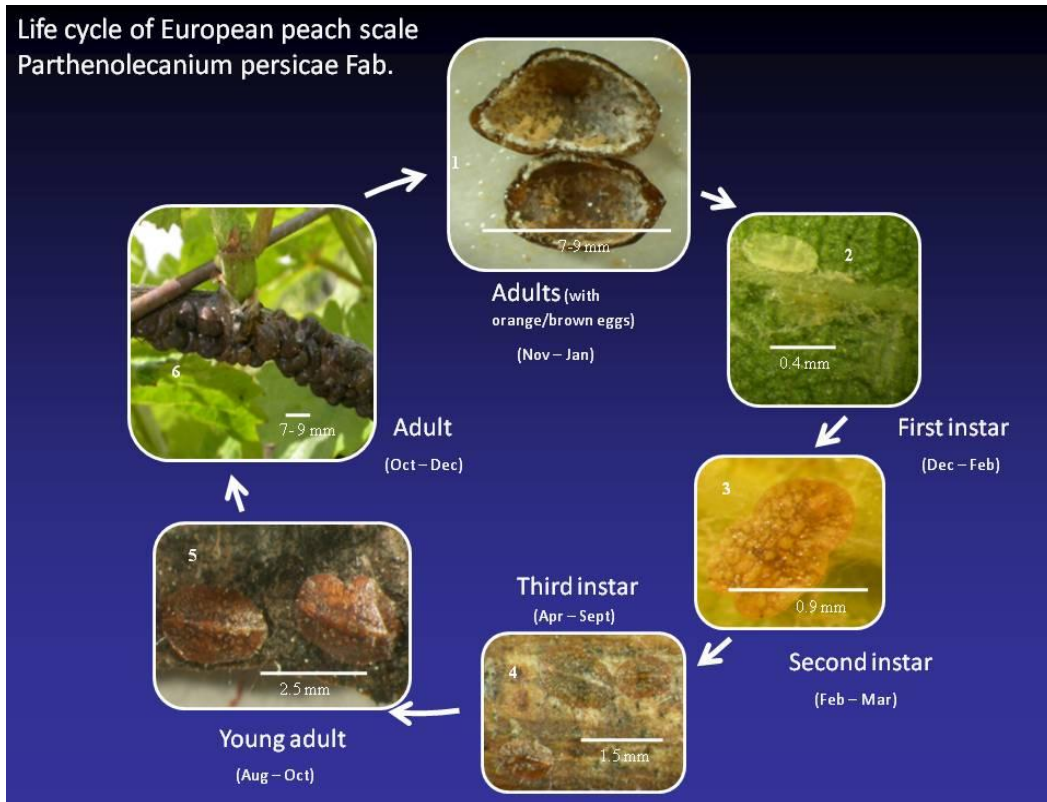
**Figure 4. Overwintering juvenile stage of grapevine scale *P. persicae*. The dorsal cuticle has tessellate black markings and raised medial ridge.**



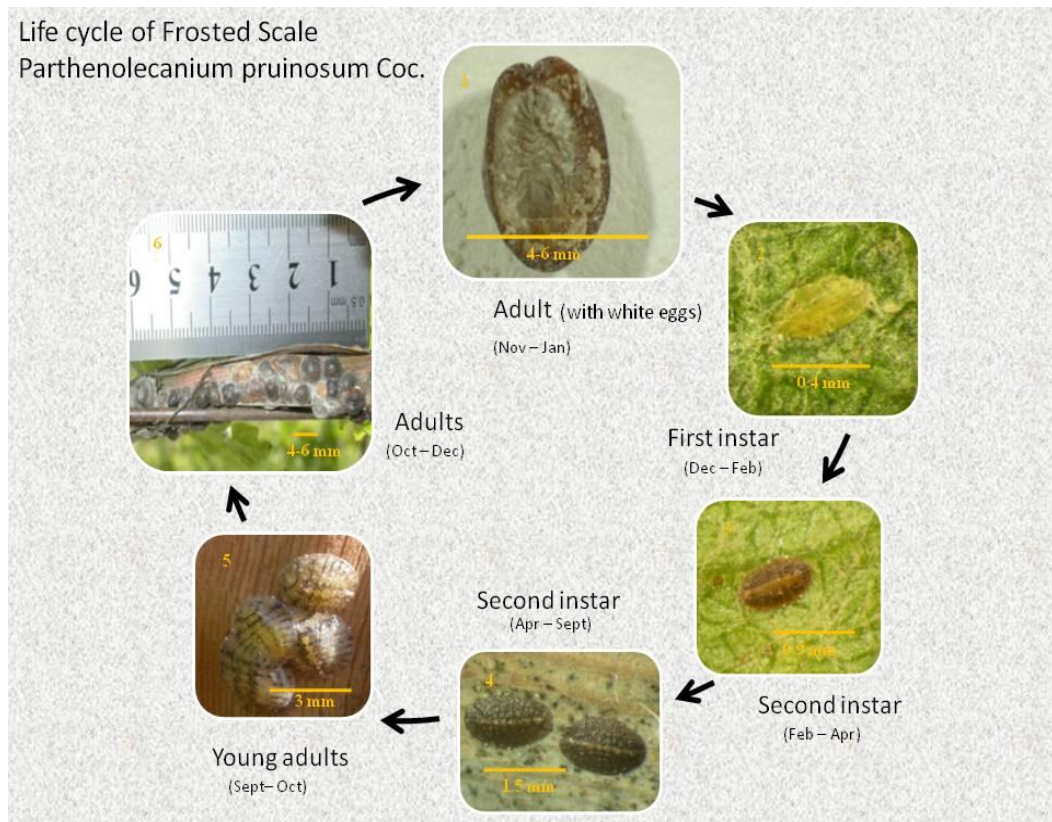
**Figure 5. Overwintering juvenile stage of frosted scale *P. pruinosum*. The dorsal cuticle is purplish brown with raised medial ridge.**

(range 106-3252) (Rakimov 2010). The emergence of first instars (crawlers) of *P. persicae* occurred in October to December (Austral summer) (Rakimov 2010). Crawlers disperse to feeding sites, moving from the stem/branch where adult females were located, to the leaves and soft succulent shoots (Rakimov 2010). Before leaves of deciduous hosts fall (end of summer and/or early autumn), nymphs of *P. persicae* moved from the leaves to the branches or other woody parts (Greathead, 1997, Rakimov 2010). During the cold months (Autumn and Winter), the remaining nymphs (mostly third instars) overwinter under the bark of the vines (Brittin 1940, Rakimov 2010). In Spring, the scales will moult into adults and rapidly develop eggs. *P. persicae* lay eggs in Austral Spring (October and November) (Brittin 1940, Rakimov 2010).

*P. persicae* develop from egg to adult in approximately 12 months in Australia, with three nymphal instars occurring between December and September of the following year; third instars are the overwintering stage (Rakimov 2010) (Figure 6). There is little information on the biology and morphological features of *P. pruinosum* under the Australian climate, although it has univoltine biology in temperate climate of Northern Hemisphere; either the second or third instar has been reported as the overwintering stage (Michelbacher and Hitchcock 1956, Gill 1988, Pfeiffer 1997) (Figure 7). Although, sexual reproduction does occur in the genus *Parthenolecanium* (Coccidae), males were only found in the Hunter Valley (2 individuals) and possibly Western Australia in the Margaret River region in an extensive survey of grapevines (Rakimov 2010). As a result of the rarity of males, reproduction is considered to be parthenogenetic in *P. persicae* in Australia.



**Figure 6. Life stage development of grapevine scale *P. persicae* in a typical Australian vineyard**



**Figure 7. Life stage development of frosted scale *P. prunosum* in a typical Australian vineyard**

With the asexual (parthenogenesis) reproduction inherent in scale insects, population growth can increase as a result of a single insect, with rapid adaptation to plant chemical traits (Hoffmann et al. 2008, Ross et al. 2013). Although grapevine and frosted scales may feed on several grapevine varieties, prolonged feeding and adaptation on certain varieties may increase population more than others, potentially leading to economic effects if scales seriously affect those varieties' yield or health.

## **2.0 Feeding Behaviour of Grapevine and Frosted scales**

Evolution of sedentary life mode results from prolonged adaptation to specific food environment that has varied over space and time (Gullan and Kosztarab 1997, Bernays 1998). With the sedentary life mode, larval stages and adults may feed on the same food sources resulting in local adaptation (Alstad and Edmunds Jr 1983, Hanks and Denno 1994, Hanks and Denno 1998, Spitzer 2004, Spitzer 2006). Potential for self-selection of food is limited in sedentary insects and takes whatever the environment offers. Food selection therefore occurs at finer scale and is driven in part by plant chemistry (nutrition and toxins) (Dethier 1954, Barrett and Heil 2012, Mithöfer and Boland 2012). Declining food quality affect sedentary insects profoundly since dispersal is restricted, thus population growth rate is reduced on poor host plants (Washburn et al. 1985). Severe effect of plant sap feeders are observed in less defended and/or highly nutritious plants (Cates 1980), especially with increased number of insects.

### **2.1 Feeding Structure in Scale Insects**

Morphology of stylets in relation to food source has been studied for zoophagous, phytophagous and zoophytophagous hemipterans as these insects can be pests and transmission vectors of diseases of plants and animals (Boyd Jr et al. 2002, Douglas 2006, Powell et al. 2006). Sap-feeding insects are predominantly from the subclasses Cicadomorpha, Fulgoromorpha, Sternorrhyncha and some Heteroptera (Hemiptera). They have highly specialized stylet structures and sensory organs for isolating host plants and plant tissues. Improved knowledge of the stylet morphology and feeding behaviour have been made possible through various techniques of histology and electrical penetration graph (Tjallingii and Esch 1993, Annan et al. 2000, Tjallingii, 2001, Leopold et al. 2003, Backus et al. 2005, Jin and Baoyu 2007, Backus et al. 2009, Xue et al. 2009, Boquel et al. 2011). These studies have led to a better understanding of insect-plant relationships, resistance/tolerance and co-evolution that have been utilised for pest management.

Scale insects (Hemiptera; Sternorrhyncha:Coccoidea) inhabit various organs of plants from the roots up to the leaves. Two phase evolutionary history of scale insects were: a primary phase of soil dwellers feeding by sucking roots, rotten plants and fungal hyphae and a secondary phase of aboveground dwelling on leaves and stems (Gullan and Kosztarab 1997). Aerial-dwelling families evolved recently, with the largest groups in Diaspididae (armored scales, about 2400 species), Pseudococcidae (mealybugs, about 2200 species) and Coccidae (soft scales, about 1150 species) (Ben-Dov and Hodgson 1997).

Coccoidea (synonym coccoid) is closely related to sister group of aphids (Aphidoidea), jumping lice (Psylloidea) and white flies (Alleyrodoidea) (Hemiptera: Sternorhycha) (Gullan and Kosztarab 1997). The majority of the coccoids have functional legs amongst

the first instar stages (Ben-Dov and Hodgson 1997, Gullan and Kosztarab 1997). As a consequence, many species exhibit restricted mobility between various organs of the host plant, thus utilising first instar stages for selection of feeding sites (Greathead 1997). The coccoids are effective colonisers simply by adjusting their biology (adaptation) to the host plant phenology.

## **2.2 Relationship Between Feeding Structure and Feeding Behaviour**

There is considerable variability in stylet shape and size among hemipterans (Goodchild 1966). Stylet length and width have been used to identify feeding location within plant cells of several sap-sucking insects (Ben-Dov and Hodgson 1997, Freeman et al. 2001, Cid and Fereres 2010, Garzo et al. 2012, Zhao et al. 2012, Sandanayaka et al. 2013, Zhao et al. 2013). In rice brown plant hopper *Nilaparvata lugens* Stal (Delphacidae), stylet length ranged between 650 and 700  $\mu\text{m}$  which enable feeding in the xylem tissue of rice plants (Foster et al. 1983). Hemipterans feeding in the phloem cells of the leaves may have stylet lengths ranging between 110 and 200  $\mu\text{m}$  (Freeman et al. 2001). Freeman et al. (2001), observed stylet length of *Bemisia argentifolii* Bellows and Perring (Aleyrodidae) increased with each moult to maintain the feeding location in the phloem cells as leaf cells became thicker.

Scale insects feed extensively in the vascular tissues of host plant. Small insects may obtain plant fluid from just a few cells below the leaf epidermis (or even within leaf epidermis) in the mesophyll and parenchyma cells, while larger insects may utilise cells of the vascular bundle such as phloem and xylem cells. Studies have shown that stylet sizes are



proportional to body size depending on each development stage, thus the stylet structure may represent how plant sap feeders obtain their nutrients from plants (Fold, 1990, Foldi 1997, Cid and Fereres 2010, Zhao et al. 2013). For example male nymphs of Chinese white wax scale insect (CWWS), *Ericerus pela* (Chavannes) (Coccidae) frequently feed on parenchyma cells by lodging on the adaxial side of leaves (lower surface of leaf) while female nymphs feed in the phloem cells by lodging in the abaxial side of leaves (upper leaf surface) (Zhao et al. 2013). Mealybugs (Pseudococcidae) feed mostly on phloem cells, however they occasionally feed on xylem cells if reduced humidity (or moisture) is detected within vascular tissues (Cid and Fereres 2010, Sandanayaka et al. 2013).

Although most appendages of Coccids decrease as body size increases, stylet bundle size may vary depending on feeding sites (Gullan and Kosztarab 1997). Stylet length may thus vary with insect size, the requirement for different nutrients and the location of plant sap or cells.

### **3.0 Interactions between Grapevine and Grapevine and Frosted scales**

*P. persicae* and *P. prunosum* on grapevines may develop rapidly in warmer regions than in cooler regions. For example *P. persicae* can develop a partial second generation in warmer climates but in cool temperate regions have one annual generation (Masten-Milek et al. 2007, Rakimov 2010). *Parthenolecanium corni* Bouche has up to three generations in warmer areas and a single generation in cool climates (Danzig 1997). Further changes in temperature and effect on grapevine growth with climate change may affect seasonal development and voltinism of these scales (Kivelä et al. 2013).

Univoltine biology of *P. persicae* and *P. pruinosum* is closely associated with grapevine phenology. Overwintering stage of grape scales resume feeding when grapevine sap flow (active plant metabolism) commences in grapevine with warm spring temperatures (Mullins et al. 1992, Lebon et al. 2008, Holzzapfel et al. 2010, Zufferey et al. 2012). Since plant nutrition is stored in parenchyma cells of vine trunks (Lebon et al. 2008, Zufferey et al. 2012), overwintering stages on cordons and trunks may feed on these tissues. For target growth and reproduction, balanced nutrition may be obtained from the phloem and mesophyll cells. Crawler and juvenile stages emerge and feed during the advanced stage of maturity in fruit bunch, leaf and stem which are declining in nutritional and biomass composition (Greer and Sicard 2009). Adaptation for food resource which has low nutritional quality is widespread in polypahgous insects including scales. Shoot growth and leaf flush consist of relatively high nutrition and plant chemical defences and overtime declines to lower levels in matured leaves (Cizek 2005, Cizek et al. 2006, Blüthgen and Metzner 2007). *P. persicae* and *P. pruinosum*, although present together on single grapevines, may differ in development rate and population size, simply because of differences in feeding or phenology.

#### **4.0 Management of Grape scales**

Grapevine and frosted scales can be managed using natural enemies (parasites and predators) (Sands and Van Driesche 2004), pesticides (Hely et al. 1982), viticulture practices such as pruning and intercropping with different grapevine varieties and non-host plants. Cultivation of mixed crop varieties can affect insect population dynamics in

agricultural cropping system depending on the resistant traits of host plants (Utsumi et al. 2011, Ratnadass et al. 2012, Yang et al. 2012, McArt and Thaler 2013). This aspect of pest management against grapevine and frosted scale has not been considered, although mixed grapevine varieties are cultivated.

There are several predators and parasites of grape scales (Pellizzari 1997). These organisms can be present throughout late spring and summer months. *Metaphycus maculipennis* (Timberlake) (Hymenoptera: Chalcidoidea: Encyrtidae) is an endoparasite and highly host specific which attacks only *P. persicae* (Sands and Van Driesche 2004). *M. maculipennis* is released in vineyards for the control of *P. persicae* in Australia (Rakimov 2010). For biological control of *P. prunosum*, an endoparasite *Metaphycus californicus* (Hymenoptera: Chalcidoidea: Encyrtidae) is an effective agent in California, USA (Michelbacher and Swift 1954, Michelbacher and Hitchcock 1956), but this wasp has not been imported and released in Australia. Both parasites have several generations in a year and parasitize (oviposits) juvenile stages. They may overwinter inside the scale and develop rapidly in spring. *M. californicus* emerge from adult scales by the end of spring (Michelbacher and Swift 1954). *M. maculipennis* also emerge from the adults by end of spring (Rakimov 2010), and parasitised females have a large reduction in their viable offspring. Parasite oviposition inside the scales in early autumn is an important adaptation for refuge/protection during the winter. Negative impact on the survival of parasites is high when pesticides are used for the control of fungus diseases such as powdery mildew, downy mildew and botrytis during the summer months (Bostanian et al. 2009). Fungicides are ineffective against grape scales although sulphur based fungicides may be effective on the crawlers. Intensive use of pesticides and other antagonistic agrochemicals can deprive

the vineyard of important beneficial insects (parasites and predators) (Duelli et al. 1990, Hardin et al. 1995, Corino and Calo 2001, Altieri and Nicholls 2002, Altieri et al. 2005, Tesic et al. 2007, Thomson and Hoffmann 2007a, Thomson and Hoffmann 2007b, Thomson et al. 2007, Thomson and Hoffmann 2010).

Pruning of grapevine is carried out every year in winter months for next season growth. However, the value of spur and cane pruning and/or recycle pruning on the control of grape scale is unclear, although this mechanism may suppress population build up on an annual basis. Grapevines may consist of parasitized scales and can be removed during pruning. Thus, parasite activity may be seriously curtailed by pruning and pesticides.

Alternate forms of crop protection and conservation of beneficial insects is needed to reduce occasional outbreak of *P. persicae* and *P. pruinosum*. Integrated pest management (IPM) is the widely used practice in agricultural pest management with the option of using less disruptive pesticides and increase vegetative biodiversity for the benefit of natural enemy function (Matthews 2003, Radcliffe et al. 2009). IPM requires a thorough knowledge of pest-natural enemy interaction so that pest density is maintained below the economic injury level and the economic return is maximised from the use of several pest control techniques (biological, cultural and chemical) (Tang and Cheke 2008). Decisions on the use of pest control techniques depend on the pest density reaching the economic threshold (ET); above this point, economic loss is imminent. In order to maintain a suitable IPM program in vineyards against scale insects, knowledge on pest population dynamics and the potential of various grapevine varieties to affect scale populations is a necessary part of pest monitoring system.

Presence of sooty mould fungus on the grapevines and fruits can occur during the grapevine fruit bearing period. This is associated with the actively feeding juvenile instars, producing honey dew, a sugary faeces excreted by the scales. The presence of ants (especially *Iridomyrmex* sp.) feed on the sugary faeces (Figure 8) (Chong et al. 2010, Rakimov, 2010, Chong et al. 2011). Ants are providing sanitation, transportation and protection for honey dew producing insects, as well as reduce the cause of sooty mould fungus growth (Gullan and Kosztarab 1997, Chong et al. 2010). However, the presence of ants may limit the ability of natural control by other insects, as the ants will protect the scales from attack.

## **5.0 Pest Status of Grapevine and Frosted scales**

The genus *Vitis* (Vitaceae) has 60 species, with origins from Europe, Asia and North America ((Mullins et al. 1992). Cultivars of *Vitis vinifera* (approximately 10,000 genotypes/varieties) which is of European origin are grown for production of wine, table grapes and raisins. *V. vinifera* is cultivated in the southern hemisphere, especially South Africa, South America, Australia and New Zealand (Mullins et al. 1992). There are 94 commercial grape species under cultivation, 52 red wine species and 42 white wine species (Kerridge and Antcliff 1999, Dry 2004). The selection of grapevine varieties for cultivation is determined by climate, soil and market demand (Schamel and Anderson 2003, Patchell 2008, Hall and Jones 2010, Jones et al. 2010). Generally, grapes are cultivated under narrow temperature range 10 – 24 °C, that requires vineyards to be located between 30-35 degree latitude in the northern hemisphere and 30 – 40 degree altitude in the southern

hemisphere (Petrie and Sadras 2008, Hall and Jones 2010). For wine grape species, the temperature range between 13 – 21 °C and for table grape species and fortified wines



**Figure 8. Ants attending to young adult frosted scale *P. pruinosum* on *V. vinifera* Riesling variety**

between 21 – 24 °C. Australian climate is suitable for grape cultivation consisting of dry, warm and cool nights (Dry and Coombe 2005, Soar et al. 2008). There are 63 wine regions in Australia based on geographic indications (GI), with majority of GIs (28) located in warm regions (17 - 19 °C median Growing Season Temperature (GST) over 7 months, October -April). Several regions are in the intermediate (15 – 17 °C GST) and hot categories (19 – 21 °C GST) (consisting of 14 and 16 GIs respectively). Few GIs are in the cool (13 – 15 °C) and very hot (21 – 24 °C) categories (2 and 3 regions respectively) (Hall and Jones 2010). There is considerable overlap between regions for GST and topography (elevation) for suitability of cultivated variety (Hall and Jones 2010). Regions with broad elevation and temperature range may cultivate several grapevine varieties, although certain varieties do better at specific locations because of climate.

Grapevine variety finds its expression of wine quality in a unique climate and region supplemented by history and culture of wine growing. This pattern impacts on the decision of farmers on the cultivation of grapevines. Individual vineyards and estate growers build a collective reputation with their locality to provide them with a platform to market their own differentiated product (Patchell 2008). Regional and varietal differentiation relative to consumer demand is evident in Australia (Schamel and Anderson 2003). Therefore cultivation of mixed grapevine varieties is a common practice leading to spatial availability of grapevines that can affect distribution and pest status of grapevine and frosted scales across wine growing zones and districts.

The vineyard environment is spatially heterogeneous (Bramley and Hamilton 2004, Panten and Bramley 2011, Santesteban et al. 2013), consisting of diverse crop varieties, non-crop plants and natural enemy composition (Altieri and Nicholls 2002, Altieri et al. 2005, Bruggisser et al. 2010, Thomson et al. 2010, Trivellone et al. 2012). Cultivation of intra-species mix of grapevine varieties may generate favourable and/or unfavourable environments for vineyard arthropods. Studies have shown that cultivation of mixed crop varieties can reduce pest populations (Yang et al. 2012, McArt and Thaler, 2013, Yang et al. 2014), but whether such a response occurs in vineyards is unknown. However, Alteri et al. (2005) have reported that pest populations may increase in vineyards with a monoculture structure compared with mixed cropping of vine varieties.

Although pest insects may feed on several grapevine varieties, few varieties can support high pest populations (Moreau et al. 2006, Moreau et al. 2007, Tesic et al. 2007). Grapevines may have different levels of secondary plant chemicals, such as phenolic and volatile organic compounds, that can be detrimental and/or attractive respectively (Moreau et al. 2006, Moreau et al. 2007, Muganu and Paolucci 2013). Grapevine varieties with lower phenolic content or certain phenolics may be more susceptible to pest insects and diseases (Moreau et al. 2006, Moreau et al. 2007, Muganu and Paolucci 2013).

## **6.0 Outline of Research Undertaken**

*P. persicae* and *P. pruinosum* are significant pests of perennial crops, including grapes. Seedling and young grapevines may be especially affected by the presence of scale insects, whereas the mature grapevines may have only limited responses, but can act as reservoirs



for further scale population growth in subsequent seasons. There is considerable variation in the prevalence rate and intensity of infestation in vineyards across Australia (Rakimov 2010). In order to devise appropriate management strategies, a fundamental understanding of the biology (including morphological changes) and ecology of these insects relative to the varieties of grapevine infested is needed. The role of this thesis is to expand on what is known regarding aspects of biology of these insects and how grapevine variety may influence these processes.

Using both the laboratory and field studies several questions on the life stage development, population growth, host plant preference and distribution, population dynamics and feeding effect on grapevine performance were evaluated. The research in chapters 2, 3, 4, 5, and 6 were produced as standalone chapters with an introduction, methods, results, discussion and conclusion. These are original studies with new contributions to knowledge in feeding behaviour, growth and biology of grapevine and frosted scales.

Chapter 2 describes the number of instars in both grapevine and frosted scales using morphological characters and the duration of each life stage were determined for both scale insects using laboratory and field measurements.

Chapter 3 determined reproduction and population growth rate in both species and provides knowledge on the reproductive and outbreak potential of the two scale insects (Prepared for publication in *Annals of Entomological Society of America*).

Chapter 4 uses responses from a survey to growers in the Northeast Victoria and Hunter Valley grape growing regions to investigate the distribution and prevalence of scale insects on grapevine varieties and vineyards. This provides new information how scale insects interact with grapevines and how scales as a pest are perceived by growers.

Chapter 5 examines population dynamics in selected vineyards in the Canberra wine region near Australian Capital Territory. Intensity and pattern of infestation among vineyards and between grapevine varieties is presented.

Chapter 6 is a greenhouse study that uses selected grapevine varieties identified as either susceptible or resistant in chapters 4 and 5 to be evaluated for host preference and effect on feeding on grapevine growth under controlled environment. This research helps validate the host specificity and damage that can be caused by the feeding activity of grapevine and frosted scales.

The concluding chapter 7 summarises and integrates the information from the other chapters, and identifies future research directions.

## 7.0 References

- Alstad DN and Edmunds Jr GF. 1983. Adaptation, host specificity, and gene flow in the black pineleaf scale. In: *Variable Plants and Herbivores in Natural and Managed Systems*. (eds R Denno and MS Mclure) . pp. 413-426. Academic Press.
- Altieri MA and Nicholls CI. 2002. The simplification of traditional vineyard based agroforests in northwestern Portugal: some ecological implications. *Agroforestry Systems* **56**, 185-191.
- Altieri MA, Ponti L and Nicholls CI. 2005. Manipulating vineyard biodiversity for improved insect pest management: case studies from northern California. *International Journal of Biodiversity Science and Management* **1**, 191-203.
- Annan IB, Tingey WM, Schaefers GA, Tjallingii WF, Backus EA and Saxena KN. 2000. Stylet penetration activities by *Aphis craccivora* (Homoptera: Aphididae) on plants and excised plant parts of resistant and susceptible cultivars of cowpea (Leguminosae). *Annals of the Entomological Society of America* **93**, 133-140.
- Backus EA, Habibi J, Yan F and Ellersieck M. 2005. Stylet penetration by adult *Homalodisca coagulata* on grape: electrical penetration graph waveform characterization, tissue correlation, and possible implications for transmission of *Xylella fastidiosa*. *Annals of the Entomological Society of America* **98**, 787-813.

- Backus EA, Holmes WJ, Schreiber F, Reardon BJ and Walker GP. 2009. Sharpshooter X wave: correlation of an electrical penetration graph waveform with xylem penetration supports a hypothesized mechanism for *Xylella fastidiosa* inoculation. *Annals of the Entomological Society of America* **102**, 847-867.
- Barrett LG and Heil M. 2012. Unifying concepts and mechanisms in the specificity of plant–enemy interactions. *Trends in Plant Science* **17**, 282-292.
- Ben-Dov Y and Hodgson CJ. 1997. World crop pests. soft scale insects: their biology, natural enemies and control. Vol 7A. Elsevier Science, Amsterdam, Netherlands.
- Bernays EA. 1998. Evolution of feeding behavior in insect herbivores. *BioScience* **48**, 35-44.
- Bianchi FJJA, Booij CJH and Tscharntke T. 2006. Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and natural pest control. *Proceedings of the Royal Society B Series: Biological Sciences* **273**, 1715-1727.
- Blüthgen N and Metzner A. 2007. Contrasting leaf age preferences of specialist and generalist stick insects (Phasmida). *Oikos* **116**, 1853-1862.
- Boquel S, Giordanengo P and Ameline A. 2011. Probing behavior of apterous and alate morphs of two potato colonizing aphids. *Journal of Insect Science* **11**, 1-10.

- Boratynski K. 1970. On some species of 'Lecanium' (Homoptera, Coccidae) in the collection of the Naturhistorisches Museum in Vienna; with description and illustration of the immature stages of *Parthenolecanium persicae*. *Analen des Naturhistorischen Museums Wien* **74**, 63-76.
- Bordeu E, Troncoso DO and Zaviezo T. 2012. Influence of mealybug (*Pseudococcus* spp.)-infested bunches on wine quality in Carmenere and Chardonnay grapes. *International Journal of Food Science and Technology* **47**, 232-239.
- Bostanian N, Thistlewood H, Hardman J and Racette G. 2009. Toxicity of six novel fungicides and sulphur to *Galendromus occidentalis* (Acari: Phytoseiidae). *Experimental and Applied Acarology* **47**, 63-69.
- Boyd Jr DW, Cohen AC and Alverson DR. 2002. Digestive enzymes and stylet morphology of *Deraeocoris nebulosus* (Hemiptera: Miridae), a predacious plant bug. *Annals of the Entomological Society of America* **95**, 395-401.
- Bramley RGV and Hamilton RP. 2004. Understanding variability in winegrape production systems. *Australian Journal of Grape and Wine Research* **10**, 32-45.
- Brittin G. 1940. The life history of *Lecanium (Eulecanium) persicae* (Fabricius), and descriptions of the different instars. *Transactions and Proceedings of the Royal Society of New Zealand* **69**, 413-421.

- Bruggisser OT, Schmidt-Entling MH and Bacher S. 2010. Effects of vineyard management on biodiversity at three trophic levels. *Biological Conservation* **143**, 1521-1528.
- Cates R. 1980. Feeding patterns of monophagous, oligophagous, and polyphagous insect herbivores: The effect of resource abundance and plant chemistry. *Oecologia* **46**, 22-31.
- Chong CS, D'alberto CF, Thomson LJ and Hoffmann AA. 2010. Influence of native ants on arthropod communities in a vineyard. *Agricultural and Forest Entomology* **12**, 223-232.
- Chong CS, Thomson LJ and Hoffmann AA. 2011. High diversity of ants in Australian vineyards. *Australian Journal of Entomology* **50**, 7-21.
- Cid M and Fereres A. 2010. Characterization of the probing and feeding behavior of *Planococcus citri* (Hemiptera: Pseudococcidae) on grapevine. *Annals of the Entomological Society of America* **103**, 404-417.
- Cizek L. 2005. Diet composition and body size in insect herbivores: Why do small species prefer young leaves? *European Journal of Entomology* **102**, 675-681.
- Cizek L, Fric Z and Konvicka M. 2006. Host plant defences and voltinism in European butterflies. *Ecological Entomology* **31**, 337-344.

- Corino L and Calo A. 2001. Sustainable viticulture: current practices and future developments. *Agriculturae Conspectus Scientificus* **66**, 3-11.
- Cretazzo E, Padilla C, Carambula C, Hita I, Salmerón E and Cifre, J. 2010. Comparison of the effects of different virus infections on performance of three major grapevine cultivars in field conditions. *Annals of Applied Biology* **156**, 1-12.
- Danzig EM. 1997. Intraspecific variation of taxonomic characters. In: *World Crop Pests. Soft Scale Insects: Their Biology, Natural Enemies and control. Vol 7A.* (eds Y Ben-Dov and CJ Hodgson). pp. 203-212. Elsevier Science, Amsterdam, Netherlands.
- Dethier V. 1954. Evolution of feeding preferences in phytophagous insects. *Evolution* 33-54.
- Douglas AE. 2006. Phloem-sap feeding by animals: problems and solutions. *Journal of Experimental Botany* **57**, 747-754.
- Dry P and Coombe B. 2005. *Viticulture volume 1–Resources*. 2nd ed. Adelaide, Australia: Winetitles.
- Dry PR. 2004. Grapevine varieties. In: *Viticulture Volume 1–Resources* (eds P. Dry and B Coombe). Winetitles. pp. 132-149. Adelaide, Australia.

- Duelli P, Studer M, Marchand I and Jakob, S. 1990. Population movements of arthropods between natural and cultivated areas. *Biological Conservation* **54**, 193-207.
- Foldi I. 1990. Internal anatomy. In: *Armored Scale Insects. Their Biology, Natural Enemies and Control. Volume 4A.* (ed. D Rosen). pp 65 – 80. Elsevier, Amsterdam, Netherlands.
- Foldi I. 1997. Internal anatomy of the adult female. In: *World Crop Pests. Soft Scale Insects: Their Biology, Natural Enemies and control. Vol 7A.* (eds Y Ben-Dov and CJ Hodgson) pp. 73-90. Elsevier Science, Amsterdam, Netherlands.
- Foster S, Goodman LJ and Duckett JG. 1983. Sensory receptors associated with the stylets and cibarium of the rice brown planthopper, *Nilaparvata lugens*. *Cell and Tissue Research* **232**, 111-119.
- Freeman TP, Buckner JS, Nelson DR, Chu CC and Henneberry TJ. 2001. Stylet penetration by *Bemisia argentifolii* (Homoptera: Aleyrodidae) into host leaf tissue. *Annals of the Entomological Society of America* **94**, 761-768.
- Garzo E, Bonani JP, Lopes JRS and Fereres A. 2012. Morphological description of the mouthparts of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). *Arthropod Structure and Development* **41**, 79-86.



- Gill RJ. 1988. The scale insects of California, Part 1: the soft scales (Homoptera: Coccoidea: Coccidae). In: *Analysis and Identification Branch, California Dept. of Food and Agriculture*. Sacramento, California, USA.
- Goodchild A. 1966. Evolution of the alimentary canal in the Hemiptera. *Biological Reviews* **41**, 97-139.
- Gill RJ and Kosztarab M. 1997. Pest status of soft scale insects: Economic importance. In: *World Crop Pests. Soft scale insects: their biology, natural enemies and control. Vol. 7B*. (eds Y. Ben-Dov and CJ Hodgson). pp. 161-163. Elsevier Science, Amsterdam, Netherlands.
- Greathead DJ. 1997. Crawler behaviour and dispersal. In: *World Crop Pests. Soft scale insects: their biology, natural enemies and control. Vol. 7A*. (eds Y. Ben-Dov and CJ Hodgson). pp. 339-342. Elsevier Science, Amsterdam, Netherlands.
- Greer DH and Sicard SM. 2009. The net carbon balance in relation to growth and biomass accumulation of grapevines (*Vitis vinifera* cv. Semillon) grown in a controlled environment. *Functional Plant Biology* **36**, 645-653.
- Gullan PJ and Kosztarab M. 1997. Adaptations in scale insects. *Annual Review of Entomology* **42**, 23-50.

- Hall A and Jones GV. 2010. Spatial analysis of climate in winegrape-growing regions in Australia. *Australian Journal of Grape and Wine Research* **16**, 389-404.
- Hanks L and Denno R. 1998. Dispersal and adaptive deme formation in sedentary coccoid insects. In: *Genetic Structure and Local Adaptation in Natural Insect Populations*. (eds S Mopper and S Strauss). pp 239-262. Springer, US.
- Hanks LM and Denno RF. 1994. Local adaptation in the armored scale insect *Pseudaulacaspis pentagona* (Homoptera: Diaspididae). *Ecology*. 2301-2310.
- Hardin MR, Benrey B, Coll M, Lamp WO, Roderick GK and Barbosa P. 1995. Arthropod pest resurgence: an overview of potential mechanisms. *Crop Protection* **14**, 3-18.
- Hely PC, Pasfield G and Gellatley JG. 1982. *Insect pests of fruit and vegetables in NSW*, Inkata Press.
- Hoffmann AA, Reynolds TK, Nash MA and Weeks AR. 2008. A high incidence of parthenogenesis in agricultural pests. *Proceedings of Royal Society of Biological Science* **275**, 2473-2481.
- Holzapel BP, Smith JP, Field SK and Hardie WJ. 2010. Dynamics of carbohydrate reserves in cultivated grapevines. *Horticultural Reviews* **37**. John Wiley and Sons, Inc.

- Jin M and Baoyu H. 2007. Probing behavior of the tea green leafhopper on different tea plant cultivars. *Acta Ecologica Sinica* **27**, 3973-3982.
- Jones GV, Duff AA, Hall A and Myers JW. 2010. Spatial analysis of climate in winegrape growing regions in the western United States. *American Journal of Enology and Viticulture* **61**, 313-326.
- Jonsson M, Buckley HL, Case BS, Wratten SD, Hale RJ. and Didham RK. 2012. Agricultural intensification drives landscape-context effects on host–parasitoid interactions in agroecosystems. *Journal of Applied Ecology* **49**, 706-714.
- Kerridge G and Antcliff AJ. 1999. Wine grape varieties. CSIRO, Australia.
- Kivelä SM, Välimäki P and Gotthard K. 2013. Seasonality maintains alternate life-history phenotypes. *Evolution* **67**, 3145-3160.
- Lebon G, Wojnarowicz G, Holzapfel B, Fontaine F, Vaillant-Gaveau N and Clément C. 2008. Sugars and flowering in the grapevine (*Vitis vinifera* L.). *Journal of Experimental Botany* **59**, 2565-2578.
- Leopold R, Freeman T, Buckner J and Nelson D. 2003. Mouthpart morphology and stylet penetration of host plants by the glassy-winged sharpshooter, *Homalodisca coagulata*, (Homoptera: Cicadellidae). *Arthropod Structure and Development* **32**, 189-199.

Masten-Milek T, Šimala M, Korić B and Bjeliš M. 2007. Status of scale insects (Coccoidea), family Coccidae, on grapes in 2006. In Croatia with emphasis on rarity of second generation of *Parthenolecanium corni* (Bouche) and *Parthenolecanium persicae* (Fabricius). *Predavanj in Referatov* 326.

Matthews G. 2003. Integrated Pest Management Principles. In: *Encyclopedia of Applied Plant Sciences*. (ed. T Brian) Oxford, Elsevier.

McArt SH and Thaler JS. 2013. Plant genotypic diversity reduces the rate of consumer resource utilization. *Proceedings of the Royal Society Biological Sciences* **280**. doi:[10.1098/rspb.2013.0639](https://doi.org/10.1098/rspb.2013.0639):20130639.

Médiène S, Valantin-Morison M, Sarthou JP, De Tourdonnet S, Gosme M, Bertrand M, Roger-Estrade J, Aubertot JN, Rusch A, Motisi N, Pelosi C and Doré T. 2011. Agroecosystem management and biotic interactions: A review. *Agronomy for Sustainable Development* **31**, 491-514.

Michelbacher A and Hitchcock S. 1956. Frosted scale on walnuts. *California Agriculture* **10**, 11-14.

Michelbacher A and Swift JE. 1954. Parasites of the frosted scale. *California Agriculture* **8**, 9 -10.

- Miller DR and Kosztarab M. 1979. Recent advances in the study of scale insects. *Annual Review of Entomology* **24**, 1-27.
- Mithöfer A and Boland W. 2012. Plant defense against herbivores: Chemical aspects. *Annual Review of Plant Biology* **63**, 431-450.
- Moreau J, Benreya B and Thiéry D. 2006. Grape variety affects larval performance and also female reproductive performance of the European grapevine moth *Lobesia botrana* (Lepidoptera: Tortricidae). *Bulletin of Entomological Research* **96**, 205-212.
- Moreau J, Thiery D, Troussard JP and Benrey B. 2007. Grape variety affects female but also male reproductive success in wild European grapevine moths. *Ecological Entomology* **32**, 747-753.
- Muganu M and Paolucci M. 2013. Adaptation of local grapevine germplasm: exploitation of natural defence mechanisms to biotic stresses. In: *The Mediterranean Genetic Code - Grapevine and Olive* (ed. B Sladonja) <http://www.intechopen.com/books/the-mediterranean-genetic-code-grapevine-and-olive/adaptation-of-local-grapevine-germplasm-exploitation-of-natural-defence-mechanisms-to-biotic-stresse>.
- Mullins MG, Bouquet A and Williams LE. 1992. *Biology of the grapevine*. UK, Cambridge University Press.

- Nash MA, Hoffmann AA and Thomson LJ. 2010. Identifying signature of chemical applications on indigenous and invasive nontarget arthropod communities in vineyards. *Ecological Applications* **20**, 1693-1703.
- Panten K and Bramley RGV. 2011. Viticultural experimentation using whole blocks: evaluation of three floor management options. *Australian Journal of Grape and Wine Research* **17**, 136-146.
- Patchell J. 2008. Collectivity and differentiation: a tale of two wine territories. *Environment and Planning* **40**, 2364.
- Pellizzari G. 1997. Grapevine. In: *World Crop Pests. Soft Scale Insects: Their Biology, Natural Enemies and control. Vol 7B.* (eds Y Ben-Dov and CJ Hodgson) pp. 323-331. Elsevier, Amsterdam.
- Petrie PR and Sadras VO. 2008. Advancement of grapevine maturity in Australia between 1993 and 2006: putative causes, magnitude of trends and viticultural consequences. *Australian Journal of Grape and Wine Research* **14**, 33-45.
- Pfeiffer, DG. 1997. Deciduous fruit trees. In: *World Crop Pests. Soft Scale Insects: Their Biology, Natural Enemies and control. Vol 7B.* (eds Y Ben-Dov and CJ Hodgson) pp. 293-322. Elsevier, Amsterdam

- Powell G, Tosh CR and Hardie J. 2006. host plant selection by aphids: Behavioral, evolutionary, and applied perspectives. *Annual Review of Entomology* **51**, 309-330.
- Radcliffe EB, Hutchison WD and Cancelado RE. 2009. Integrated pest management: Concepts, tactics, strategies and case studies. Cambridge University Press, UK
- Rakimov, A. 2010. Aspects of the biology, ecology and biological control of soft scale insects (Coccidae) in Australian vineyards. PhD Thesis, University of Melbourne, Dept. of Zoology. Melbourne, Australia
- Rakimov A, Ben-Dov Y, White V and Hoffmann AA. 2013. Soft scale insects (Hemiptera: Coccoidea: Coccidae) on grapevines in Australia. *Australian Journal of Entomology* **52**, 371-378.
- Ross L, Hardy NB, Okusu A and Normark BB. 2013. Large population size predicts the distribution of asexuality in scale insects. *Evolution* **67**, 196-206.
- Sandanayaka WRM, Blouin AG, Prado E and Cohen D. 2013. Stylet penetration behaviour of *Pseudococcus longispinus* in relation to acquisition of grapevine leafroll virus 3. *Arthropod-Plant Interactions* **7**, 137-146.
- Sands D and Van Driesche R. 2004. Using the scientific literature to estimate the host range of a biological control agent. In: *Assessing host ranges for parasitoids and predators used for classical biological control: a guide to best practice*. USDA

*Forest Health Technology Enterprise Team* (eds RG Van Driesch and R Reardon)  
pp. 15-23. Morgantown, West Virginia, US.

Santesteban LG, Guillaume S, Royo JB and Tisseyre B. 2013. Are precision agriculture tools and methods relevant at the whole-vineyard scale? *Precision Agriculture* **14**, 2-17.

Schamel G and Anderson K. 2003. Wine quality and varietal, regional and winery reputations: hedonic prices for Australia and New Zealand. *Economic Record* **79**, 357-369.

Scopel E, Triomphe B, Affholder F, Da Silva FAM, Corbeels M, Xavier JHV, Lahmar R, Recous S, Bernoux M, Blanchart E, De Carvalho Mendes I and De Tourdonnet S. 2013. Conservation agriculture cropping systems in temperate and tropical conditions, performances and impacts. A review. *Agronomy for Sustainable Development* **33**, 113-130.

Segoli M and Rosenheim JA. 2012. Should increasing the field size of monocultural crops be expected to exacerbate pest damage? *Agriculture, Ecosystems and Environment* **150**, 38-44.

Soar CJ, Sadras VO and Petrie PR. 2008. Climate drivers of red wine quality in four contrasting Australian wine regions. *Australian Journal of Grape and Wine Research* **14**, 78-90.



- Speight MR. 1994. Reproductive capacity of the horse chestnut scale insect, *Pulvinaria regalis* Canard (Hom., Coccidae). *Journal of Applied Entomology* **118**, 59-67.
- Spitzer BW. 2006. Local maladaptation in the soft scale insect *Saissetia coffea* (Hemiptera: Coccidae). *Evolution* **60**, 1859-1867.
- Spitzer BW. 2004. Maternal effects in the soft scale insect *Saissetia coffea* (Hemiptera: Coccidae). *Evolution*, 58, 2452-2461.
- Swirski E, Ben-Dov Y and Wysoki M. 1997. Persimmon. In: *World Crop Pests. Soft Scale Insects: Their Biology, Natural Enemies and control. Vol 7B.* (eds Y Ben-Dov and CJ Hodgson) pp. 265-270. Elsevier, Amsterdam
- Tang S and Cheke RA. 2008. Models for integrated pest control and their biological implications. *Mathematical Biosciences* **215**, 115-125.
- Tesic D, Keller M and Hutton RJ. 2007. Influence of vineyard floor management practices on grapevine vegetative growth, yield, and fruit composition. *American Journal of Enology Viticulture* **58**, 1-11.
- Thomson LJ and Hoffmann AA. 2007a. Ecologically Sustainable Chemical Recommendations for Agricultural Pest Control? *Journal of Economic Entomology* **100**, 1741-1750.

- Thomson LJ and Hoffmann AA. 2007b. Effects of ground cover (straw and compost) on the abundance of natural enemies and soil macro invertebrates in vineyards. *Agricultural and Forest Entomology* **9**, 173-179.
- Thomson LJ and Hoffmann AA. 2010. Natural enemy responses and pest control: Importance of local vegetation. *Biological Control* **52**, 160-166.
- Thomson LJ, Mckenzie J, Sharley DJ, Nash MA, Tsitsilas A and Hoffmann AA. 2010. Effect of woody vegetation at the landscape scale on the abundance of natural enemies in Australian vineyards. *Biological Control* **54**, 248-254.
- Thomson LJ, Sharley DJ and Hoffmann AA. 2007. Beneficial organisms as bioindicators for environmental sustainability in the grape industry in Australia. *Australian Journal of Experimental Agriculture* **47**, 404-411.
- Tjallingii W. 2001. Plant penetration by aphids as revealed by electrical penetration graphs. *Aphids and other Homopterous Insects* **8**, 105-120.
- Tjallingii WF and Esch TH. 1993. Fine structure of aphid stylet routes in plant tissues in correlation with EPG signals. *Physiological Entomology* **18**, 317-328.

- Trivellone V, Paltrinieri LP, Jermini M and Moretti M. 2012. Management pressure drives leafhopper communities in vineyards in Southern Switzerland. *Insect Conservation and Diversity* **5**, 75-85.
- Tsai CW, Rowhani A, Golino DA, Daane KM and Almeida RPP. 2010. Mealybug Transmission of Grapevine Leafroll Viruses: An Analysis of Virus–Vector Specificity. *Phytopathology* **100**, 830-834.
- Tscharntke T, Klein AM, Kruess A, Steffan-Dewenter I and Thies C. 2005. Landscape perspectives on agricultural intensification and biodiversity – ecosystem service management. *Ecology Letters* **8**, 857-874.
- Tscharntke T, Steffan-Dewenter I, Kruess A and Thies C. 2002. Characteristics of insect populations on habitat fragments: A mini review. *Ecological Research* **17**, 229-239.
- Tscharntke T, Tylianakis JM, Rand TA, Didham RK, Fahrig L, Batáry P, Bengtsson J, Clough Y, Crist TO, Dormann CF, Ewers RM, Fründ J, Holt RD, Holzschuh A, Klein AM, Kleijn D, Kremen C, Landis DA, Laurance W, Lindenmayer D, Scherber C, Sodhi N, Steffan-Dewenter I, Thies C, Van Der Putten WH and Westphal C. 2012. Landscape moderation of biodiversity patterns and processes - eight hypotheses. *Biological Reviews* **87**, 661-685.

- Utsumi S, Ando Y, Craig TP and Ohgushi T. 2011. Plant genotypic diversity increases population size of a herbivorous insect. *Proceedings of the Royal Society Biological Sciences* **278**, 3108-3115.
- Wardhaugh CW and Didham RK. 2005. Density-dependent effects on the reproductive fitness of the New Zealand beech scale insect (*Ultracoelostoma assimile*) across multiple spatial scales. *Ecological Entomology* **30**, 733-738.
- Washburn JO, Frankie GW and Grace JK. 1985. Effects of density on survival, development, and fecundity of the soft scale, *Pulvinariella mesembryanthemi* (Homoptera: Coccidae), and its host plant. *Environmental Entomology* **14**,: 755-761.
- Xue K, Wang XY, Huang CH, Wang RJ, Liu B, Yan FM and Xu CR. 2009. Stylet penetration behaviors of the cotton aphid *Aphis gossypii* on transgenic Bt cotton. *Insect Science* **16**, 137-146.
- Yang B, Ge F, Ouyang F and Parajulee M. 2012. Intraspecies mixture alters pest and disease severity in cotton. *Environmental Entomology* **41**, 1029-1036.
- Yang B, Parajulee M, Ouyang F, Wu G and Ge F. 2014. Intraspecies mixture exerted contrasting effects on nontarget arthropods of *Bacillus thuringiensis* cotton in northern China. *Agricultural and Forest Entomology* **16**, 24-32

Zhao JJ, Chen XM, Wand ZL, Ye SD, Chen Y and Wang SY. 2012. Ultrastructure of *Ericerus pela* (Chavannes) nymph mouthparts and ecological adaptations. *Zoological Research* **33**, 447-454.

Zhao JJ, Wang ZL, Chen XM and Chen, Y. 2013. Sex differences in piercing–sucking sites on leaves of *Ligustrum lucidum* (Oleaceae) infested by the chinese white wax scale insect, *Ericerus pela* (Chavannes) (Hemiptera: Coccidae). *Neotropical Entomology* **42**, 158-163.

Zufferey V, Murisier F, Vivin P, Belcher S, Lorenzini F, Spring JL and Viret O. 2012. Carbohydrate reserves in grapevine (*Vitis vinifera* L.'Chasselas'): the influence of the leaf to fruit ratio. *Vitis* **51**, 103-110.

# CHAPTER 2

## Life History Structure of Grapevine Scale

### *Parthenolecanium persicae* Fab. and Frosted Scale

### *Parthenolecanium pruinosum* Cocq. On Wine Grapes

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## Summary

The number of life stages and timing of overwintering (or cold resistant) stage may differ between *P. persicae* and *P. pruinosum* when present on grapevines. Insects of all life stages present were collected monthly in vineyards near Canberra, Australian Capital Territory between September 2010 and November 2011, and morphological characters were described from frozen and slide-mounted specimens. Three life stages in *P. pruinosum* and four life stages in *P. persicae* were recognised from non-hierarchical cluster analysis of linear characters. Although antennal and tibia length increased by 137% and 210% between first instar and adult for *P. pruinosum*, stylet length increased by only 58% between first and adult stage. Feeding position changes from leaves and soft branches in first instars to woody stems in second instars and later life stages, however the relatively slight change in stylet length may indicate that feeding location may not change with development. *P. pruinosum* was larger than *P. persicae* at each life stage, although the body shape of adult female *P. persicae* was more ovoid than adult female *P. pruinosum*, where length was only slightly greater than width. Further work is necessary to establish how environment and plant nutrition may account for the size differences in the two species of scales observed in this study.

Keywords. Frosted scale, Grapevine scale, Growth Rate, Life stages, Stylet Bundle

## 1.0 Introduction

Growth rates of scale insects (Hemiptera; Coccidea) will vary as a result of plant resources ingested, environment and genetic effects that cause differences in time of development from first instar to adult (Nijhout et al. 2010, Pöykkö and Hyvärinen 2012, Prasad et al. 2012, Kivelä et al. 2013, Nijhout et al. 2014). Some monocyclic scale species (one generation per year) can achieve polycyclic life style (multiple generations per year) if environmental conditions are favourable (Danzig 1997). For example, *Parthenolecanium corni* (Bouche') has a monocyclic life cycle in cool temperate climates but is polycyclic under warm temperate conditions (Danzig 1997). Rapid growth to increase number of generations per year can result in reduced body size compared with a monocyclic pattern that includes diapause (Kivelä et al. 2013). *P. persicae* (common name Grapevine scale) and *P. pruinosum* (common name frosted scale) both have monocyclic life cycles, however a prolonged summer season may allow *P. persicae* to have a partial second generation (Rakimov 2010). *P. persicae* and *P. pruinosum* feed on several grapevine varieties in Australia but only limited information about their biology and life history structure is available. In this study, numbers of life stages and morphological changes of these two species of scale insects present on grapevines in the Canberra wine region near Australian National Capital are described.

*P. persicae* has four instars (Brittin 1940, Boratynski 1970) and *P. pruinosum* may have two or three instars (Michelbacher and Hitchcock 1956, Michelbacher and Hitchcock 1957, Gill 1988, Pfeiffer 1997). Instars entering overwintering diapause during cold months may differ between these species, as second instars in *P. pruinosum* (Gill 1988, Michelbacher



and Hitchcock 1956, Michelbacher and Hitchcock 1957, Pfeiffer 1997) and third instars of *P. persicae* (Pellizzari 1997) may be the overwintering stages. First instar crawlers of both scales feed on the grapevine leaves in summer and the larger juveniles return to cryptic sites on the vine branch in autumn where they remain and develop into adults.

The deteriorating condition of grapevines associated with approaching winter is the signal for movement from leaves to woody refuges necessary for survival of the scale insects. Development of larval stages to the next life stage after winter suggests energy for growth is obtained from either the stored body fat or by feeding on woody parts of grapevines in late winter/early spring. Grapevines store carbohydrate in the roots, stems and branches during autumn and winter months (Mullins et al. 1992). Since, plant nutrition is stored in parenchyma cells of vine trunks (Lebon et al. 2008, Zufferey et al. 2012), overwintering stages on cordons and trunks may feed on these tissues. Soluble nutrients become available to the scale insects in early Spring, as plants transport the nutrients to support the meristem tip growth and bud break (Mullins et al. 1992, Lebon et al. 2008, Holzapfel et al. 2010, Zufferey et al. 2012). Once the nutrients become available, the insects are able to end their diapause and continue their development. Thus the life history strategies and population dynamics of grapevine and frosted scales may be dependent upon the temporal availability of nutrients.

Insects increase in body mass between moults (Hutchinson et al. 1997), but most external morphology only changes at the moult. In scale insects, appendages may become shorter with age, as leg appendages become non-functional with adulthood (Gullan and Kosztarab 1997). Although mobility of scale insects is reduced with age, feeding location and

mouthparts may be modified as older insects become more sessile. Sap-sucking insects obtain nutrients within hosts through a modified straw-like mouth structure (stylet bundle) that penetrates cells or vascular bundles and allows ingestion of fluids. The stylet bundle is composed of two interlocking mandibulatory and maxillary stylets; two maxillary stylets interlocked on the inside and the two mandibulatory stylets on the outside (Le Rü et al. 1995, Rosell et al. 1995, Foldi 1997, Ahmad et al. 2012, Garzo et al. 2012). Within the two interlocking maxillary stylets are located the food and salivary canals. Release of saliva during the penetration process helps to construct a salivary sheath and/or feeding canal (Morgan et al. 2013). In scale insects, stylet bundles are enclosed in the crumena when not extended (Foldi 1997). In this study stylet refers to the stylet bundle.

Growth in insects is discontinuous for hard body structures and moulting permits both body size and many cuticularly lined morphological structures to change in shape and form. The body size of each succeeding instar after a moult is usually larger than the preceding instar. However, not all body parts may grow and/or develop at the same rate. For example size of the stylet bundle is unchanged between life stages in *Daktulosphaira vitifoliae* Fitch (Phylloxeridae – grape phylloxera), possibly as feeding was mainly from parenchyma cells of grapevine (Kingston et al. 2007). Changes in the mouth parts may depend both on food requirements and growth of host plant (Freeman et al. 2001, Zhao et al. 2012). For example, Freeman et al. (2001) observed that stylet length of *Bemisia argentifolii* Bellows and Perring (Aleyrodidae) increased with each instar to maintain feeding location in the phloem cells as the leaf cells grew. Scale insects (Coccoidea) feeding on the woody stems may have longer and thicker stylet lengths (Gullan and Kozstarab 1997, Zhao et al. 2012) to allow access to vascular bundles or other feeding locations.

Two questions are investigated in relation to the changes in plant development and how that impacts on the two scale species. Firstly is the life history structure and scheduling of life stages of *P. persicae* and *P. pruinosum* on grapevines similar to what has been previously reported? Secondly does the stylet bundle differ with different life stages as a result of nutrient availability requiring changes in access to the nutrients as the insects vary their location of feeding?

## **2.0 Materials and Methods**

### **2.1 Study Site**

The growth patterns of grapevine and frosted scales were monitored only on *V. Vinifera* Riesling or Chardonnay grapevines respectively at three different vineyards near Canberra, A.C.T, Australia. Canberra has a cool temperate climate with grapevine growing season temperatures of 13 – 20 °C (Hall and Jones 2010). Bud burst typically occurs in mid-September, while harvesting is completed by April.

### **2.2 Insect Samples**

Insect samples were collected monthly between September 2010 and November 2011. This period covered development of each life stage from oviposition to adult. Infested vine twigs and leaves were removed from the vine and brought to the lab in plastic bags. Plastic bags

and twigs were stored in styrofoam cooler boxes for transport from field to the laboratory (~30 minute drive). Adults with eggs were collected into Eppendorf tubes with pin-size holes made on the cap for aeration. The tubes were labelled and stored in the cooler box. Insects in the Eppendorf tubes were snap frozen in liquid nitrogen and stored in freezer (-80 °C), until used for morphological measurements.

### **2.3 Insect Preparation Technique and Identification**

In the laboratory, scale insects were defrosted and mounted on slides for morphological measurements. The standard slide preparation technique for soft scale insects (Ben-Dov and Hodgson 1997) was followed with some minor adjustments. First instars were directly mounted using Stroyan's mountant (Gullan 1984, Cook et al. 2000). Other stages were fixed in an acetic acid alcohol (two parts 96% ethyl alcohol to one part lactic acid) for 2 minutes. The insect body was pricked with a pin or an incision was made in the abdomen and incubated in 10% KOH for 24 hrs to clear the internal body contents. The insects then were transferred to reverse osmosis water (dH<sub>2</sub>O), and the internal body tissues removed. Insects were transferred to clean dH<sub>2</sub>O, and acetic acid (AA) was added progressively to the dH<sub>2</sub>O with insect until the solution was 70% AA. Then, the insect was transferred to 100% AA. To stain the insect, acid fushin was added (one drop per two millilitres of 100% AA). Excess stain was removed transferring the insects to 100% AA. Excess waxy material was removed by adding a drop of xylene (100%) into the AA solution. The xylene was retained in the AA solution for 30 minutes.

Dehydration of insects was completed by transferring the insects from the AA solution into clove oil for 24 hrs. A small drop of Canada balsam was added to the slide and the insect specimen was transferred to the mountant and flattened to be fully immersed in the Canada balsam. A cover slip was placed over the insect and the slide was placed on hot plate (30 °C) for 24 hours to remove air bubbles.

Morphological measurements were made either directly using a light microscope (Leica DMLB) or using ImageJ from pictures (Nikon 4500 Coolpix). Stage micrometers were used to calibrate both methods. Scale species were determined by using keys provided by Brittin (1940), Boratynski (1970) and Gill (1988).

## **2.4 Scanning Electron Microscopy**

For comparison with the prepared material, some frozen scales were examined directly using cold stage scanning electron microscopy (Hitachi S-4300 Cold field Emission SEM) at 3 kV.

Frozen insects were directly mounted onto cold stage stubs with a mounting paste of (1:1) liquid graphite and cryo-protectant (Tissue Tek<sup>®</sup>), and then frozen in liquid nitrogen. The insect and stub were coated with 10 nm gold, and immediately transferred into the cold stage SEM. Measurements of external body features were made from the SEM images collected digitally using ImageJ<sup>®</sup>.

## 2.5 Data Analysis

Morphological measurements of body length, body width, antennal length, tibia length, tarsus length, and stylet length and width were determined for *P. pruinorum*, but only body length and width was measured for *P. persicae*. Measurements were made on micrographs using ImageJ (ImageJ 1.45 m, Bethesda, MD) and all measurements were compared with images of a calibrated stage micrometer ( $\pm 10 \mu\text{m}$ ) taken at the same magnifications. One way ANOVA was used to calculate the differences in Natural logarithm transformed body length, body width, antennae length, tarsus length, tibia length, stylet length and stylet width between the life stages of *P. pruinorum*, and t-tests were used to compare body length and width between *P. pruinorum* and *P. persicae* at each developmental stage.

The linear measurements for the body and appendages of *P. pruinorum* and the body lengths and widths of *P. persicae* were used in a non-hierarchical cluster analysis to determine the number of life stages present. Non-hierarchical cluster of groups (K-means) was analysed in Genstat® version 16. A cluster analysis attempts to find natural groupings in the data by clustering variables into groups (clusters). In non-hierarchical cluster, data is partitioned into predetermined non-overlapping groups or K-means clusters (Krzanowski et al. 1985, Steinley 2006). According to Steinley (2006) N objects, each having measurements on P variables, are allocated into K classes ( $C_1, C_2, \dots, C_K$ ), where  $C_k$  is the set of  $n_k$  objects in cluster k, and K is stated as number of potential classes. The aim is to minimise the error sums of squares between K-mean cluster groups. The overall variability (or maximal variability) across the K mean groups is the objective criterion value. The maximal variability (or criterion value) was determined for each pre-specified groups (K-

mean cluster groups) two, three, four, five, six and seven. As the K cluster groups increase the error sums of square decreases until this decrease cannot be detected with increasing K-cluster groups (Steinley 2006). The best number of clusters was chosen based on the “elbow” of a scree plot. The cluster group beyond the “elbow” showed reduced variability. The number of life stages of each insect species is determined by the best number of clusters or number where “elbow” is present in the criterion value (scree) plot.

The means of best cluster group in non-hierarchical cluster analysis is used to calculate the growth ratio. Growth ratio of *P. persicae* and *P. pruinousm* is determined from equation 1:

$$\text{Growth ratio} = \text{natural logarithm } (L_{x+1}/L_x) \text{ or } \text{Ln } (L_{x+1}) - \text{Ln}(L_x), \quad (1)$$

where  $L_x$ , is the body length of preceding instar stage and  $(L_{x+1})$ , is the body length of the successive instar. Growth rate is determined from the slope of the simple linear regression analysis between the number of life stages and K-means of the various morphological measurements (body length and body width for both species) and length of antennae, tibia and tarsi and length and width of stylet bundle only for *P. pruinousum*.

To determine the changes in feeding structures of *P. pruinousum*, length and width of stylet bundle was regressed with body size using simple regression analysis with natural logarithm transformed stylet bundle length and width as response variables and log transformed body size as explanatory variable.

## 3.0 Results

### 3.1 Growth Patterns of Life Stages

*P. persicae* adults are the smaller of the two species (Tables 1, 2 and 3) (Mann-Whitney-Wilcoxon test, U value = 2404.5,  $p < 0.014$ ,  $n_{\text{pruinorum}} = 89$ ,  $n_{\text{persicae}} = 70$ ). *P. pruinorum*, first instar (Mann-Whitney-Wilcoxon test, U value = 1,  $p < 0.001$ ,  $n_{\text{pruinorum}} = 29$ ,  $n_{\text{persicae}} = 13$ ), and second instar (Mann-Whitney-Wilcoxon test, U value = 1700,  $p < 0.001$ ,  $n_{\text{pruinorum}} = 197$ ,  $n_{\text{persicae}} = 72$ ) are also larger than the same instar of *P. persicae*. Average stylet length of third instar stage of *P. persicae* is not significantly different to the adult stage of *P. pruinorum* (Mann-Whitney-Wilcoxon test, U value = 14.5,  $p < 0.638$ ,  $n_{\text{(P. pruinorum)}} = 4$ ,  $n_{\text{(P. persicae)}} = 9$ ). However, average stylet width of third instar stage of *P. persicae* is significantly smaller than the adult stage of *P. pruinorum* (Mann-Whitney-Wilcoxon test, U value = 0.5,  $p < 0.003$ ,  $n_{\text{(P. pruinorum)}} = 4$ ,  $n_{\text{(P. persicae)}} = 9$ ). In *P. pruinorum* stylet length is twice as long as body length in first instars (crawlers), the same as body length in second instars and 30% shorter than body length in adults (Table 1 and Figure 3). The body length increased 826% between first and adult stage, antennae increased by 137%, tibia length increased by 210% between first and adult stages and stylet length increased by 58% between first and adult stage (Figures 3, 4 and 5). Stylet width doubled in size between first and adult stages (Figure 3).

### 3.2 Insect K groups



The non-hierarchical cluster analysis identified the number of natural groupings using the morphological data of *P. pruinorum* and *P. persicae* (Figure 1). Measurements of antennae, body length, body width, tibia and tarsus length of *P. pruinorum* indicated only three groups, tentatively identified as first instar, second instar and adults. Body length and width of *P. persicae* indicated four groups, identified as first, second and third instars and adult. The inflection point (elbow) is not clearly defined for *P. persicae* as only two morphological variables body length and width were used in the analysis.

ANOVA indicated that significant differences are present between the three K groups of *P. pruinorum* in body length ( $F = 99.8$ ,  $df = 2, 15$ ,  $p < 0.001$ ), body width ( $F = 76.5$ ,  $df = 2, 15$ ,  $p < 0.001$ ), antennal length ( $F = 204.8$ ,  $df = 2, 15$ ,  $p < 0.001$ ), tarsus length ( $F = 663.46$ ,  $df = 2, 15$ ,  $p < 0.001$ ), tibia length ( $F = 249.9$ ,  $df = 2, 15$ ,  $p < 0.001$ ), stylet length ( $F = 150.4$ ,  $df = 2, 15$ ,  $p < 0.001$ ) and stylet width ( $F = 100.8$ ,  $df = 2, 15$ ,  $p < 0.001$ ). *P. pruinorum* may have a third instar stage, not identified by the cluster analysis, but intermediate in body size between second instars and adults (Table 3).

The growth ratio between first and second stages of *P. persicae* and *P. pruinorum* were similar (Table 1). Between species the growth ratio of the adult stages is different, with a higher ratio in *P. pruinorum*. Body length growth rate of *P. persicae* between first instars and adults changed by a factor of 1.01 (Figure 2) and *P. pruinorum* growth rate between first instars and adults changed by a factor of 1.1. Different rates of growth between first instars and adults of *P. pruinorum* were shown for antennae, tarsus and tibia, being 0.44, 0.51 and 0.56 respectively (Figure 2), all being lower than body length changes (Table 1), indicating that the appendages were becoming relatively smaller compared to the body.

Growth rate of stylet length of *P. pruinorum* changed by a factor of 0.24 between first instars and adults (Figure. 2), demonstrating that stylet growth did not match body size changes. Stylet length to body length ratio decreased at each stage of insect moult between first instar and adult stage for *P. pruinorum*, although absolute length did increase by 58%.

## **4.0 Discussion**

### **4.1 Identification of Life Stages**

Previously, the number of life stages of *P. persicae* were reported as four (Boratynski 1970, Brittin 1940), and that is the number of K groups determined for this insect on grapevines in Australia. The number of life stages of *P. pruinorum* was unclear (Gill 1988, Michelbacher and Hitchcock 1956, Pfeiffer 1997), but at least in the populations that were examined in this study, only three K groups were clearly present. The overwintering life stages of each species were the second instars and third instars in *P. pruinorum* and *P. persicae* respectively (Figure 6).

Scale insects in the genus *Parthenolecanium* have been reported to have two to three nymphal stages (Kozár and Ben-Dov 1997, Rainato and Pellizari 2009, Meineke et al. 2013). Although some insect species have been reported to vary the number of instars depending upon quality or quantity of food, no evidence was present to suggest that such variation occurred in these two species over the course of this study.

Both species were monocyclic (univoltine), with time to leaf fall limiting the time that insects could develop prior to having to seek shelter and feed on the woody branches (Chapters 5 and 6). Rakimov (2010) reported a second partial generation in *P. persicae* on grapevines in one locale and observed short development time and reduced fecundity in sub-tropical regions (long summer season). As observed in *P. corni*, environment can influence the phenotypic variation in life history structure and microscopic body features among multivoltine genotypes (Kozár and Ben-Dov 1997). If warming occurs with climate change, the environment may become suitable so that these species could develop polycyclic (multivoltine) life history traits (Danzig 1997, Pellizzari 1997). As this study was only done within a cool wine growing location, regions that are farther north should be studied to see if their longer growing season permits a second generation. Having only two juvenile stages may then become advantageous for *P. pruinorum*, as that could reduce total development time to adulthood under the warmer conditions compared with three juvenile stages of *P. persicae*.

In Australian vineyards *P. persicae* and *P. pruinorum* can coexist on grapevine hosts (Rakimov 2010, Rakimov et al. 2013). All life stages of *P. persicae* and *P. pruinorum* feed on grapevines, but location of feeding may differ, as length and thickness of the stylet suggest first instars are restricted to feeding on the soft succulent tissues of new branches and leaves whereas the subsequent instars can feed on the woody stems. The limitation on location of feeding as a result of the stylet dimensions may determine when first instars can be present, as their feeding would be restricted to the spring and summer periods when the grapevine leaves are present.

## 4.2 Development of Life Stages

This study showed that growth rate between the life stages was faster in *P. pruinorum* than *P. persicae*. Part of the difference in growth rate was associated with the one less instar in *P. pruinorum*, although the final size of adults was similar. Growth of appendages for *P. pruinorum* such as antennae, leg structures and stylet, was reduced compared with the growth in body size at each moult, resulting in relatively smaller appendages with later life stage. Growth of different body parts may be influenced differently by the hormones controlling growth at each moult (Nijhout et al. 2013). As the adult is comparatively sessile, the appendages are not necessary for movement, location of food or accessing the food, as the settlement site must meet their food requirements for egg production (Gullan and Kozstarab 1997). Because of their sessile nature (parasitic living), scale insects have become functional specialists, crawlers (first instars) select feeding sites and are responsible for dispersal; the second and third instars can store food for further development and for diapause, and the adult females are solely responsible for reproduction (Gullan and Kozstarab 1997). Differences in growth patterns in *P. persicae* and *P. pruinorum* may result from their different sensitivity to environmental conditions (e.g. temperature and host plant quality). Selection may have caused rapid growth and early hardening (sclerotization) of cuticle in *P. pruinorum* under stressful environmental conditions, in association with declining nutritional conditions of grapevine leaves (Wermelinger and Koblet, 1990, Mullins et al. 1992). Although both study insects develop into adults straight after their respective diapausing stage, the pattern of adjustment to environment may encourage survival in *P. pruinorum* over winter, but allow increased reproduction in *P. persicae* in the

following spring typical in diapausing insects (Etilé and Despland 2008, Kivelä et al. 2013, Pöykkö et al. 2012, Vargas et al. 2013).

### **4.3 Feeding Structure**

There is considerable variability in stylet shape and size among hemipterans (Goodchild 1966). Stylet length and width have been used to identify feeding location within plant cells of several sap-sucking insects (Garzo et al. 2012, Zhao et al. 2012, Sandanayaka et al. 2013). Freeman et al. (2001) observed that stylet length of *B. argentifolii* increased with each stage to maintain the feeding location in phloem cells as leaf cells became thicker. Stylet growth pattern in *P. prunosum* also increases as insects grow, however, resource acquisition between *P. prunosum* and *B. argentifolii* differs, as *B. argentifolii* completes its nymphal development entirely on the leaves of host plants, whereas *P. prunosum* feeds on the leaves during the first instar and later feeds on the stems of grapevines during second instar and adult. Stylet length of first instars of *P. prunosum* was nearly twice as long as body length, suggesting possible feeding location within the phloem and parenchyma cells. Stylet length remained unchanged among the nymphal instars of *D. vitifoliae* and doubled in size in the adult stage; nymphal instars ranged between 149 and 182 um and in adult 349 um (Kingston et al. 2007). Despite this, grape phylloxera obtained nutrients from parenchyma cells of grapevine roots as the phloem cells are located 200 – 300 um deep.

Change in stylet length and width may be associated with the feeding site and plant physiological changes with growth. Differentiation and thickening of plant cells or changes in location in feeding may increase the distance or force required to access feeding sites,

and hence increases stylet length and width with each moult are necessary for continued feeding. Stylet length and width of *P. pruinosum* increases between first instars feeding on the leaf and the second and adult stages feeding on woody stems of vines. First instars may settle on the soft succulent branches of grapevines but as cells in the stems divide cambium layer thickens (Mullins et al. 1992) hence thickening and increase in length of stylet bundle size may be necessary to track feeding location. Similar observations were reported on Chinese White Wax Scale Insect, *Ericerus pela* (Chavannes) (Coccidae) (Zhao et al. 2012). According to Zhao et al. (2012) change in stylet morphology (length and width) between feeding sites by Chinese white wax scale may be associated with the feeding sites; nymphs feeding on leaves had short and soft stylet length between 414 um and 415 um and diameter of 1.6 um whereas nymphs feeding on the stems had stylet length between 595 um and 616 um and diameter of 5.2 um.

## **5.0 Conclusion**

*P. persicae* and *P. pruinosum* grow to achieve a large enough adult size to ensure reproduction by developing through third or second instars. With each stage of insect development, the antennae, leg structures and stylet bundle develop at a slower rate than body growth. In first instars of *P. pruinosum*, stylet length is twice the body length, which allows feeding in the grapevine leaf. The length of this structure is equivalent to the body length and slightly thicker in the second instar. Adult stylet length and width increased by 58% and 50%, respectively, between first instar and adult stage. This increase in size may allow feeding on the woody stems and the increased distance from the feeding site within the plant.

## **6.0 Acknowledgements**

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## 7.0 References

- Ahmad A, Kaushik S, Ramamurthy VV, Lakhanpaul S, Ramani R, Sharma KK and Vidyarthi AS. 2012. Mouthparts and stylet penetration of the lac insect *Kerria lacca* (Kerr) (Hemiptera: Tachardiidae). *Arthropod Structure and Development* **41**, 435-441.
- Ben-Dov Y and Hodgson CJ. 1997. World crop pests. Soft scale insects: Their biology, natural enemies and control. Vol 7A. Elsevier, Elsevier Science.
- Boratynski K. 1970. On some species of 'Lecanium' (Homoptera, Coccidae) in the collection of the Naturhistorisches Museum in Vienna; with description and illustration of the immature stages of *Parthenolecanium persicae*. *Analen des Naturhistorischen Museums Wien* **74**, 63-76.
- Brittin G. 1940. The life history of *Lecanium (Eulecanium) persicae* (Fabricius), and descriptions of the different instars. *Transactions and Proceedings of the Royal Society of New Zealand* **69**, 413-421.
- Cook LG, Gullan PJ and Stewart AC. 2000. First-instar morphology and sexual dimorphism in the gall-inducing scale insect *Apiomorpha Rubsamen* (Hemiptera: Coccoidea: Eriococcidae). *Journal of Natural History* **34**, 879-894.



- Danzig EM. 1997. Intraspecific variation of taxonomic characters. In: *World Crop Pests. Soft Scale Insects: Their Biology, Natural Enemies and control. Vol 7A.* (eds Y Ben-Dov and CJ Hodgson). pp. 203-212. Elsevier Science, Amsterdam, Netherlands.
- Etilé E and Despland E. 2008. Developmental variation in the forest tent caterpillar: life history consequences of a threshold size for pupation. *Oikos* **117**, 135-143.
- Foldi I. 1997. Internal anatomy of the adult female. In: *World Crop Pests. Soft Scale Insects: Their Biology, Natural Enemies and control. Vol 7A.* (eds Y Ben-Dov and CJ Hodgson) pp. 73-90. Elsevier Science, Amsterdam, Netherlands.
- Freeman TP, Buckner JS, Nelson DR, Chu CC and Henneberry TJ. 2001. Stylet penetration by *Bemisia argentifolii* (Homoptera: Aleyrodidae) into host leaf tissue. *Annals of the Entomological Society of America* **94**, 761-768.
- Garzo E, Bonani JP, Lopes JRS and Fereres A. 2012. Morphological description of the mouthparts of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). *Arthropod Structure and Development* **41**, 79-86.
- Gill RJ. 1988. The scale insects of California, Part 1: the soft scales (Homoptera: Coccoidea: Coccidae). In: *Analysis and Identification Branch, California Dept. of Food and Agriculture.* Sacramento, California, USA.

- Goodchild A. 1966. Evolution of the alimentary canal in the Hemiptera. *Biological Reviews* **41**, 97-139.
- Gullan P. 1984. A revision of the gall-farming coccoid genus *Apiomorpha* Randuuml; bsaamen (Homoptera : Eriococcidae : Apiomorphinae). *Australian Journal of Zoology Supplementary Series* **32**, 1-203.
- Gullan PJ and Kosztarab M. 1997. Adaptations in scale insects. *Annual Review of Entomology* **42**, 23-50.
- Hall A and Jones GV. 2010. Spatial analysis of climate in winegrape-growing regions in Australia. *Australian Journal of Grape and Wine Research* **16**, 389-404.
- Holzapfel BP, Smith JP, Field SK and Hardie WJ. 2010. Dynamics of carbohydrate reserves in cultivated grapevines. *Horticultural Reviews* **37**. John Wiley and Sons, Inc.
- Hutchinson JMC, Mcnamara JM, Houston AI and Vollrath F. 1997. Dyar's rule and the investment principle: optimal moulting strategies if feeding rate is size-dependent and growth is discontinuous. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **352**, 113-138.
- Kingston KB, Powell KS and Cooper PD. 2007. Grape phylloxera external morphology observations under scanning electron microscope. In: *Proceedings of the 111rd*

*International Grapevine Phylloxera Symposium. II International Grapevine Phylloxera Symposium 733.* (eds KS Powell and CJ Trethowan). pp. 107 – 114. Acta Horticulturae, Fremantle, Australia.

Kivelä SM, Välimäki P and Gotthard K. 2013. Seasonality maintains alternate life-history phenotypes. *Evolution* **67**, 3145-3160.

Kozár F and Ben-Dov Y. 1997. Zoogeographical considerations and status of knowledge of the family. In: *World Crop Pests. Soft Scale Insects: Their Biology, Natural Enemies and control. Vol 7A.* (eds Y Ben-Dov and CJ Hodgson) pp. 213-228. Elsevier Science, Amsterdam, Netherlands.

Krzanowski W J and Lai Y. 1985. A criterion for determining the number of groups in a data set using sum-of-squares clustering. *Biometrics* **44**, 23-34.

Le Rü B, Renard S, Allo MR, Le Lannic J and Rolland JP. 1995. Ultrastructure of sensory receptors on the labium of the cassava mealybug, *Phenacoccus manihoti* Matile Ferrero. *Entomologia Experimentalis et Applicata* **77**, 31-36.

Lebon G, Wojnarowicz G, Holzapfel B, Fontaine F, Vaillant-Gaveau N and Clément C. 2008. Sugars and flowering in the grapevine (*Vitis vinifera* L.). *Journal of Experimental Botany* **59**, 2565-2578.

- Meineke EK, Dunn RR, Sexton JO and Frank SD. 2013. Urban warming drives insect pest abundance on street trees. *PLoS ONE* **8**, e59687.
- Michelbacher A and Hitchcock S. 1956. Frosted scale on walnuts. *California Agriculture* **10**, 11-14.
- Michelbacher A and Hitchcock, S. 1957. Soft scales on walnut in 1956: Increase in soft scale populations on walnuts in northern California effected by several factors in complex problem. *California Agriculture* **11**, 7-14.
- Morgan JK, Luzio GA, Ammar ED, Hunter WB, Hall DG and Shatters Jr RG. 2013. Formation of stylet sheaths in *āere* (in air) from eight species of phytophagous hemipterans from six families (Suborders: Auchenorrhyncha and Sternorrhyncha). *PLoS ONE* **8**, e62444.
- Mullins MG, Bouquet A and Williams, L. E. 1992. *Biology of the grapevine*. UK, Cambridge University Press.
- Nijhout HF, Riddiford LM, Mirth C, Shingleton AW, Suzuki Y and Callier V. 2014. The developmental control of size in insects. *Wiley Interdisciplinary Reviews: Developmental Biology* **3**, 113 - 134.

- Nijhout HF, Roff DA and Davidowitz G. 2010. Conflicting processes in the evolution of body size and development time. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**, 567-575.
- Pellizzari G. 1997. Grapevine. In: *World Crop Pests. Soft Scale Insects: Their Biology, Natural Enemies and control. Vol 7B.* (eds Y Ben-Dov and CJ Hodgson) pp. 323-331. Elsevier Science, Amsterdam, Netherlands.
- Pfeiffer DG. 1997. Deciduous fruit trees. In: *World Crop Pests. Soft Scale Insects: Their Biology, Natural Enemies and control. Vol 7B.* (eds Y Ben-Dov and CJ Hodgson) pp. 293-322. Elsevier Science, Amsterdam, Netherlands.
- Pöykkö H and Hyvärinen M. 2012. To grow fast or to grow big? Time-limited larvae of *Eilema depressum* speed up their growth and reduce number of instars. *Entomologia Experimentalis et Applicata* **142**, 145-152.
- Prasad Y, Prabhakar M., Sreedevi G, Ramachandra RG and Venkateswarlu B. 2012. Effect of temperature on development, survival and reproduction of the mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) on cotton. *Crop Protection* **39**, 81-88.
- Rainato A and Pellizzari G. 2009. Observations on the biology of *Parthenolecanium rufulum* in northeastern Italy, with a redescription of the first and second instar females. *Bulletin of Insectology* **62**, 85 - 91.

- Rakimov A. 2010. Aspects of the biology, ecology and biological control of soft scale insects (Coccidae) in Australian vineyards. PhD Thesis, University of Melbourne, Dept. of Zoology. Melbourne, Australia
- Rakimov A, Ben-Dov Y, White V and Hoffmann AA. 2013. Soft scale insects (Hemiptera: Coccoidea: Coccidae) on grapevines in Australia. *Australian Journal of Entomology* **52**, 371-378.
- Rosell RC, Lichty JE and Brown JK. 1995. Ultrastructure of the mouthparts of adult sweetpotato whitefly, *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae). *International Journal of Insect Morphology and Embryology* **24**, 297-306.
- Sandanayaka WRM, Blouin AG, Prado E and Cohen D. 2013. Stylet penetration behaviour of *Pseudococcus longispinus* in relation to acquisition of grapevine leafroll virus 3. *Arthropod-Plant Interactions* **7**, 137-146.
- Steinley D. 2006. K-means clustering: A half-century synthesis. *British Journal of Mathematical and Statistical Psychology* **59**, 1-34.
- Vargas G, Michaud JP, Nechols JR and Moreno CA. 2013. Age-specific maternal effects interact with larval food supply to modulate life history in *Coleomegilla maculata*. *Ecological Entomology* **39**, 39 -46.

Wermelinger B and Koblet W. 1990. Seasonal growth and nitrogen distribution in grapevine leaves, shoots and grapes. *Vitis* **29**, 15-26.

Zhao JJ, Chen XM, Wand ZL, Ye SD, Chen Y and Wang SY. 2012. Ultrastructure of *Ericerus pela* (Chavannes) nymph mouthparts and ecological adaptations. *Zoological Research* **33**, 447-454.

Zufferey V, Murisier F, Vivin P, Belcher S, Lorenzini F, Spring JL and Viret O. 2012. Carbohydrate reserves in grapevine (*Vitis vinifera* L.'Chasselas'): the influence of the leaf to fruit ratio. *Vitis* **51**, 103-110.

**Table 1. Measurements of morphological structures of life stages of *P. persicae* and *P. pruinorum***

| <i>P. pruinorum</i>  | Number of    | Body length                   | Antennae                   | Tibia length             | Tarsus length             | Stylet length               | Stylet width            | Stylet length |
|----------------------|--------------|-------------------------------|----------------------------|--------------------------|---------------------------|-----------------------------|-------------------------|---------------|
| Life stage           | observations | (mm)                          | length (um)                | (um)                     | (um)                      | (um)                        | (um)                    | to body ratio |
|                      |              | (± SEM)                       | (± SEM)                    | (± SEM)                  | (± SEM)                   | (± SEM)                     | (± SEM)                 | (mm)          |
| <b>First instar</b>  | 21           | 0.38 ± 0.003<br>(0.34 - 0.42) | 110.5 ± 3.9<br>(100 – 139) | 36.5 ± 1.3<br>(29 – 41)  | 28.4 ± 0.45<br>(27 – 30)  | 665 ± 9.6<br>(630 – 696)    | 2.2 ± 0.07<br>(2 – 2.7) | 1.75          |
| <b>Second instar</b> | 57           | 0.91 ± 0.02<br>(0.5 – 1.1)    | 173 ± 1.3<br>(170 – 176)   | 67.4 ± 1.3<br>(64 – 71)  | 55.4 ± 1.4 (52<br>– 58)   | 864 ± 10.4 (836<br>– 897)   | 3 ± 0.02<br>(3 – 3.1)   | 0.96          |
| <b>Adult</b>         | 36           | 5.19 ± 0.13<br>(2.12 – 7.33)  | 263 ± 3.5<br>(256 – 272)   | 115 ± 3.5<br>(105 – 120) | 79 ± 2.0<br>(76 – 85)     | 1050 ± 41.6<br>(958 – 1134) | 4.4 ± 0.22<br>(4 – 5)   | 0.22          |
| <i>P. persicae</i>   |              |                               |                            |                          |                           |                             |                         |               |
| <b>Life stage</b>    |              |                               |                            |                          |                           |                             |                         |               |
| <b>First instar</b>  | 14           | 0.33 ± 0.01<br>(0.31 – 0.42)  | na                         | na                       | na                        | na                          | na                      | na            |
| <b>Second instar</b> | 30           | 0.8 ± 0.04<br>(0.46 – 1.08)   | na                         | na                       | na                        | na                          | na                      | na            |
| <b>Third instar</b>  | 75*          | 1.4 ± 0.03<br>(1.1 – 2.1)     | 227 ± 3.1<br>(214 – 246)   | 100 ± 1.9<br>(90 – 111)  | 76 ± 1.8<br>(60.6 – 73.9) | 1027 ± 18.5<br>(920 – 1134) | 3.2 ± 0.12<br>(2.4 – 4) | 0.73          |
| <b>Adult</b>         | 35           | 4.48 ± 0.26<br>(2.12 - 9.00)  | na                         | na                       | na                        | na                          | na                      | na            |

\* - In the third instar of *P. persicae*, body length and width were measured from 75 insect samples. Antennae, Tibia, Tarsus and Stylet were measured from 11 insect samples. na – data not measure



**Table 2. Mean ( $\pm$  SEM) and range of body length and width of life stages of *P. persicae* and phenology of life stages over a year**

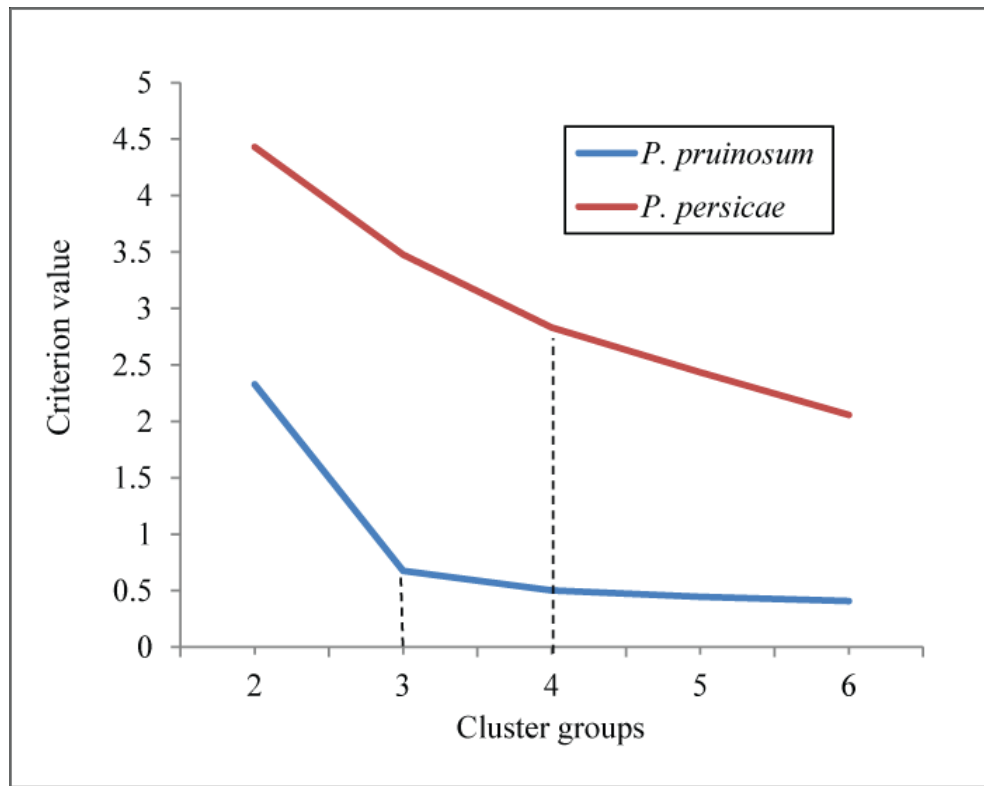
| <b>Life stages</b>   | <b>No of observations</b> | <b>Body length* (mm)</b>          | <b>Body width (mm)</b>            | <b>Time of occurrence</b> |
|----------------------|---------------------------|-----------------------------------|-----------------------------------|---------------------------|
| <b>Egg</b>           | 22                        | 0.27                              | 0.16 $\pm$ 0.006<br>(0.15 – 0.19) | Nov - Dec                 |
| <b>First instar</b>  | 64                        | 0.31 $\pm$ 0.004<br>(0.31 - 0.35) | 0.19 $\pm$ 0.004<br>(0.19 - 0.2)  | Nov - Jan                 |
| <b>Second instar</b> | 36                        | 0.65 $\pm$ 0.04<br>(0.39 - 0.92)  | 0.35 $\pm$ 0.03<br>(0.19 - 0.5)   | Jan - Mar                 |
| <b>Third instar</b>  | 157                       | 1.46 $\pm$ 0.02<br>(1.00 – 2.04)  | 0.84 $\pm$ 0.02<br>(0.57 - 1.35)  | Mar - Sept                |
| <b>Adult</b>         | 69                        | 4.48 $\pm$ 0.26<br>(2.12 - 9.00)  | 2.80 $\pm$ 0.18<br>(2.15 - 6.86)  | Aug - Dec                 |

**Table 3. Mean ( $\pm$  SEM) and range of body length and width of life stages of *P. pruinosum* and phenology of life stages over a year**

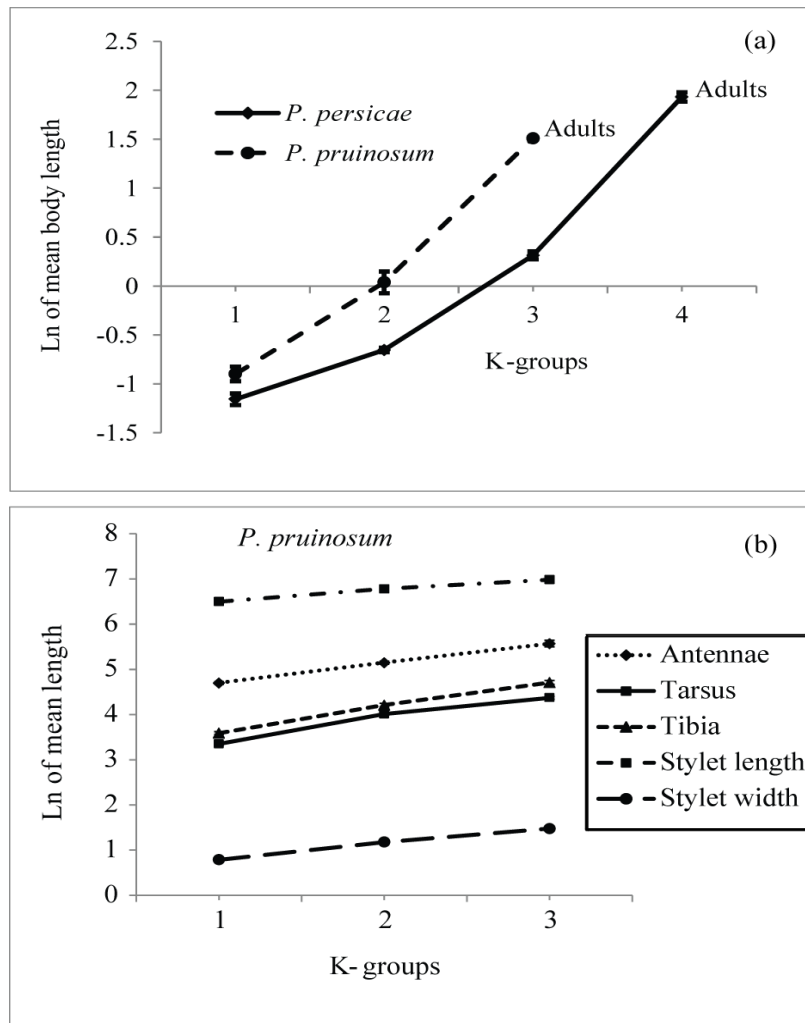
| <b>Life stages</b>   | <b>No of observations</b> | <b>Body length* (mm)</b>           | <b>Body width (mm)</b>            | <b>Time of occurrence</b> |
|----------------------|---------------------------|------------------------------------|-----------------------------------|---------------------------|
| <b>Egg</b>           | 24                        | 0.308                              | 0.18 $\pm$ 0.005<br>(0.15 - 0.19) | Nov – Dec                 |
| <b>First instar</b>  | 62                        | 0.39 $\pm$ 0.006 (<br>0.34 - 0.42) | 0.19 $\pm$ 0.005<br>(0.19 - 0.23) | Dec and Jan               |
| <b>Second instar</b> | 251                       | 0.91 $\pm$ 0.01<br>(0.46 - 1.08)   | 0.49 $\pm$ 0.01<br>(0.23 - 0.62)  | Jan - Sept                |
| <b>Third instar?</b> | 74                        | 1.55 $\pm$ 0.02<br>(1.19 - 1.81)   | 0.88 $\pm$ 0.01<br>(0.65 - 1.10)  | Aug - Oct                 |
| <b>Adult</b>         | 89                        | 5.19 $\pm$ 0.13<br>(2.12 – 7.33)   | 3.78 $\pm$ 0.13<br>(1.38 – 6.67)  | Oct - Dec                 |

\* – data in brackets represent the range

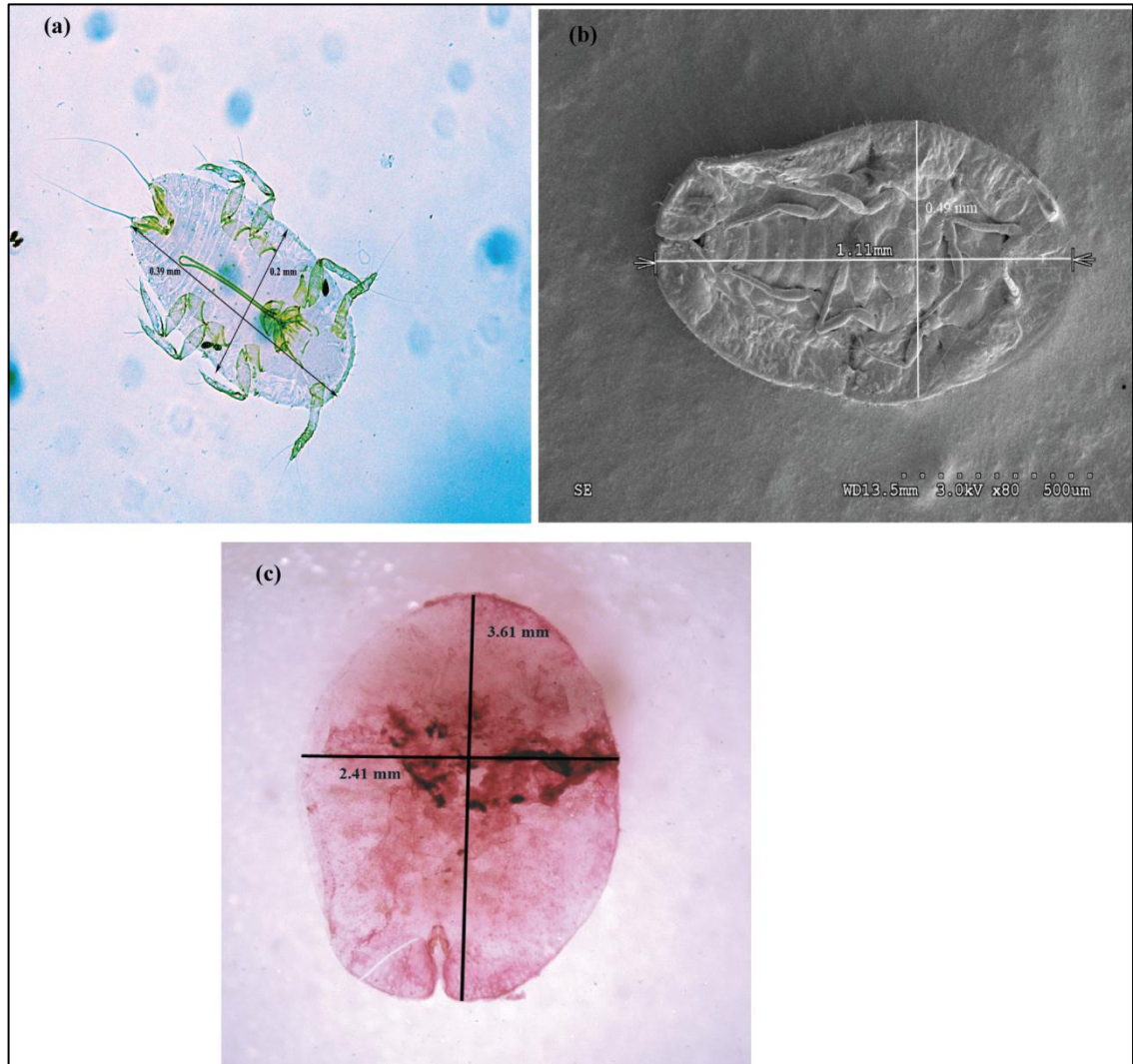
? – third instar stage may be present



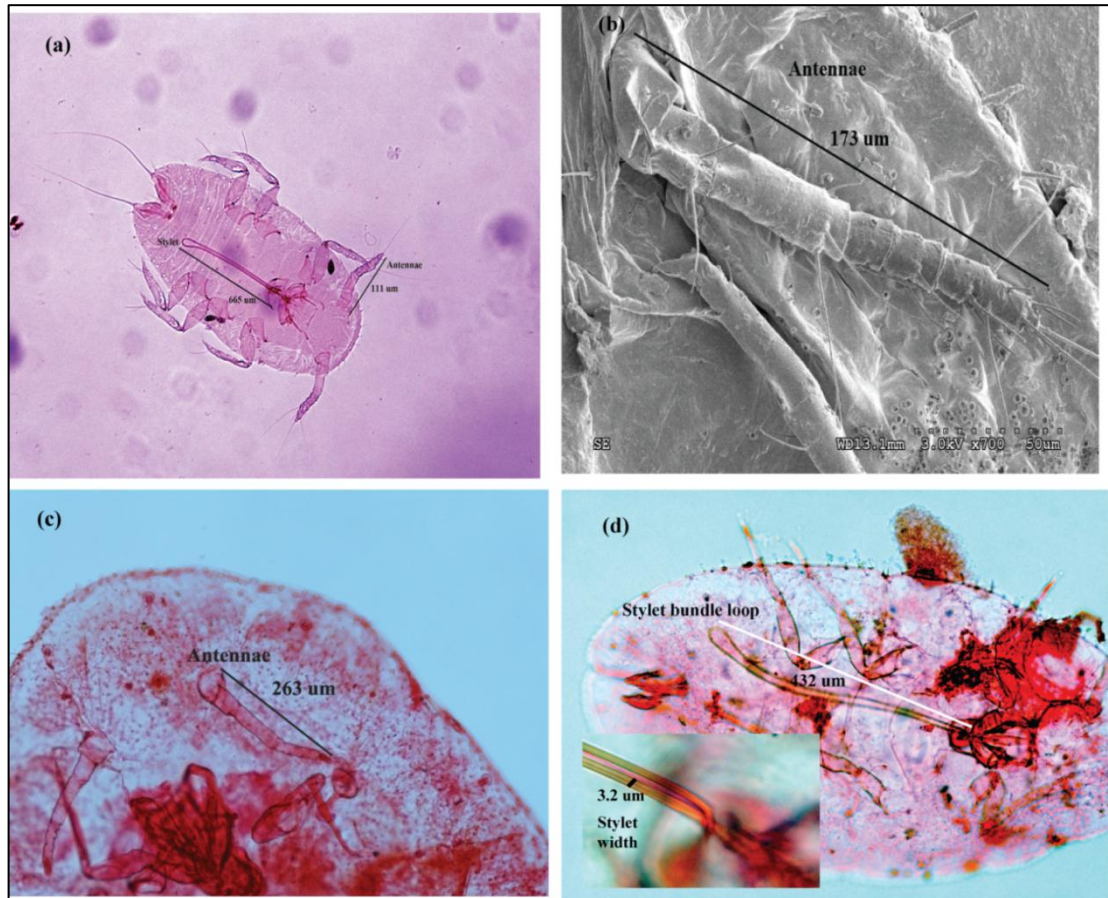
**Figure 1. Optimal criterion value of pre-defined cluster groups for *P. pruinosa* and *P. persicae*. The dotted vertical line indicates the inflection point (elbow) of the criterion value which is used to identify the number of life stages (refer to text in materials and methods section for definition of natural grouping of K-means and criterion value).**



**Figure 2. Natural logarithm of (a) *P. persicae* mean body length (mm) ( $y = -2.45 + 1.02x$ ,  $r^2 = 0.97$ ) and *P. pruinorum* mean body length (mm) ( $y = -2.04 + 1.1x$ ,  $r^2 = 0.99$ ) and (b) *P. pruinorum* mean antennae length ( $\mu\text{m}$ ) ( $y = 4.27 + 0.44x$ ,  $R^2 = 0.99$ ), mean tarsus length ( $\mu\text{m}$ ) ( $y = 2.89 + 0.51x$ ,  $r^2 = 0.99$ ), mean tibia length ( $\mu\text{m}$ ) ( $y = 3.05 + 0.56x$ ,  $r^2 = 0.99$ ), mean stylet length ( $\mu\text{m}$ ) ( $y = 6.27 + 0.24x$ ,  $r^2 = 0.99$ ) and mean stylet width ( $\mu\text{m}$ ) ( $y = 0.46 + 0.35x$ ,  $r^2 = 0.99$ ) plotted against instar numbers (life stages). The standard error bars in both graphs (a and b) are smaller than the dots.**



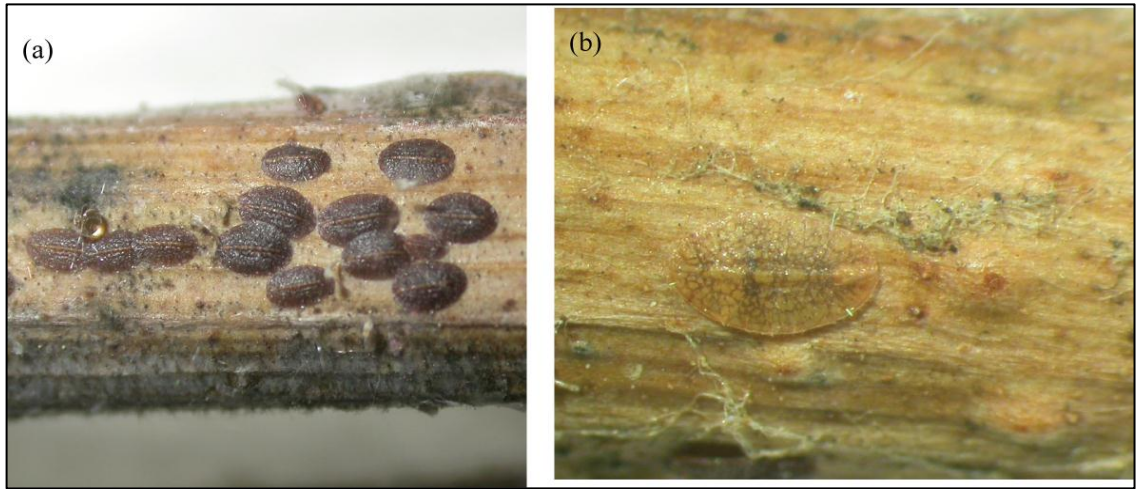
**Figure 3. Body size measurements of *P. pruinosum* (a) first instar (b) SEM second instar (c) adult**



**Figure 4. Antennae and stylet bundle measurements of *P. pruinorum* (a) first instar (b) SEM antennae of second instar (c) adult antennae (d) stylet bundle of second instar (insert stylet width measurement)**



**Figure 5. Metathorax leg, tibia and tarsus measurements of *P. pruinorum* (a) first instar (b) second instar (c) adult**



**Figure 6. Overwintering life stages on branches of grapevines, (a) second instar stage of *P. prunosum* and (b) third instar stage of *P. persicae*.**



# CHAPTER 3

## **Reproductive Output and Population Growth of Grapevine Scale *Parthenolecanium persicae* Fab and Frosted Scale *Parthenolecanium pruinatum* Cocq. On Selected Grapevine Varieties**

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## Summary

Grapevine and Frosted scales cause long term physiological damage to grapevines. Although, they persist in major grape-growing regions of Australia, reproductive and population growth potential of these insect pests are poorly understood. The reproductive output of gravid adults of grapevine and frosted scales were studied under field conditions on, Riesling, Chardonnay and Sauvignon Blanc grape varieties. Intrinsic rate of increase of grapevine and frosted scales were studied on Riesling and Chardonnay varieties respectively. Gravid adult female of grapevine scale has a larger body length, body mass and higher fecundity than in gravid frosted scale. Egg size and size of first instar were smaller for grapevine scale than for frosted. Egg incubation period, post-oviposition by adult females, was affected by grapevine varieties, being 20 days on Chardonnay and 19 days on Riesling for grapevine scale and in frosted scale 18 days on Chardonnay and Riesling and 22 days on Sauvignon Blanc. Fertility was not affected by grape varieties in both scale insects, on average 94% fertile offspring by grapevine scale and 96% fertile offspring by frosted scale. Fecundity increased with body mass but it is not affected by grapevine varieties. The intrinsic rate of increase ( $r_m$ ) of grapevine scale on Riesling was 0.28 per month and the  $r_m$  for frosted scale on Chardonnay was 0.21 per month. The finite rate of increase ( $\lambda$ ) for grapevine and frosted scales were 1.28 per month and 1.23 per month respectively, and population doubling time (DT) was 3.35 months and 2.50 months for grapevine scale and frosted scale respectively. In this context, the population of grapevine and frosted scales may persist in vineyards and frequently rise to outbreak level. The information provided in this study has important implications for grapevine and frosted scale population dynamics and their effective management in vineyards.

Keywords: Body size, Fecundity, frosted scale, grapevine scale, grapevine varieties,  
intrinsic rate of increase

## **1.0 Introduction**

Reproductive output and population growth rate of insects is often influenced by the physiological and morphological conditions of the adult females. These changes are driven by both biotic (food, predation and competition) and abiotic (climate) conditions (Begon et al. 1990, Arendt 1997, Tammaru and Haukioja 1996, Tammaru et al. 2002, Chown and Nicholson 2004). By increasing adult female body size (length or mass), insect herbivores can increase reproductive output and intrinsic growth rate (Fenchel, 1974, Tammaru et al. 2002). In scale insects (Hemiptera: Sternorrhyncha: Coccoidea), adult female body size can influence the reproductive output and often these factors can be affected directly by the host plant quality (nutritional requirements) and insect population density (Washburn et al. 1985, Speight 1994, Lo 1995, Loch and Zalucki 1997, Marotta 1997, Wardhaugh and Didham 2005, Hill et al. 2009, Qin et al. 2011, Tanga et al. 2013).

Scale insects are significant pests of various horticultural crops because of high rates of population growth that allows them to persist across a range of habitats (Hoffmann et al. 2008, Ross et al. 2013). Infected plant parts are symptomless during periods of reduced scale population growth, but then plant damage increases as the population of insects reaches outbreak levels. Such rapid changes in population numbers can occur if the conditions are favourable for enhancing growth and reproductive output. The outbreak level is defined as the insect population density that exceeds the density that results in economic injury. Pest density above the outbreak level typically leads to a profit reduction, and this density is defined as an economic threshold for pest control (Tang and Cheke 2008) and insects with a high rate of population growth can frequently reach this economic threshold.

To reduce dependency upon pest control systems that result from outbreaks, the reproductive output and population growth rate of insects such as scale insects are needed as part of an integrated pest management strategy.

Grapevine and frosted scales are distributed widely in vineyards throughout Australia (Rakimov et al. 2013). Since their introductions in the early and mid-1900s, populations have persisted in vineyards with occasional outbreaks as a result of a sudden increase in population density. Grapevine and frosted scales feeding on grapevines in Australia have not been studied in relation to the rate of population growth, partially because most vineyards have control programs in place. However, feeding by scale insects can reduce photosynthetic rate (Zvereva et al. 2010) and plant vigour and may lead to secondary infection of grapevine diseases (Hommay et al. 2008, Charles et al. 2009, Bahder et al. 2013, Krüger and Douglas-Smit 2013).

Grapevine and frosted scales are sedentary with asexual (parthenogenesis) reproduction (Pellizzari 1997, Pfeiffer 1997). This has enabled the existence of highly specialised populations and functional structure (Alstad and Edmunds Jr 1983, Glynn and Herms 2004, Gullan and Kosztrab 1997, Hanks and Denno 1998). Dispersal occurs by the first instar stage which is extremely mobile in seeking feeding and refuge sites (Greathead, 1997). Sexually dimorphic forms of life stages occur at the end of second instar; males develop holometabolically into pre-pupa and pupae before moulting into winged adults (alates) while the females develop hemimetabolically into apterous wingless adults resembling morphologically the second instar stage. The difference between the juveniles and adult females is reflected in the body size and general reduction of appendages and functionality

(Gullan and Kosztrab 1997). Once the feeding site is selected subsequent larval stages expand in body size with each moulting phase. Reproduction in adult females occurs once an individual reaches a certain body size (Arendt 1997, Boggs 2009). Ovipositioning may occur over several days for each gravid female adult.

Previous studies in Australia on the biology of grapevine and frosted scales on grapevines indicated that adult females differ in body length and reproductive potential when feeding on grapevines (Rakimov 2010, Rakimov et al. 2013). Gravid grapevine scale generally has a larger body length (5.71-9.82 mm) than frosted scale (4.35-7.03 mm) (Rakimov 2010). Fecundity varied between species; with the mean number of eggs laid being slightly more for grapevine scale than for frosted scale adult females (Rakimov 2010). Fecundity may increase with female body length as in other species of coccids (Speight 1994, Lo 1995, Wakgari 2000), but whether host grape variety influences this pattern is unknown in grapevine and frosted scales. Environmental conditions, such as temperature and moisture, insect density, and nutrient availability and quality have been implicated in the correlation between insect fecundity and body and egg sizes (Washburn et al. 1985, Speight 1994, Lo 1995, Wardhaugh and Didham 2005,).

Grapevine and frosted scales have specialised feeding behaviour associated with feeding within plant vascular tissues. Penetration of plant cells and acquisition of nutrition is aided by mandibular and maxillary stylets. A salivary sheath acts as a feeding tube and is developed by the saliva released during cell penetration (Foldi 1997). The salivary sheath eliminates external osmotic pressure and plant toxins. Feeding in this manner requires detection of plant vascular cells and subsequent ingestion of specific plant chemicals and

nutrients that support growth and reproduction although these chemicals in grapevines vary heterogeneously both spatially and temporally. Concentration of plant toxins in the vascular cells may also influence the ability of grapevine and frosted scales to utilise the host plants (Ali and Agrawal 2012). In vineyards, pest outbreaks may arise from the presence of host vines with reduced plant toxins (secondary defence chemicals). Commercial vineyards usually cultivate several different varieties of grapevines for both red and white wine production. The differences in varieties can influence the population dynamic of insects, such as the grapevine and frosted scales that feed on grapevines (Martinson and Dennehy 1995, Moreau et al. 2006, Moreau et al. 2007, Peverieri et al. 2009, Sharon et al. 2009).

Reproductive output and population growth of insects can be associated with physiological attributes (feeding behaviour) and life history traits (body size variation and generation time) (Begon et al. 1990, Tammaru and Haukioja 1996, Arendt 1997, Tammaru et al. 2002, Chown and Nicholson 2004,). These aspects may determine the mechanism responsible for the prevalence and outbreak of these coccids in vineyards across Australia. This study is conducted with a view to assess the impact of grapevine varieties on population dynamics of grapevine and frosted scales and this information can be used for pest management. In particular, the following topics should be addressed: (1) with grapevine and frosted scales feeding on several different vine varieties, does reproductive output differ between vine varieties? In this context, is it possible that these vine varieties influence the relationship between fecundity and body size of adult females of both species? (2) In the case of different soft scale species, does population growth rate differ between the two study species and what is their intrinsic rate of increase?

## **2.0 Materials and Methods**

### **2.1 Insect Material**

Grapevine and frosted scales are widely distributed in vineyards across Australia (Rakimov, 2010; 2013). In a preliminary survey of vineyards near Canberra, Australian Capital Territory, Australia in March to September 2010, grapevine scale was found on two *Vitis vinifera* L. varieties Chardonnay and Riesling while frosted scale was observed on eight *V. vinifera* varieties Chardonnay, Riesling, Pinot Noir, Merlot, Pinot Gris, Viogner, Semillon and Shiraz. Of the three vineyards considered in the survey, two were infested with frosted scale and one with both grapevine scale and frosted scale present. In the vineyard with a mixed scale infestation, heavy infestation of grapevine scale and frosted scale were found on Riesling and Chardonnay varieties (refer Chapter 5). In the current study on the reproductive output, gravid adult frosted scale were collected from Chardonnay, Riesling and Sauvignon Blanc while gravid adult grapevine scale were collected on Chardonnay and Riesling varieties. A study on scale population growth was conducted on Riesling and Chardonnay varieties for grapevine and frosted scale respectively as these varieties were favoured in the field. Field studies were undertaken within one growing season from October 2010 to November 2011.

### **2.2 Fecundity Experiment**



Twenty gravid adults of each grape scale species were collected from two grapevine varieties, Chardonnay and Riesling. Gravid frosted scale females were also collected from Sauvignon Blanc. Insects were collected into a 90 mm petri-dish labelled for each grapevine variety. The cap of the petri dish was firmly held in place with sellotape. The petri-dish and its contents were kept in a cooler box with ice for storage and transported back to the laboratory (10-15 km away). In the laboratory, ten adult females from each grapevine variety were isolated, measured for body length and mass then maintained at 22°C. Body size was measured using a dissecting microscope with a calibrated eye piece lens under 100x magnification. The body mass of the insects was determined using a Mettler A260 delta range analytical microbalance with 0.1 mg readability (Mettler Toledo). Individual insects were kept in an adult brood chamber consisting of a small glass Petri-dish (d 55 mm) retained inside a larger glass Petri-dish (d 90 mm) containing moistened cotton wool. Water was added to the cotton wool to maintain high humidity in the brood chamber. Egg oviposition, egg incubation period and emergence of first instar ‘crawlers’ were monitored daily. Emerging crawlers were counted and removed from the Petri-dish until no further crawlers emerged after ten sequential days. At the end of the study, the unhatched eggs were counted and categorised as infertile.

Egg oviposition and incubation by the adult females was monitored by examining the brood chamber. Egg incubation was monitored from the start of oviposition to the first day that the first instar emerged under the adult female brood chamber. Total fecundity was determined by counting the number of crawlers emerging from the adult brood chamber and the number of unhatched eggs from individual scales. The number of fertile offspring indicates the insect reproductive potential. Hence percentage fertility was determined for

each species on each grapevine variety, according to equation 1: *Eq. 1. % fertility = 100\*(Number of crawlers per female/ Total fecundity per female).*

Egg shape of both species was oval. The egg size was determined by using an elliptical volume calculation ( $volume = 1/6 * \pi * length * width * height$ ) (Speight, 1994). Two measurements of egg size were taken, the length and width. Height was determined by the assumption that the width is equivalent to the height due to the roundness in the middle section of the oval-shaped egg. In individual insect species and grapevine variety, five eggs per adult were measured. The egg size was determined to evaluate if adult female body mass affect the egg size.

The relationship between body length and body mass for each scale species was first analysed to determine if the variance of body length is determined by body mass. In scale insects, there is considerable variation in body length and width and body width is seldom longer than the length depending on habitat (Ben-Dov et al. 1997). Therefore the body length-mass relationship should illustrate the major reason behind the variability of body length across the three grapevine varieties.

### **2.3 Population Growth Rate**

Population growth rate was determined in the field by monitoring reproduction and age-specific survival of each life stage using repeated sampling techniques. Grapevine scale feeding on Riesling and frosted scale on Chardonnay were used. A single branch with adult females was selected on a grapevine for each coccid species. The number of adult females

was determined at the start and the total insects on the branch including those on the leaves were counted monthly from October 2010 to November 2011 until the juveniles had reached the adult stage, assuming that the immigration and emigration rates were equal for juveniles during the study period. Survival rate of different life stages was determined at four time points, December, March, June and October which encompass first, second and adult stages for frosted scale and first, second, third and adult stages for grapevine scale. Gravid females were collected and reared in the laboratory to determine reproductive output as in the fecundity experiment. Life history parameters measured were the net reproductive rate ( $R_0$ ), time interval between generations ( $T$ ), the intrinsic rate of increase ( $r_m$ ), the finite rate of increase ( $\lambda$ ) and population doubling time ( $DT$ ) (Arendt, 1997, Begon et al. 1990).

Life table parameters were calculated according to the methods described by Birch (1948). These parameters were calculated by the age intervals for each life stage ( $x$ ), average population for each life stage ( $L_x$ ), and the fecundity of adults ( $m_x$ ). The life table parameters were calculated using the formulae, reproductive rate ( $R_0 = \sum F_x/x_0$ ), mean generation time ( $T_{\text{months}} = \ln R_0/r_m$ ), intrinsic rate of increase ( $r_m = \ln R_0/T$ ) (months) and doubling time ( $DT = \ln 2/r_m$ ) (months). Life table determinations for grapevine scale and frosted scale were obtained from the Riesling and Chardonnay varieties respectively.

## **2.4 Data Analysis**

A General Linear Mixed Model (GLMM) ANOVA with normal distribution and log-linked function was used for analysing differences between scale species for each life history

parameter of grapevine and frosted scales using Genstat® version 16 software (Genstat, 2013). The life history parameters used as response variables were body mass, body length, fecundity, egg incubation period, egg size and fertility in the model fitted with species as a fixed factor (explanatory factor) and variety as the random factor. Within individual insect species, a further General Linear Model (GLM) ANOVA with normal distribution and log-linked function was undertaken separately to test the effect of grapevine variety on each life history parameter. The response variables were life history parameters as in GLMM and the fixed factor was variety. The result was presented as back-transformed means ( $\pm$  SEM).

The difference between the size of eggs produced by grapevine and frosted scale was analysed using a t-test with two-sided probability test. The effect of adult body mass on egg size for individual insect species across grapevine varieties was analysed separately using a simple linear regression ANOVA; egg size was the response variable, the independent variable was body mass and grapevine variety was the group factor. Differences in the body length of first instar stage of grapevine and frosted scales was analysed using a t-test with two sided probability test.

The relationship between fecundity and fertility of each species was analysed using the body mass as the predictor (explanatory variable) and the response variables were fecundity and fertility in a simple linear regression ANOVA with grapevine variety as the grouping factor. The response variables were not transformed as conditions for normal distribution were met.

## 3.0 Results

### 3.1 Body Size

On average gravid adult females of grapevine scale were significantly ( $p < 0.001$ ) larger than frosted scale females in both body length and body mass (Table 1). Grapevine variety did not influence the body length ( $F = 0.01$ ,  $df = 1, 17$ ,  $p < 0.916$ ) or body mass ( $F = 0.01$ ,  $df = 1, 17$ ,  $p < 0.904$ ) of grapevine scale females (Table 2). Similarly, grapevine varieties did not affect body length ( $F = 1.98$ ,  $df = 2, 21$ ,  $p = 0.163$ ) or body mass ( $F = 1.10$ ,  $df = 2, 21$ ,  $p < 0.350$ ) of gravid adult females of frosted scale (Table 2).

Average egg size of frosted scale was significantly larger than grapevine scale (T-test value =  $-4.00$ ,  $df = 38$ ,  $P < 0.001$ ; Table 1). Within the female adults of grapevine scale, the effect of grapevine variety on egg size was not significant (Table 2). For frosted scale, the egg size was not significantly different between the grapevine variety (Table 2). There was no significant relationship between adult female body mass and egg size in grapevine scale ( $F = 0.62$ ,  $df = 1, 15$ ,  $p = 0.442$ ) and frosted scale ( $F = 0.16$ ,  $df = 1, 15$ ,  $p < 0.697$ ). Average first instar body length of frosted scale was significantly larger ( $0.39 \pm 0.003\text{mm}$ ) than grapevine scale ( $0.31 \pm 0.004\text{mm}$ ) (T-test value =  $-14.09$ ,  $df = 32$ ,  $p < 0.001$ ).

There is a broad overlap in body length and body mass of gravid females in the two coccids (Fig. 1). The relationship between the body length and body mass of the two coccids is positively correlated (Grapevine scale  $y = 4.8 + 0.09x$ , Frosted scale  $y = 4.2 + 0.09x$ ,  $F =$

69.75,  $df = 1, 40$ ,  $p < 0.001$ ,  $r^2 = 0.64$ ). Body mass predictor was used in the subsequent ANOVA to evaluate the effect of body mass on the reproductive output.

### **3.2 Reproductive Parameters**

Fecundity in grapevine scale was significantly higher than frosted scale fecundity ( $F = 37.8$ ,  $df = 1, 39$ ,  $p < 0.001$ ; Table 1). Mean fecundity for the grapevine and frosted scales on the various grape varieties was  $1361 \pm 128$  and  $387 \pm 113$  per adult female respectively. There was no significant difference in the fecundity of grapevine scale on Riesling or Chardonnay ( $F = 0.39$ ,  $df = 1, 17$ ,  $p = 0.542$ ; Table 2). However, the frosted scale did show significant differences in fecundity among the three varieties ( $F = 3.6$ ,  $df = 1, 21$ ,  $p < 0.045$ ), produced more eggs on Chardonnay and Riesling than on Sauvignon Blanc (Table 2). Fertility was not significantly different between the two species ( $F = 0.06$ ,  $df = 1, 39$ ,  $p < 0.805$ ). Grapevine scale produced  $93.4 \pm 3.2\%$  fertile offspring compared with  $96.4 \pm 2.9\%$  fertile eggs for frosted scale. No effect of grapevine variety was found for fertility of either grapevine scale on Chardonnay and Riesling ( $F = 3$ ,  $df = 1, 17$ ,  $p < 0.101$ ) or frosted scale on Chardonnay, Riesling and Sauvignon ( $F = 0.83$ ,  $df = 1, 21$ ,  $p < 0.452$ ).

Eggs were laid between October and November for both scale species. Grapevine and frosted scales eggs hatched around 19 days post-oviposition for both species ( $20.7 \pm 0.27$  and  $18.8 \pm 0.23$ , respectively) (Wald statistic = 31.14,  $df = 1, 41$ ,  $p < 0.002$ ). A significant varietal effect on egg incubation period was observed for grapevine scale ( $F = 5.78$ ,  $df = 1, 17$ ,  $p < 0.028$ ), with a longer time for eggs of grapevine scales to hatch on Chardonnay compared with Riesling (Table 2). Incubation of frosted scale eggs also differed

significantly between grapevine varieties ( $F=17.01$ ,  $df = 2, 21$ ,  $p < 0.001$ ), with less time for all eggs to hatch on Chardonnay and Riesling than on Sauvignon Blanc (Table 2).

Fecundity of the two coccids species varied with body mass. Fecundity increased with body mass in grapevine scale feeding on Chardonnay and Riesling, (Chardonnay  $y = -15 + 70.9x$ , Riesling  $y = 95 + 62.1x$ ,  $F = 7.78$ ,  $df = 1, 15$ ,  $p < 0.014$ ,  $r^2 = 0.23$ ; Fig. 2) and in frosted scale feeding on Chardonnay, Riesling and Sauvignon Blanc (where Chardonnay  $y = 165 + 36.6x$ , Riesling  $y = -179 + 20.6x$  and Sauvignon Blanc  $y = -44 + 17.4x$ ,  $F = 28.69$ ,  $df = 1, 16$ ,  $p < 0.001$ ,  $r^2 = 0.65$ ) (Fig. 3). No interactions between variety and body mass on fecundity was found in grapevine scale ( $F = 0.03$ ,  $df = 1, 15$ ,  $P < 0.873$ ) and in frosted scale ( $F = 1.20$ ,  $df = 2, 16$ ,  $p < 0.328$ ).

### 3.3 Population Growth Parameters

Life tables were constructed to show the number of immature stages entering adulthood as well as their survival and mortality rates (Table 3 and 4). Only 2% of immature stages in both scale species survived to adulthood. Mortality was highest in second and third instar stages of grapevine scale and in second instar and young adult stage of frosted scale. The mortality for first instar, second instar and third instar of grapevine scale was 38%, 85% and 67% respectively (Table 3). Mortality in first instar, second instar and the adult of frosted scale was 45%, 85% and 63% respectively (Table 4).

The two coccids complete their life cycle within a 12 month period on respective host varieties. The net reproductive rate ( $R_0$ ), intrinsic rate of increase  $r_m$ , and finite rate of

increase  $\lambda$  for grapevine scale were higher than the same values for frosted scale (Table 5). On average adult female grapevine scale oviposited 1372 eggs while frosted scale with 572 eggs per adult female (Table 5). There was a low survival rate of adults in both scale species.  $R_o$  was 28 and 12 fertile offspring per adult in grapevine and frosted scales respectively. However,  $R_o$  is  $>1$  for both species, implying that the population will increase rapidly for both coccids. The intrinsic rate of increase ( $r_m$ ) was 0.28 per month and 0.21 per month in grapevine and frosted scale scales respectively. There was exponential population growth ( $\lambda$ ) in grapevine scale and frosted scale which was 1.28 and 1.23 respectively. Frosted scale has slightly longer doubling time (DT) of 3.35 months compared to grapevine scale (2.50 months).

#### **4.0 Discussion**

Grapevine scale is a significant pest of wine grapes in several countries including Australia (Hely et al. 1982, Buchanan and Amos 1992, Pellizzari 1997). In addition frosted scale feeds and reproduces well on several grapevine varieties grown in Australia (Rakimov 2010, Rakimov et al. 2013). In Australian vineyards both scale insects are present and can occasionally reach outbreak levels. Although, life cycle and reproduction of these coccids has been briefly reported in relation to population dynamics (Hely et al. 1982, Pellizzari 1997, Rakimov 2010, Rakimov et al. 2013), the reproductive potential and inherent properties for population growth for these two species have not been investigated on grapevines. The current study is the first attempt at providing the biological and ecological information that is involved in population growth and hence population dynamics of grapevine scale and frosted scale.



Adult females of grapevine and frosted scales have high fecundity, produce a high proportion of viable offspring and thus have a high intrinsic growth rate. However, gravid adult female of grapevine scales were larger than gravid females of frosted scales, and had a slightly greater fecundity as a result. Females of frosted scale had reduced fecundity when present on Sauvignon Blanc compared with individuals present on Chardonnay and Riesling grapevines. The differences in fecundity were not a result of reduced body size, although smaller individuals were present on Sauvignon Blanc and Riesling. The two grapevine varieties, Chardonnay and Riesling, did not affect the fecundity, body length and body biomass of grapevine scale. The influence of grapevine variety on the reproductive output of frosted scale is similar to those of related species in the Coccoidea feeding on multiple host plants (Hill et al. 2009, Qin et al. 2011, Tanga et al. 2013). Those studies showed that a higher reproductive output and population growth rate occurred when insects fed on more favoured hosts than on less favoured or resistant varieties. High reproductive output of grapevine scale and frosted scale on Chardonnay and Riesling grapevines therefore suggests these varieties may be preferred hosts for both coccids.

Gravid females of grapevine scale have a higher reproductive output, as well as increased body length and body mass compared with frosted scale. Adult female fecundity increased with the body mass in both species (Figs. 2 and 3) with no significant effect of host plant variety. In most insects, larger females are more fecund compared with smaller individuals (Begon et al. 1990, Arendt 1997, Hutchinson et al. 1997, Roff 2002, Tammaru et al. 2002), and this relation has been shown previously in Coccoidea (Speight 1994). Body size (length or mass) of several adult female sap-sucking Hemiptera is affected by the environmental

conditions (Washburn et al. 1985, Speight 1994, Kim et al. 2008, Mathenge et al. 2009). Temperature, crowding conditions (population density) and time limitation factors can influence the adult insects' body morphology. In coccids, pest density increases on suitable host plants (or susceptible plants) (Mathenge et al. 2009, Qin et al. 2011). This increase in scale numbers can cause a reduction in plant vigour, as the insects consume the plant nutrients and this reduction in nutrients can affect future insect body morphology (Washburn et al. 1985, Wardhaugh and Didham 2005). Selection for large body size of grapevine and frosted scale on preferred host varieties, such as Chardonnay and Riesling, may favour reproductive output but the less preferred grapevine varieties, such as Sauvignon Blanc, may lead to smaller adults and reduced reproductive potential. In Chapter 2, average body length of the adult stage of grapevine scale, mean 4.48 (2.12 - 9.00) mm was significantly lower than frosted scale mean 5.19 (2.12 – 7.33) mm. Length of egg, first and second instar stages were on average longer in frosted scale than in grapevine scale. Using only the gravid adults in the current study, grapevine scale may be larger than frosted scale which is usual occurrence in the field (Rakimov 2010).

Variation in body mass and fecundity in the two coccids were observed in both between species and with host variety. Part of the body size variation results from size differences that are genetic (difference between species) and part of the variation may result from a higher density of scale insects, thus leading to variation in adult body size as suggested above (i.e. a density-dependent effect). However, on Sauvignon Blanc variation in body mass and fecundity of adult female frosted scale was lower than females feeding on other varieties (Fig. 3). Such a difference with variety suggests that Sauvignon Blanc may be

affecting the reproductive output of the scales as a result of host plant factors, or a density-independent regulation of reproduction.

Grapevine scale had a higher intrinsic rate of increase, finite rate of increase and shorter doubling time on Riesling grapevines than frosted scale feeding on Chardonnay (Table 3). The intrinsic rate of increases measured the success/fitness of individual adult females for growth, survival and reproduction (Southwood and Henderson 2000). The two coccid species on Chardonnay and Riesling grapevines can frequently achieve high population densities beyond the economic injury level in vineyards because the reproductive rate is higher than unity (value of one) (Southwood and Henderson 2000). Outbreak species usually have shorter development times and higher net reproductive rate and intrinsic rate of increase (Silvertown et al. 2002, Chevin et al. 2010, Ross et al. 2013). Grapevine and frosted scales both have univoltine life cycles and complete their development in 12 months (Table 3). Both scales are unrestricted by adult male mating and the reproduction is primarily parthenogenetic (asexual). The long development time is favoured by univoltine species in extreme climatic conditions (Arendt 1997). In grapevines, carbohydrates are stored in the roots and stems during autumn and winter months (Greer and Sicard 2009, Holzapfel et al. 2010). Carbohydrate remobilisation from stored reserves and nutrient flow for bud-break, leaf formation, and fruit development occurs in spring and throughout summer. Obtaining appropriate nutrition is essential for reproduction whilst adult feeding is timed when nutrient flow is at its highest.

The study suggests that larger gravid females have an advantage over smaller females in terms of reproduction as their higher fecundity can counteract the high mortality of these

insects. Mortality resulted in only two percent of eggs oviposited surviving to adulthood. Increased fecundity was observed in both coccid species, as egg size did not differ with the body mass for either species. Egg volume may not vary for these species of scales, however in horse chestnut scale (*Pulvinaria regalis*), large females produced larger eggs and offspring than small females (Speight 1994). Further work is required to determine whether egg size can vary in grapevine and/or frosted scale insects.

Scale egg development time varied slightly between grapevine varieties. For grapevine scale, egg maturity was on average 20 days post-oviposition on Chardonnay and 19 days on Riesling. The largest difference between oviposition and hatching was for frosted scale eggs laid on Sauvignon Blanc (22 days) compared with eggs laid on Chardonnay (18 days) and Riesling (18 days). This variation in egg maturity may indicate a physiological disparity in relation to the maternal resource allocation for egg production and offspring performance, or alternatively something associated with the plant that decreased insect development. Suboptimal developmental conditions have been recognised to reduce offspring performance (Lorioux et al. 2012). Delayed egg maturity, lower body mass and reduced fecundity of frosted scale suggests that Sauvignon Blanc affects reproductive parameters compared with Chardonnay and Riesling varieties.

The current study was conducted in the field reflecting the general environmental conditions affecting the individual fitness, offspring performance and population growth, assuming that immigration and emigration rates were equal and the mortality from natural enemies is negligent. Under this condition, the cohabitation of both coccid species on grapevines may lead to increased population density, and in turn may accelerate injury to

the host plant (Simbiken et al. submitted). Combined infestations of grapevine scale and frosted scale have been observed on grapevine varieties (Rakimov et al. 2013), so that outbreaks could involve either one or both scale species. As both grapevine and frosted scales have high intrinsic growth rates and parthenogenetic reproduction, controlling these pests from reaching outbreak levels is an important consideration for vineyard owners. One potential mechanism of control can be integration of different grapevine varieties that affect reproductive output of both scale pests. Further work is needed to clarify and elucidate what traits of grapevine varieties may affect population parameters in these insects.

## **5.0 Conclusion**

Achieving large adult body mass (and/or length) is an advantage for individual fitness and persistence of grapevine and frosted scales. In this study adult female fecundity of both species increased with body mass with no significant effect of host plant variety. Variation in body size and fecundity is partly due to genetic differences between scale species (i.e. density-independent) and plant factors affecting physiological traits and population density (i.e. density-dependent). Gravid grapevine scale has a larger body length and body mass and higher fecundity than frosted scale. Grapevine variety did not affect fecundity in grapevine scale but in frosted scale fecundity was lower on Sauvignon Blanc than on Chardonnay and Riesling.

The two coccids in the study achieved the adult female stage in 12 months and only two percent of eggs laid in the previous reproductive cycle reached the adult female stage in both coccids. High fecundity achieved in both scales may counteract high mortality in both

scale insects. This study confirms that grape scales have a high intrinsic natural increase and an exponential population growth potential and thus the possibility of populations frequently reaching outbreak levels. Under this situation, frequent pesticide application and/or augmentative release of biological control agent are needed for sufficient pest control. In addition, a slight plant (varietal) effect on fecundity of frosted scale suggests grapevine varieties may be considered as barrier crops (push-pull strategy) for as part of an integrated pest management approach.

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## 7.0 References

- Ali JG and Agrawal AA. 2012. Specialist versus generalist insect herbivores and plant defense. *Trends in Plant Science* **17**, 293-302.
- Alstad DN and Edmunds Jr GF. 1983. Adaptation, host specificity, and gene flow in the Black Pineleaf Scale. In: *Variable Plants and Herbivores in Natural and Managed Systems*. (eds R. F. Denno and M. S. McClure) pp. 413-426. Academic Press, London, UK.
- Arendt JD. 1997. Adaptive intrinsic growth rates: an integration across taxa. *The Quarterly Review of Biology* **72**, 149-177.
- Bahder BW, Poojari S, Alabi OJ, Naidu, RA and Walsh DB. 2013. *Pseudococcus maritimus* (Hemiptera: Pseudococcidae) and *Parthenolecanium corni* (Hemiptera: Coccidae) are capable of transmitting grapevine leafroll-associated virus 3 between *Vitis x labruscana* and *Vitis vinifera*. *Environmental Entomology* **42**, 1292-1298.
- Begon M, Harper J and Townsend C. 1990. *Ecology: individuals, populations and communities*. Blackwell Scientific Publications, London, UK.
- Ben-Dov Y and Hodgson CJ. 1997. *World crop pests. soft scale insects: their biology, natural enemies and control*. Vol. 7A. Elsevier Science, Amsterdam, Netherlands.

- Birch LC. 1948. The intrinsic rate of natural increase of an insect population. *Journal of Animal Ecology* **17**, 15-26.
- Boggs CL. 2009. Understanding insect life histories and senescence through a resource allocation lens. *Functional Ecology* **23**, 27-37.
- Buchanan G and Amos T. 1992. Grape pests. In: *Viticulture Volume 2 - Practices* (eds B. Coombe and P. Drys). pp. 209-231. Winetitles, Adelaide, Australia.
- Charles JG, Froud KJ, Brink R and Allan DJ. 2009. Mealybugs and the spread of grapevine leafroll-associated virus 3 (GLRaV-3) in a New Zealand vineyard. *Australian Plant Pathology* **38**, 576-583.
- Chevin LM, Lande R and Mace GM. 2010. Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. *PLOS Biology* **8**, e1000357.
- Chown SL and Nicolson S. 2004. *Insect physiological ecology: mechanisms and patterns: mechanisms and patterns*. Oxford University Press, Oxford, UK.
- Fenchel T. 1974. Intrinsic rate of natural increase: the relationship with body size. *Oecology* **14**, 317-326.



Foldi I. 1997. Internal anatomy of the adult female. In: *World Crop Pests. Soft scale insects: their biology, natural enemies and control. Vol. 7A.* (eds Y Ben-Dov and CJ Hodgson). pp. 73-90. Elsevier Science, Amsterdam, Netherlands.

Genstat Sixteenth Edition. 2013. VSN International Ltd. Hempstead, UK.

Glynn C and Herms DA. 2004. Local adaptation in Pine Needle scale (*Chionaspis pinifoliae*): natal and novel host quality as tests for specialization within and among Red and Scots pine. *Environmental Entomology* **33**, 748-755.

Greathead DJ. 1997. Crawler behaviour and dispersal. In: *World Crop Pests. Soft scale insects: their biology, natural enemies and control. Volume 7A.* (eds Y. Ben-Dov and CJ Hodgson). pp. 339-342. Elsevier Science, Amsterdam, Netherlands.

Greer DH and Sicard SM. 2009. The net carbon balance in relation to growth and biomass accumulation of grapevines (*Vitis vinifera* cv. Semillon) grown in a controlled environment. *Functional Plant Biology* **36**, 645-653.

Gullan PJ and Kosztarab M. 1997. Adaptations in scale insects. *Annual Review of Entomology* **42**, 23-50.

Hanks L and Denno RF. 1998. Dispersal and adaptive deme formation in sedentary coccoid insects. In: *Genetic structure and local adaptation in natural insect populations.* (eds S Mopper and S Strauss). pp. 239-262. Springer, US.

Hely PC, Pasfield G and Gellatley JG. 1982. Insect Pests of Fruit and Vegetables in NSW. Inkata Press, Melbourne, Australia.

Hill MG, Mauchline NA, Hall AJ and Stannard KA. 2009. Life table parameters of two armoured scale insect (Hemiptera: Diaspididae) species on resistant and susceptible kiwifruit (*Actinidia* spp.) germplasm. New Zealand Journal of Crop and Horticultural Science **37**, 335-343.

Hoffmann AA, Reynolds TK, Nash MA and Weeks AR. 2008. A high incidence of parthenogenesis in agricultural pests. Proceedings of Royal Society of Biological Science **275**, 2473-2481.

Holzapfel BP, Smith JP, Field SK and Hardie WJ. 2010. Dynamics of Carbohydrate Reserves in Cultivated Grapevines. Horticultural Reviews **37**. John Wiley and Sons, Inc.

Hommay G, Komar W, Lemaire O and Herrbach E. 2008. Grapevine virus A transmission by larvae of *Parthenolecanium corni*. European Journal of Plant Pathology **121**, 185-188.

Hutchinson JMC, Mcnamara JM, Houston AI and Vollrath F. 1997. Dyar's Rule and the Investment Principle: optimal moulting strategies if feeding rate is size-dependent

and growth is discontinuous. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **352**, 113-138.

Kim SC, Song JH and Kim DS. 2008. Effect of temperature on the development and fecundity of the Cryptic Mealybug, *Pseudococcus cryptus*, in the laboratory. *Journal of Asia-Pacific Entomology* **11**, 149-153.

Krüger K and Douglas-Smit N. 2013. Grapevine leafroll-associated Virus 3 (GLRaV-3) transmission by three soft scale insect species (Hemiptera: Coccidae) with notes on their biology. *African Entomology* **21**, 1-8.

Lo P. 1995. Size and fecundity of soft wax scale (*Ceroplastes destructor*) and Chinese wax scale (*C. sinensis*)(Hemiptera: Coccidae) on citrus. *New Zealand Entomology* **18**, 63.

Loch AD and Zalucki MP. 1997. Variation in length, fecundity and survival of pink wax scale, *Ceroplastes rubens* Maskell (Hemiptera: Coccidae), on umbrella trees. *Australian Journal of Zoology* **45**, 399-407.

Lorioux S, Denardo DF, Gorelick R and Lourdais O. 2012. Maternal influences on early development: preferred temperature prior to oviposition hastens embryogenesis and enhances offspring traits in the Children's python, *Antaresia childreni*. *Journal of Experimental Biology* **215**, 1346-1353.

- Marotta S. 1997. General life history. In: *World Crop Pests. Soft scale insects: their biology, natural enemies and control. Vol. 7A.* (eds Y. Ben-Dov and CJ Hodgson) pp. 251-256. Elsevier Science, Amsterdam, Netherlands.
- Martinson TE and Dennehy TJ. 1995. Varietal preferences of *Erythroneura* leafhoppers (Homoptera: Cicadellidae) feeding on grapes in New York. *Environmental Entomology* **24**, 550-558.
- Mathenge CW, Holford O, Hoffmann JH, Spooner-Hart R, Beattie GAC and Zimmermann HG. 2009. The biology of *Dactylopius tomentosus* (Hemiptera: Dactylopiidae). *Bulletin of Entomological Research* **99**, 551-559.
- Moreaua J, Benreya B and Thiérya D. 2006. Grape variety affects larval performance and also female reproductive performance of the European grapevine moth *Lobesia botrana* (Lepidoptera: Tortricidae). *Bulletin of Entomology Research* **96**, 205-212.
- Moreaua J, Thiery D, Troussard JP and Benrey B. 2007. Grape variety affects female but also male reproductive success in wild European grapevine moths. *Ecology Entomology* **32**, 747-753.
- Pellizzari, G. 1997. Grapevine. In: *World Crop Pests. Soft scale insects: their biology, natural enemies and control. Vol. 7B.* (eds Y. Ben-Dov and CJ Hodgson) pp. 323-331. Amsterdam, Netherlands.

- Peverieri GS, Simoni S, Goggioli D, Liguori M and Castagnoli M. 2009. Effects of variety and management practices on mite species diversity in Italian vineyards. *Bulletin of Insectology* **62**, 53-60.
- Pfeiffer DG. 1997. Deciduous fruit trees. In: *World Crop Pests. Soft scale insects: Their Biology, Natural Enemies and Control. Vol. 7B.* (eds Y. Ben-Dov and CJ Hodgson). pp. 293-322. Elsevier Science, Amsterdam, Netherlands.
- Qin Z, Wu J, Qiu BL, Ren S and Ali S. 2011. Effects of host plant on the development, survivorship and reproduction of *Dysmicoccus neobrevipes* Beardsley (Hemiptera: Pseudococcidae). *Crop Protection* **30**, 1124-1128.
- Rakimov A. 2010. Aspects of the biology, ecology and biological control of soft scale insects (Coccidae) in Australian vineyards. PhD Thesis. Dept. of Zoology. University of Melbourne, Melbourne, Australia
- Rakimov A, Ben-Dov Y, White V and Hoffmann AA. 2013. Soft scale insects (Hemiptera: Coccoidea: Coccidae) on grapevines in Australia. *Australian Journal of Entomology* **52**, 371-378.
- Roff DA. 2002. Life history evolution. Sinauer Associates, Sunderland, USA.
- Ross L, Hardy NB, Okusu A and Normark BB. 2013. Large population size predicts the distribution of asexuality in scale insects. *Evolution* **67**, 196-206.

- Sharon R, Zahavi T, Soroker V and Harari AR. 2009. The effect of grapevine cultivars on *Lobesia botrana* (Lepidoptera: Tortricidae) population levels. *Journal of Pest Science* **82**, 187-193.
- Silvertown J, McConway KJ, Hughes Z, Biss P, Macnair M and Lutman P. 2002. Ecological and genetic correlates of long-term population trends in the park grass experiment. *American Naturalist* **160**, 409-420.
- Simbiken NA, Cooper PD and Powell KS. 2014. Host suitability and population dynamics of grapevine scale *Parthenolecanium persicae* (Fabricius) and frosted scale *Parthenolecanium pruinosum* (Cocquillet) (Hemiptera: Coccidae) on grapevine varieties in the Canberra wine region. *Austral Entomology* (submitted).
- Southwood, T., and P. Henderson. 2000. *Ecological methods*. Blackwell Science, Oxford.
- Speight MR. 1994. Reproductive capacity of the horse chestnut scale insect, *Pulvinaria regalis* Canard (Hom., Coccidae). *Journal of Applied Entomology* **118**, 59-67.
- Tammaru T, Esperk T and Castellanos I. 2002. No evidence for costs of being large in females of *Orgyia* spp. (Lepidoptera, Lymantriidae): larger is always better. *Oecology* **133**, 430-438.

- Tammaru T and Haukioja E. 1996. Capital breeders and income breeders among Lepidoptera: consequences to population dynamics. *Oikos*. **77**, 561-564.
- Tang S and Cheke RA. 2008. Models for integrated pest control and their biological implications. *Mathematical Bioscience*. **215**, 115-125.
- Tanga CM, Ekesi S, Govender P and Mohamed SA. 2013. Effect of six host plant species on the life history and population growth parameters of *Rastrococcus iceryoides* (Hemiptera: Pseudococcidae). *Florida Entomology* **96**, 1030-1041.
- Wakgari W. 2000. Effect of scale density on female fecundity, fertility and body size of *Ceroplastes destructor* and on one of its host plants, *Syzygium malaccensis*. *African Plant Protection* **6**, 1-7.
- Wardhaugh CW and Didham RK. 2005. Density-dependent effects on the reproductive fitness of the New Zealand beech scale insect (*Ultracoelostoma assimile*) across multiple spatial scales. *Ecological Entomology* **30**, 733-738.
- Washburn JO, Frankie GW and Grace JK. 1985. Effects of density on survival, development, and fecundity of the soft scale, *Pulvinariella mesembryanthemi* (Homoptera: Coccidae), and its host plant. *Environmental Entomology* **14**,: 755-761.

Zvereva EL, Lanta V and Kozlov MV. 2010. Effects of sap-feeding insect herbivores on growth and reproduction of woody plants: a meta-analysis of experimental studies. *Oecology* **163**, 949-960.



**Table 1. Mean ( $\pm$  SEM) of life history parameters of grapevine scale *Parthenolecanium persicae* and frosted scale *P. pruinosum***

|                       | <b>Adult body length (mm)</b> | <b>Body mass (g)</b> | <b>Egg incubation (days)</b> | <b>Fecundity (Eggs/female)</b> | <b>Egg size (mm<sup>2</sup>)</b> | <b>% Fertility</b> |
|-----------------------|-------------------------------|----------------------|------------------------------|--------------------------------|----------------------------------|--------------------|
| <i>P. persicae</i>    | 6.4 $\pm$ 0.17                | 17.5 $\pm$ 1.2       | 20.7 $\pm$ 0.27              | 1361 $\pm$ 128                 | 0.0025 $\pm$ 0.0002              | 93.2 $\pm$ 2.8     |
| <i>P. pruinosum</i>   | 5.1 $\pm$ 0.15                | 10.4 $\pm$ 1.0       | 19.5 $\pm$ 0.23              | 387 $\pm$ 113                  | 0.0034 $\pm$ 0.0002              | 95.1 $\pm$ 2.5     |
| <b>GLMM ANOVA</b>     |                               |                      |                              |                                |                                  |                    |
| <b>Wald statistic</b> | 31.14                         | 18.20                | 11.45                        | 16.59                          | 14.87                            | 0.24               |
| <b>df</b>             | 1                             | 1                    | 1                            | 1                              | 1                                | 1                  |
| <b>Wald df</b>        | 41                            | 41                   | 41                           | 41                             | 38                               | 40.9               |
| <b>p-value</b>        | <0.001                        | <0.001               | 0.002                        | <0.001                         | <0.001                           | 0.623              |

**Table 2. Mean of body length, body mass, fecundity, fertility egg size and egg incubation period of grapevine scale *Parthenolecanium persicae* and frosted scale *P. pruinosum* on selected *V. vinifera* varieties**

| Grapevine variety      | Insect no. | Body mass (mg)<br>(± SEM) |                          | Body size (mm)<br>(± SEM) |                        | Egg incubation (days)  |                       | Fecundity (eggs)<br>(± SEM) |                       | Egg size (mm <sup>3</sup> )<br>(± SEM) |                               | % fertility<br>(± SEM)   |                          |
|------------------------|------------|---------------------------|--------------------------|---------------------------|------------------------|------------------------|-----------------------|-----------------------------|-----------------------|--|-------------------------------|--------------------------|--------------------------|
|                        |            | <i>P. persicae</i>        | <i>P. pruinosum</i>      | <i>P. persicae</i>        | <i>P. pruinosum</i>    | <i>P. persicae</i>     | <i>P. pruinosum</i>   | <i>P. persicae</i>          | <i>P. pruinosum</i>   | <i>P. persicae</i>                     | <i>P. Pruinosum</i>           | <i>P. persicae</i>       | <i>P. Pruinosum</i>      |
| <b>Chardonnay</b>      | 9          | 17.6 ± 2.1<br>(6.4-27.5)  | 11.9 ± 1.3<br>(7.3-20.3) | 6.4 ± 0.3<br>(4.3-7.9)    | 4.8 ± 0.2<br>(4.3-6)   | 20 ± 0.21<br>(19 - 20) | 18.1 ± 0.5<br>(17-19) | 1236 ± 263<br>(278-2608)    | 562 ± 85<br>(258-809) | 0.0023 ±<br>0.0003 (n=5)               | 0.0032 ±<br>0.0003 (n=5)      | 90.5 ± 3.0<br>(63-100)   | 95.5 ± 5.0<br>(75.4-100) |
| <b>Riesling</b>        | 10         | 17.3 ± 2.0<br>(11.4-29.3) | 10.3 ± 1.3<br>(4.5-16.5) | 6.4 ± 0.3<br>(5.7-7.1)    | 5.4 ± 0.2<br>(4.3-6.1) | 19.2 ±<br>0.20 (18-20) | 18.4 ± 0.4<br>(17-21) | 1473 ± 251<br>(292-2372)    | 362 ± 76<br>(44-862)  | 0.0026 ±<br>0.0003<br>(n=5)            | 0.0034 ±<br>0.0003<br>(n=5)   | 98.1 ± 2.9<br>(91.8-100) | 99.6 ± 4.5<br>(97-100)   |
| <b>Sauvignon Blanc</b> | 6          | -                         | 8.6 ± 1.7 (3.8-15)       | -                         | 5.2 ± 0.3<br>(4.7-6)   | -                      | 22 ± 0.5<br>(21-23)   | -                           | 196 ± 98<br>(18-709)  | -                                      | 0.0035 ±<br>0.0003<br>(n = 5) | -                        | 88.8 ± 5.8<br>(33-100)   |

**Table 3. Life table grapevine scale *P. persicae***

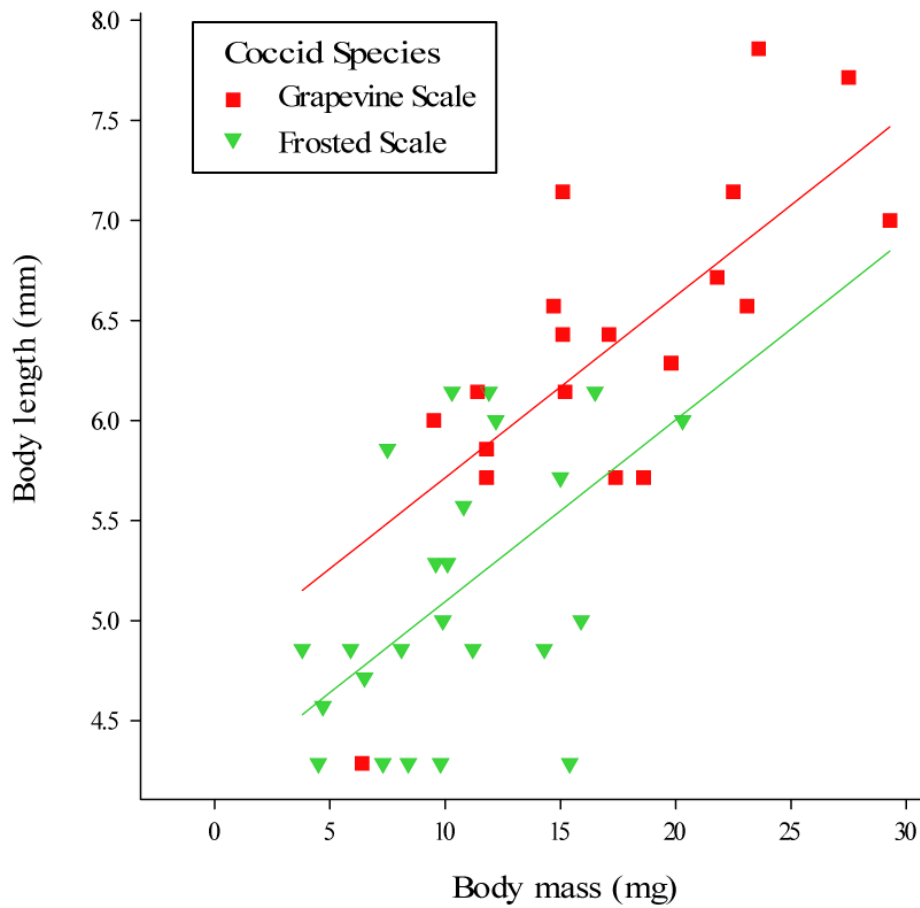
| Age interval            | Number surviving at the beginning of age interval (x) | Proportion surviving between age interval ( $l_x$ ) | Proportion dying at age interval ( $d_x = (x_n - x_{n+1})/x_0$ ) | Percentage mortality ( $100*d_x/l_x$ ) | Eggs produced in each stage ( $F_x$ ) | Eggs produced per surviving adult ( $m_x$ ) |
|-------------------------|---|---|--|--|---------------------------------------|---|
| Egg ( $x_0$ )           | 6860  | 1   | 0.418  | 41.8                                   | NA                                    | -   |
| First instar ( $x_1$ )  | 3995  | 0.582   | 0.221  | 37.9                                   | NA                                    | -   |
| Second instar ( $x_2$ ) | 2480  | 0.362   | 0.308  | 85.1                                   | NA                                    | -   |
| Third instar ( $x_3$ )  | 370   | 0.054   | 0.034  | 63.0                                   | NA                                    | -   |
| Adult ( $x_4$ )         | 140   | 0.020   |  |  | 192080                                | 1372  |

**Table 4. Life table of frosted scale *P. prunosum***

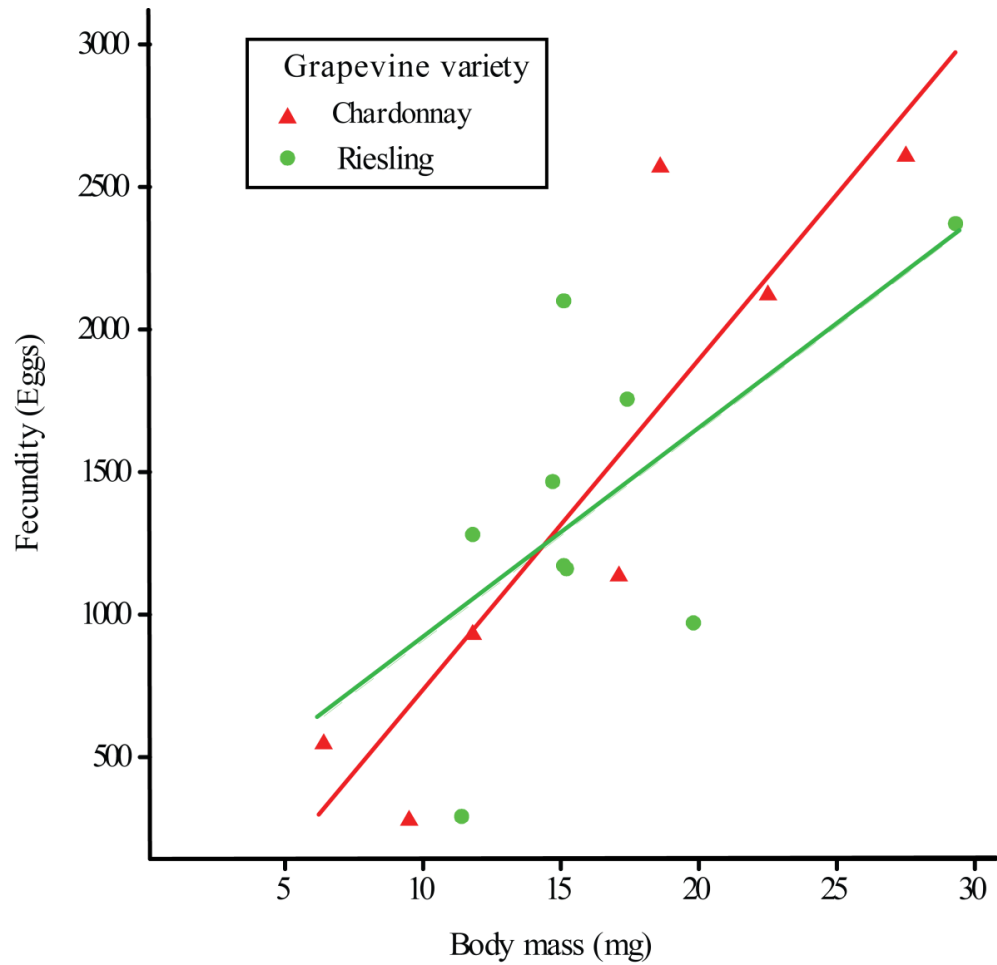
| Age interval            | Number surviving at the beginning of age interval (x) | Proportion surviving between age interval ( $l_x$ ) | Proportion dying at age interval ( $d_x = (x_n - x_{n+1})/x_0$ ) | Percentage mortality ( $100*d_x/l_x$ ) | Eggs produced in each stage ( $F_x$ ) | Eggs produced per surviving adult stage ( $m_x$ ) |
|-------------------------|---|---|--|--|---------------------------------------|---|
| Egg ( $x_0$ )           | 2810  | 1   | 0.269  | 26.67                                  |                                       |   |
| First instar ( $x_1$ )  | 2055  | 0.731   | 0.33   | 45.50                                  |                                       |   |
| Second instar ( $x_2$ ) | 1120  | 0.399   | 0.320  | 80.20                                  |                                       |   |
| Adult ( $x_3$ )         | 220   | 0.08  |  |  | 123640                                | 562   |

**Table 5. Population growth parameters (demography) of grapevine scale *P. persicae* and frosted scale *P. pruinosum***

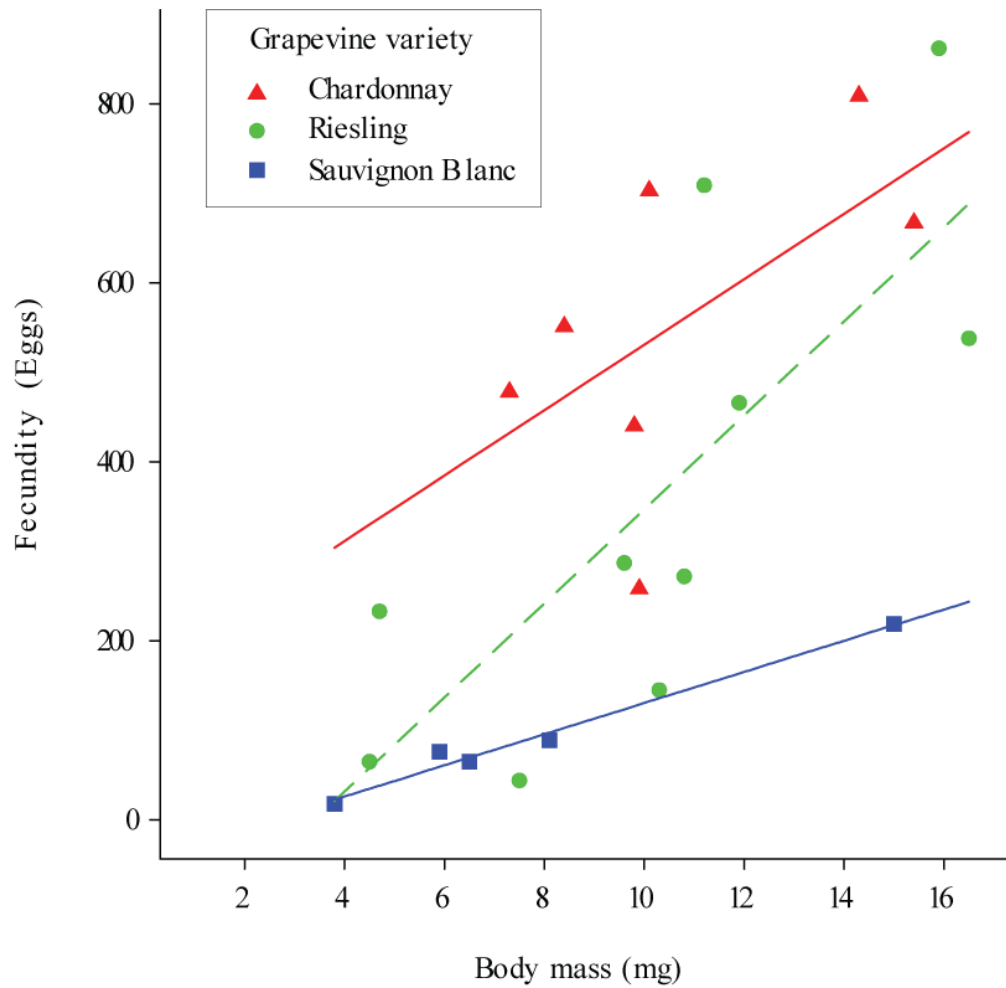
| Grape scale         | Reproductive rate<br>( $R_0 = \Sigma F_x/x_0$ ) | Intrinsic rate of increase<br>( $r_m = \ln R_0/T$ )<br>(months) | Finite rate of increase ( $\lambda = e^r$ )<br>(months) | Mean generation time<br>( $T = \ln R_0/r_m$ )<br>(months) | Doubling time<br>( $DT = \ln 2/r_m$ )<br>(months) |
|---------------------|---|---|---|---|---|
| <i>P. persicae</i>  | 28  | 0.28  | 1.28  | 11.90   | 2.50  |
| <i>P. pruinosum</i> | 12  | 0.21  | 1.23  | 11.83   | 3.35  |



**Figure 1. Relationship between the body length (mm) and body mass (mg) of grapevine Scale *P. persicae* and frosted scale *P. pruinosum* feeding on *V. vinifera* grapevines. (grapevine scale  $y = 4.8 + 0.09x$ , frosted scale  $y = 4.2 + 0.09x$ ,  $F = 69.75$ ,  $df = 1, 40$ ,  $p < 0.001$ ,  $r^2 = 0.64$ ).**



**Figure 2. Relationship between fecundity and body mass of grapevine scale *P. persicae* on Chardonnay and Riesling grapevine varieties. (Chardonnay,  $y = -15 + 70.9x$  and Riesling  $y = 95 + 62.1x$ ,  $F = 7.78$ ,  $df = 1, 15$ ,  $p < 0.014$ ,  $r^2 = 0.23$ )**



**Figure 3. Relationship between fecundity and body mass of frosted scale on Chardonnay, Riesling and Sauvignon Blanc grapevine varieties. (Chardonnay  $y = 165 + 36.6x$ , Riesling  $y = -179 + 20.6x$ , Sauvignon Blanc  $y = -44 + 17.4x$ ,  $F = 28.69$ ,  $df = 1, 16$ ,  $p < 0.001$ ,  $r^2 = 0.65$ )**



# CHAPTER 4

**Preliminary survey of infestation and pest status of  
Grape scale complex, *Parthenolecanium persicae* Fab.  
and *Parthenolecanium pruinosum* Cocq. (Hemiptera:  
Coccidae) in two wine grape-growing zones of South East  
Australia**

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## Summary

The Grape scale complex, refers to two insect species *Parthenolecanium persicae* Fab and *P. pruinosum* Cocq., and is present in all major winegrape growing regions of Australia. The economic importance of this pest complex in different winegrape regions is unclear, as various control procedures are used routinely for scales. A survey of wine grape growers in two wine zones (the warm climate zone of the Hunter Valley, New South Wales and the cool climate zone of Northeast Victoria) was carried out to determine the relative incidence, varietal interactions and pest status of the Grape scale complex. A total of 63 vineyard survey forms were mailed to wine grape growers in Northeast Victoria and 139 to growers in the Hunter Valley, New South Wales. Overall 37% of respondents reported the presence of grape scale with 39% and 36% infestation in vineyards of Northeast Victoria and Hunter Valley, respectively. The pest status of Grape scale complex across the wine zones was not associated with climate zone, vineyard size or intra-varietal cropping system. Population differences on a zonal level were more apparent on white wine grape varieties compared to red wine grape varieties. Although Grape scale incidence was relatively low in both study zones, they were more prevalent on white wine grape varieties than red wine grape varieties. Despite this, red wine grape varieties may be sites for scale survival and they may eventually disperse to white wine grape varieties where they become problematic. The study contributes fundamental knowledge to our overall understanding of the distribution and pest status of Grape scales in vineyards across Australia.

Keywords: Grape scale complex, pest status, survey, varieties, wine grape wine zones.

## 1.0 Introduction

Grapevine scale (*Parthenolecanium persicae*) and frosted scale (*P. pruinosum*), collectively referred to as Grape scale complex, can occasionally become economic important pests of grapevines, if routine control measures are relaxed. Wine quality is drastically reduced by *Pseudococcus* spp (Hemiptera; Pseudococcidae) (Bordeu et al. 2012), indicating that scales can be an economic problem and periodically vineyards can experience heavy infestation levels. Control measures in Australia include insecticide application, during periods that are either well before or post grape harvest (Chong et al. 2007, Hely et al. 1982, Nash et al. 2010, Thomson et al. 2007a, 2007b), or cultural control methods including pruning and fertilization (Bernard et al. 2007). These methods typically ensure that scales do not become an economic problem.

Recent field and controlled environment studies have demonstrated that scale occurrence varies with wine grape variety (Simbiken, in prep.), suggesting that plant traits, such as secondary metabolites and essential nutrients, may affect scale population growth and/or limit their geographical distribution and spread. Several recent studies have documented that the relative abundance of different crop genotypes (i.e. varieties/cultivar) may alter pest status within and across geographic regions (Chateil et al. 2013, McArt and Thaler 2013, Parsa et al. 2011, Yang et al. 2012). For example, intra-mixtures of cotton genotypes have been shown to reduce incidence of immobile insects and also increase incidence of host alternating or mobile insects (Yang et al. 2012, 2014). In Australia, cultivation of number of grapevine varieties in individual vineyards is a widespread practice. However, there is a

lack of information on whether such viticulture practices (intra-cultivar mixes/polycultures) may influence the degree of infestation and pest status of Grape scale complex.

Grapevine genotypes in cultivar mixes, can affect population dynamics of pest insects (English-Loeb et al. 2005, Sharon et al. 2009). Grapevine and frosted scales are polyphagous and feed on several red and white wine grape varieties, although the extent of feeding on the different varieties is unknown. Differences between red wine and white wine are associated with the degree skin pigmentation and tannin content in grape berries. Red wine grape varieties have higher phenolic compounds in the berries' skins than white wine grape varieties (Bisson et al. 2002, González-Centeno et al. 2012, Nicholas et al. 2011, Stockley et al. 2005). In wine production phenolic compounds are associated with wine quality such that high quality wines may have high concentrations of phenolic compounds (Arcari et al. 2013, Granato et al. 2011), but whether difference in phenolic contents in wine grape varieties can affect Grape scale abundance and population dynamics is unknown.

Besides providing flavours for wine, phenolic and tannin compounds can prevent development of some insects (Barbehenn and Constabel 2011, Mithöfer et al. 2012, War et al. 2012). For example, phenolic compounds found in white spruce *Picea glauca* (Moench) Voss (Pinaceae) significantly reduced the survival of spruce budworm *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae) (Delvas et al. 2011). Relatively high concentrations of phenolic compounds in carrots reduced population growth of carrot fly *Psila rosae* (Simlat et al. 2013).

In our study, growers were surveyed regarding the prevalence and perceived pest status of scale insects in two wine grape zones: the warm climate zone of Hunter Valley, New South Wales and the cool climate zone of Northeast Victoria. The survey was designed to gather data on the following:

1. Does the severity of scale infestation differ between the two wine zones?
2. Do wine grape varieties infested with Grape scale differ between the two wine zones?
3. Does Grape scale infestation differ between red and white wine grape varieties?
4. Is infestation correlated with vineyard size and mixed genotype cropping?

## **2.0 Materials and Methods**

### **2.1 Survey Zones**

The North East Victoria wine zone includes the King Valley, Alpine Valley, Glenrowan, Beechworth and Rutherglen wine regions. The Hunter Valley zone includes both the Lower Hunter and the Upper Hunter wine regions. Northeast Victoria and Hunter Valley, New South Wales wine zones differ in several aspects of topography and climatic variables for grape cultivation and wine production (Hall et al. 2010, Anon. 2012a). Typically Northeast Victoria has an average growing season temperature of 18<sup>0</sup>C (range 11– 0<sup>0</sup>C), which is defined as a cool climate zone. The Hunter Valley region is relatively warm, with an average of 21<sup>0</sup>C (range 13– 23<sup>0</sup>C) during the growing season and is classed as warm climate zone (Hall et al. 2010).

## **2.2 Vineyard Survey**

To collect information on the prevalence of Grape scale in the vineyards in the two zones we developed a farm survey questionnaire in consultation with the Statistical Consulting Unit of Australian National University, Canberra. Descriptions of the survey questions are provided in Table 1. Growers were provided with a grapevine scale pest identification brochure (Appendix 1) together with the survey questionnaires. These documents were mailed to all the vineyards as listed in two reference sources James Halliday Australian Wine Companion (Anon. 2012b) and the Australian and New Zealand Wine Directory (Anon, 2012c). Sixty-three questionnaires were distributed to vineyards in Northeast Victoria and 139 to the Hunter Valley. From these, 25 completed forms from the Northeast Victoria and 13 from the Hunter Valley were received, a total of 38 questionnaires returned. Study by Rakimov (2010) and also field observation by the author showed that grapevine and frosted scales were present on vines in the Hunter Valley and North East Wine zones. It was assumed that farmer responses consisted of both scale species, therefore the scales were defined as the Grape scale complex.

## **2.3 Data Analysis**

Infested grape varieties for individual vineyards were used as the response variate and the fixed factors were wine zones, vineyard size and mixed cropping (1 or >1 variety of grape grown). General Linear Model (GLM) with binomial distribution and logit-link function (Logistic regression with binomial errors) ANOVA was used to determine statistical significance of the main factors and interactions.

Vineyard size and mixed cropping of grapevine varieties were arbitrarily categorised into small (<10 ha) and larger (>10 ha) vineyards, and monoculture ( $\leq 3$  varieties) and polyculture (>4 varieties). Vineyard size and grapevine variety mix (polyculture) were used to determine the influence of biodiversity (plant variety) and resource concentration (plant abundance and quality) on the relative pest status of Grape scales. The Wald statistic was used to assess statistical significance level of fixed factors, with significance assumed if  $p < 0.05$ . The results were presented as back-transformed means and standard error of means for the fixed factors.

Severity ratings of Grape scale infestation were based on a scale of 1-5 with 1 being no infestation and 5 being >75% infestation. The scale severity between the two wine zones was analysed using General Linear Model (GLM) with binomial distribution and logit-link function (Logistic regression with binomial errors). The infested grape variety was used as the response variate and wine zone and severity rating as the fixed factors.

If the wine grape variety mix that was infested with Grape scale was significant for the two wine zones, we further tested for the difference between the white and red wine grape varieties. Red wine grape varieties contain higher levels of phenolic compounds than white wine grape varieties (Carando et al. 1999). As phenolics are plant secondary compounds and can be used as defence chemicals by plants against invading pests (Mithöfer et al. 2012), a difference between the red and white wine grape varieties could indicate whether scale insects were affected by the presence of phenolics. The difference between red and white wine grape varieties was compared using a Restricted Maximum Likelihood (REML)

form of a generalised linear mixed model (GLMM) for analysing the count data of grape varieties infested with Grape scale. The difference between the severity ratings between zones was examined using a Pearson Chi-Square test on the count data for grape varieties infested with Grape scale. Genstat 16<sup>th</sup> edition (VSN International) was used for all statistical analyses.

## **3.0 Results**

### **3.1 Geographical Incidence of Grape scale**

The overall response to the survey was 19 %. Of the 38 survey forms returned from the two survey zones, 37% (14/38) of vineyards were infested with Grape scales. Of the 13 survey forms returned from the Hunter Valley, 39% (5/13) of vineyards were infested and in North East Victoria, 36% (9/25) of vineyards were infested (25 forms returned).

### **3.2 Grapevine Varieties Attacked by Grape scale**

Several major grapevine varieties were infested with Grape scale (Table 2). Overall the severity of scale infestation was generally <25% although higher infestation levels were recorded on some varieties and zones. A higher scale infestation was reported for white wine grape varieties compared with the red wine grape varieties (Wald statistic = 11.72, df = 238, p < 0.001, Figure 1). The pest severity rating differed between wine zones ( $\chi^2 = 14.06$ , df = 3, p < 0.003, n = 240, Figure 2), as more grapevines were infested with the



highest severity rating ( $>3$ ) in the Hunter Valley than in North East Victoria. The proportion of wine grape varieties infested with Grape scale was also higher in the Hunter Valley (22.4%, 17/76) than Northeast Victoria (9.8%, 16/164).

### **3.3 Correlation of Grapevine Infestation Between Wine Zones, Vineyard Size and Cropping Type**

There were significant interactions between wine zone and vineyard size (Wald statistic = 11.02,  $df = 1, 29$ ,  $p < 0.05$ , Figure 3) and between wine zone and cropping system (Wald statistic = 12.33,  $df = 1, 29$ ,  $p < 0.002$ , Figure 4). Small vineyards in the Hunter Valley reported a higher incidence of scale, while monoculture vineyards appeared to have a higher infestation in Northeast Victoria than other vineyards.

## **4.0 Discussion**

From our data Grape scales were present in approximately one-third of the vineyards surveyed in both the Hunter Valley and North East Victoria wine zones, with several varieties of grapevine infested. Recent work showed that Grape scales are present in all major wine growing regions of Australia (Rakimov 2010, Rakimov et al. 2013). But the economic importance of this as a pest remains unclear, as various methods are routinely used for the control of scales (Chong et al. 2007, Hely et al. 1982, Nash et al. 2010, Thomson et al. 2007a, 2007b). Pest severity rating differed between the two regions, with a higher scale severity in the Hunter Valley zone than North East Victoria zone. Whether this difference is a result of real Grape scale population differences in the two zones, or a result

of growers' perception of differences cannot be determined. As white wine grape varieties appear to be more likely hosts for Grape scale, the proportion of white to red grapes may influence this observation as well. These findings have important implications for our understanding of the influence that viticulture cropping system may have on Grape scale management, as use of red grape varieties may aid in scale control.

The interaction between viticulture zone and vineyard size suggests that the effect of vineyard size on Grape scale incidence is zone-specific. Infestation in small and large vineyards may be associated with the tri-trophic interaction between pest-plants diversity and natural enemies. A positive correlation between vineyard size and grapevine infestation is likely to occur when large field size inhibits migration of natural enemy populations (Segoli *et al.*, 2012). Conversely, a negative relationship can occur in vineyards under conditions when pest population does not change with an increase in vineyard size. This typifies a pest dilution effect when using a spatially extensive monoculture (Parsa *et al.*, 2011). However, the effect of local environmental conditions influencing scale populations cannot be discounted, with larger vineyards potentially having a greater variation in habitat types.

Cultivation of crop genotypes has been reported to either promote or reduce pest population (McArt and Thaler 2013, Ratnadass *et al.*, 2012, Utsumi *et al.*, 2011, Yang *et al.*, 2012). Yang *et al.* (2012) reported that cultivation of crop genotypes may reduce the population of sedentary insects due to barrier plants or lack of alternate host. Conversely, crop genotype can increase pest population through complimentary effect where pest population increase in a host genotype can move onto the other (Utsumi *et al.*, 2011, Yang *et al.*, 2012). Therefore availability of preferred host genotype may support persistence and population of

Grape scale. The survey results showed that infestation of grapevines vary with the cropping system (higher incidence in monocropping versus polyculture of several grape varieties), however this effect was only present in Northeast Victoria. Thus other factors such as quality of the climate, host variety and/or natural enemy effects are as important in the persistence and pest severity of Grape scales in the vineyards. Scale insects are sedentary with short dispersal ability hence depend on what host plants can offer. Migration and pest population on the neighbouring variety may depend on the nutritional value of the plant, as well as the environmental conditions present during migration.

Higher infestations may occur on white wine grape than on red wine grape varieties. The disparity between red and white wine grape varieties points to the quality of host plants (plant chemistry) such that less defended grapevine varieties may be preferred by the Grape scales. Red wine grape varieties have been reported to produce high phenolic compounds than the white wine grape varieties (Bisson *et al.*, 2002, Stockley *et al.*, 2005). Host plants with high phenolic and tannin compounds were found to reduce development in certain groups of insects and plant pathogens (Delvas *et al.*, 2011, Krischik *et al.*, 1991, Simlat *et al.*, 2013). For example, phenolic compounds extracted from plants prolong aphid pre-reproductive period by 1.5–3.0 days and reduced daily fecundity by 1–1.5 offspring (Chrzanowski *et al.*, 2012). However, some specialised feeders may adapt to plant toxins and then utilise the toxins as feeding stimulants (Bernays, 1998). Although a difference was present between the red and white wine grape varieties, Grape scale still can be considered to have a severe infestation in certain red and white wine grape varieties. For example, the red wine grape variety Shiraz was considered to have a severe infestation in one vineyard surveyed in the Hunter Valley. The white wine grape varieties, Chardonnay, Semillon,

Verdelho and Muscat Orange, also were classified as having severe infestations, but even these levels were present in only 20-25% of the vineyards. Some of the differences may result as fewer red varieties were grown in the vineyards from which responses were obtained. Study on the feeding behaviour of mealy bugs (*Pseudococcus* sp.) on grapevines highlighted that phenolic compounds may decrease with increasing pest density thus leading to low wine quality (Bordeu *et al.*, 2012). This study did not assess the effect of phenolic compounds in grapevines *per se*, therefore the direct effect of phenolic compounds on Grape scale populations is unknown, and the difference between red and white varieties may be only indicative of other chemical differences. Further study is needed to understand the quality of individual variety for resistance/tolerance traits (antibiosis and antixenosis). In other field studies conducted in Canberra wine region, Southern NSW wine zone (Anon. 2012a, Wine Australia, <http://www.winecompanion.com.au/wineries/new-south-wales>), Grape scale population on Pinot Noir and Sauvignon Blanc was relatively lower than Riesling and Chardonnay (Chapter 5). Glasshouse study has shown that the grapevines infested with Grape scale may vary in either their ability to reduce scale population growth and establishment or to tolerate the increase numbers of scale without obvious effects on plant growth and health (Chapter 6).

Disparity between zones on pest severity ratings suggests that climate may have some effect on population dynamics or apparency of Grape scale. Pest severity may increase if both grapevine scale *P. persicae* and frosted scale *P. pruinosum* are found in mixed population on grapevines than individually (Chapter 5). Difference in pest severity observed in the survey reflects the response of grapevines to Grape scale infestation where

a high Grape scale population in red varieties (e.g Pinot Noir) may be present to show visible damage symptoms while low population in other varieties e.g. Riesling and Sauvignon Blanc (Chapter 6) Climate effect may act directly on the insect and indirectly on the host variety. The interaction between wine grape variety, temperature and insect feeding behaviour on the population dynamics and pest status is still unknown and future studies should consider these factors.

## **5.0 Conclusion**

The survey showed that Grape scales are prevalent in both the warm and cool climatic zones of Australia. Significant interactions between wine zone and vineyard area and between wine zone and cropping system imply that vineyard area and cropping of mix wine grape varieties are not important factors in the outbreak of insects. However, Grape scale severity was generally higher on white wine grape varieties than on red wine grape varieties suggesting that at landscape (or regional) level uniform cultivation of white wine grape varieties may increase the Grape scale pest problem.

## **6.0 Acknowledgements**

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## 7.0 References

- Anonymous. 2012a. Wine Australia [Online]. <http://www.wineaustralia.net.au/en/wine-zones>. [Accessed 2012].
- Anonymous. 2012b. James Halliday Australian Wine Companion [Online]. <http://www.winecompanion.com.au>. [Accessed 2012].
- Anonymous. 2012c. The Australian and New Zealand Wine Industry Directory. In: *Winetittles* (ed.). South Australia: Winetittles.
- Arcari SG, Chaves ES, Vanderlinde R, Rosier JP and Bordignon-Luiz MT. 2013. Brazilian fortified wines: Chemical composition, chromatic properties and antioxidant activity. *Food Research International* **53**, 164-173.
- Barbehenn RV and Constabel PC. 2011. Tannins in plant–herbivore interactions. *Phytochemistry* **72**, 1551-1565.
- Bernard M, Wepler R, Kourmouzis T, Yen A, Horne P, Papacek D, Jacometti M, Wratten S, Evans K and Herbert K. 2007. Guidelines for environmentally sustainable

- winegrape production in Australia: IPM adoption self-assessment guide for growers. Australian and New Zealand Grapegrower and Winemaker. pp 24-35.
- Bernays EA. 1998. Evolution of feeding behavior in insect herbivores. *BioScience* **48**, 35-44.
- Bisson LF, Waterhouse AL, Ebeler SE, Walker MA and Lapsley JT. 2002. The present and future of the international wine industry. *Nature* **418**, 696-699.
- Boratynski K. 1970. On some species of 'Lecanium' (Homoptera, Coccidae) in the collection of the Naturhistorisches Museum in Vienna; with description and illustration of the immature stages of *Parthenolecanium persicae*. *Analen des Naturhistorischen Museums Wien* **74**, 63-76.
- Bordeu E, Troncoso DO and Zaviezo T. 2012. Influence of mealybug (*Pseudococcus* spp.)-infested bunches on wine quality in Carmenere and Chardonnay grapes. *International Journal of Food Science and Technology* **47**, 232-239.
- Brittin G. 1940. The life history of *Lecanium (Eulecanium) persicae* (Fabricius), and descriptions of the different instars. *Transactions and Proceedings of the Royal Society of New Zealand* **69**, 413-421.
- Carando S, Teissedre PL, Pascual-Martinez L and Cabanis JC. 1999. Levels of Flavan-3-ols in French Wines. *Journal of Agricultural and Food Chemistry* **47**, 4161-4166.

- Chateil C, Goldringer I, Tarallo L, Kerbiriou C, Le Viol I, Ponge JF, Salmon S, Gachet S and Porcher E. 2013. Crop genetic diversity benefits farmland biodiversity in cultivated fields. *Agriculture, Ecosystems and Environment* **171**, 25-32.
- Chong CS, Hoffmann AA and Thomson LJ. 2007. Commercial agrochemical applications in vineyards do not influence ant communities. *Environmental Entomology* **36**, 1374-1383.
- Chrzanowski G, Leszczyński B, Czerniewicz P, Sytykiewicz H, Matok H, Krzyżanowski R and Sempruch C. 2012. Effect of phenolic acids from black currant, sour cherry and walnut on grain aphid (*Sitobion avenae* F.) development. *Crop Protection* **35**, 71-77.
- Delvas N, Bauce É, Labbé C, Ollevier T and Bélanger R. 2011. Phenolic compounds that confer resistance to spruce budworm. *Entomologia Experimentalis et Applicata* **141**, 35-44.
- English-Loeb G, Norton AP, Gadoury D, Seem R and Wilcox W. 2005. Tri-trophic interactions among grapevines, a fungal pathogen and a mycophagous mite. *Ecological Applications* **15**, 1679-1688.
- González-Centeno MR, Jourdes M, Femenia A, Simal S, Rosselló C and Teissedre PL. 2012. Proanthocyanidin composition and antioxidant potential of the stem winemaking byproducts from 10 different grape varieties (*Vitis vinifera* L.). *Journal of Agricultural and Food Chemistry* **60**, 11850-11858.



- Granato D, Katayama FCU and De Castro, I. A. 2011. Phenolic composition of South American red wines classified according to their antioxidant activity, retail price and sensory quality. *Food Chemistry* **129**, 366-373.
- Hall A and Jones GV. 2010. Spatial analysis of climate in winegrape-growing regions in Australia. *Australian Journal of Grape and Wine Research* **16**, 389-404.
- Hely PC, Pasfield G and Gellatley JG. 1982. *Insect pests of fruit and vegetables in NSW*, Inkata Press.
- Krischik V, Goth R and Barbosa P. 1991. Generalized plant defense: effects on multiple species. *Oecologia* **85**, 562-571.
- McArt SH and Thaler JS. 2013. Plant genotypic diversity reduces the rate of consumer resource utilization. *Proceedings of the Royal Society Series B: Biological Sciences* **280**. doi:[10.1098/rspb.2013.0639](https://doi.org/10.1098/rspb.2013.0639):20130639.
- Mithöfer A and Boland W. 2012. Plant defense against herbivores: Chemical aspects. *Annual Review of Plant Biology* **63**, 431-450.
- Nash MA, Hoffmann AA and Thomson LJ. 2010. Identifying signature of chemical applications on indigenous and invasive nontarget arthropod communities in vineyards. *Ecological Applications* **20**, 1693-1703.

- Nicholas KA, Matthews MA, Lobell DB, Willits NH and Field CB. 2011. Effect of vineyard-scale climate variability on Pinot Noir phenolic composition. *Agricultural and Forest Meteorology* **151**, 1556-1567.
- Parsa S, Ccanto R and Rosenheim JA. 2011. Resource concentration dilutes a key pest in indigenous potato agriculture. *Ecological Applications* **21**, 539-546.
- Rakimov A. 2010. Aspects of the biology, ecology and biological control of soft scale insects (Coccidae) in Australian vineyards. PhD Thesis, University of Melbourne. Melbourne
- Rakimov A, Ben-Dov Y, White V and Hoffmann AA. 2013. Soft scale insects (Hemiptera: Coccoidea: Coccidae) on grapevines in Australia. *Australian Journal of Entomology* **52**, 371-378.
- Ratnadass A, Fernandes P, Avelino J and Habib R. 2012. Plant species diversity for sustainable management of crop pests and diseases in agroecosystems: a review. *Agronomy for Sustainable Development* **32**, 273-303.
- Segoli M and Rosenheim JA. 2012. Should increasing the field size of monocultural crops be expected to exacerbate pest damage? *Agriculture, Ecosystems and Environment* **150**, 38-44.

- Sharon R, Zahavi T, Soroker V and Harari AR. 2009. The effect of grape vine cultivars on *Lobesia botrana* (Lepidoptera: Tortricidae) population levels Journal of Pest Science **82**, 187 - 193.
- Simlat M, Stobiecki M and Szklarczyk M. 2013. Accumulation of selected phenolics and expression of PAL genes in carrots differing in their susceptibility to carrot fly (*Psila rosae* F.). Euphytica **190**, 253-266.
- Stockley CS and Høj PB. 2005. Better wine for better health: Fact or fiction? Australian Journal of Grape and Wine Research **11**, 127-138.
- Thomson LJ and Hoffman AA. 2007a. Grape production in Australia: integrated strategies and bioindicators for sustainability. Encyclopedia of Pest Management **2**, 200.
- Thomson LJ and Hoffmann AA. 2007b. Ecologically sustainable chemical recommendations for agricultural pest control? Journal of Economic Entomology **100**, 1741-1750.
- Utsumi S, Ando Y, Craig TP and Ohgushi T. 2011. Plant genotypic diversity increases population size of a herbivorous insect. Proceedings of the Royal Society Series B: Biological Sciences **278**, 3108-3115.

- War AR, Paulraj MG, Ahmad T, Buhroo AA, Hussain B, Ignacimuthu S and Sharma HC. 2012. Mechanisms of plant defense against insect herbivores. *Plant Signaling and Behavior* **7**, 1306-1320.
- Yang B, Ge F, Ouyang F and Parajulee M. 2012. Intraspecies mixture alters pest and disease severity in cotton. *Environmental Entomology* **41**, 1029-1036.
- Yang B, Parajulee M, Ouyang F, Wu G and Ge F. 2014. Intraspecies mixture exerted contrasting effects on nontarget arthropods of *Bacillus thuringiensis* cotton in northern China. *Agricultural and Forest Entomology* **16**, 24-32

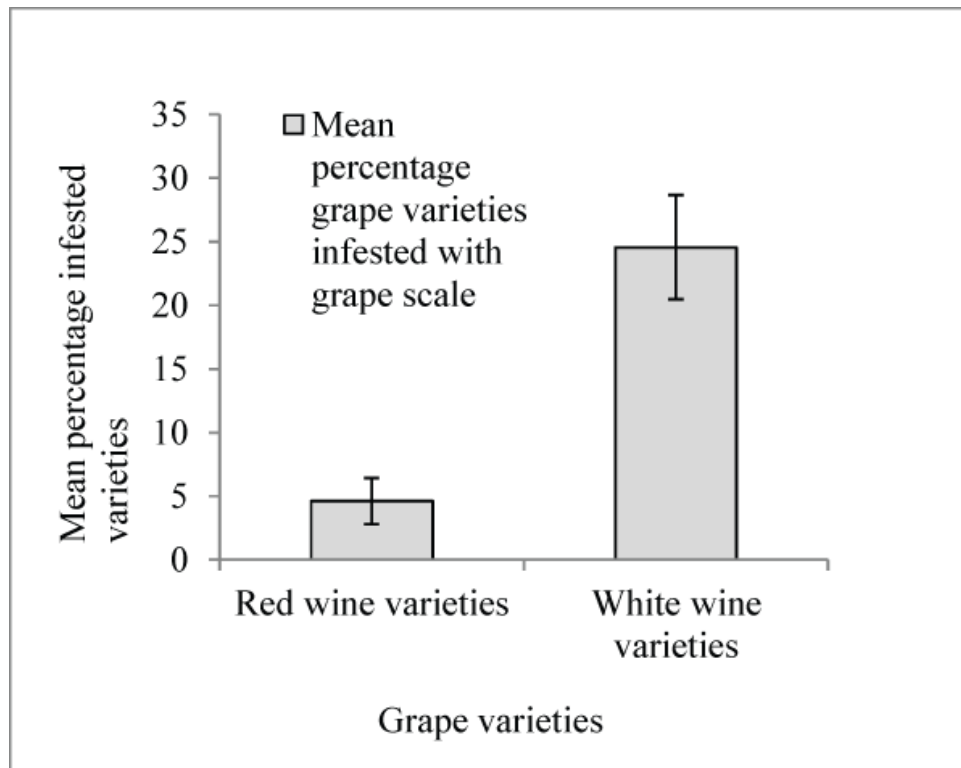
**Table 1. Description of survey questionnaire used in the farm survey to collect information on Grape scale infestation and management**

| <b>Question type</b>                      | <b>Descriptions</b>  |
|---|--|
| 1.Farm Characteristic                     | Vineyard name<br>Location: district and region<br>Vineyard size (hectares)   |
| 2. Grape scale prevalence                 | Present/absent   |
| 3. Grapevine infestation with Grape scale | Total grapevine varieties<br>Infested grapevine varieties<br>Pest rating for varieties infested with Grape scale,<br>1=nil infestation, 2= <25% infestation, 3 = 25-50%<br>infestation, 4 = 51-5% infestation, 5 = >75%<br>infestation |

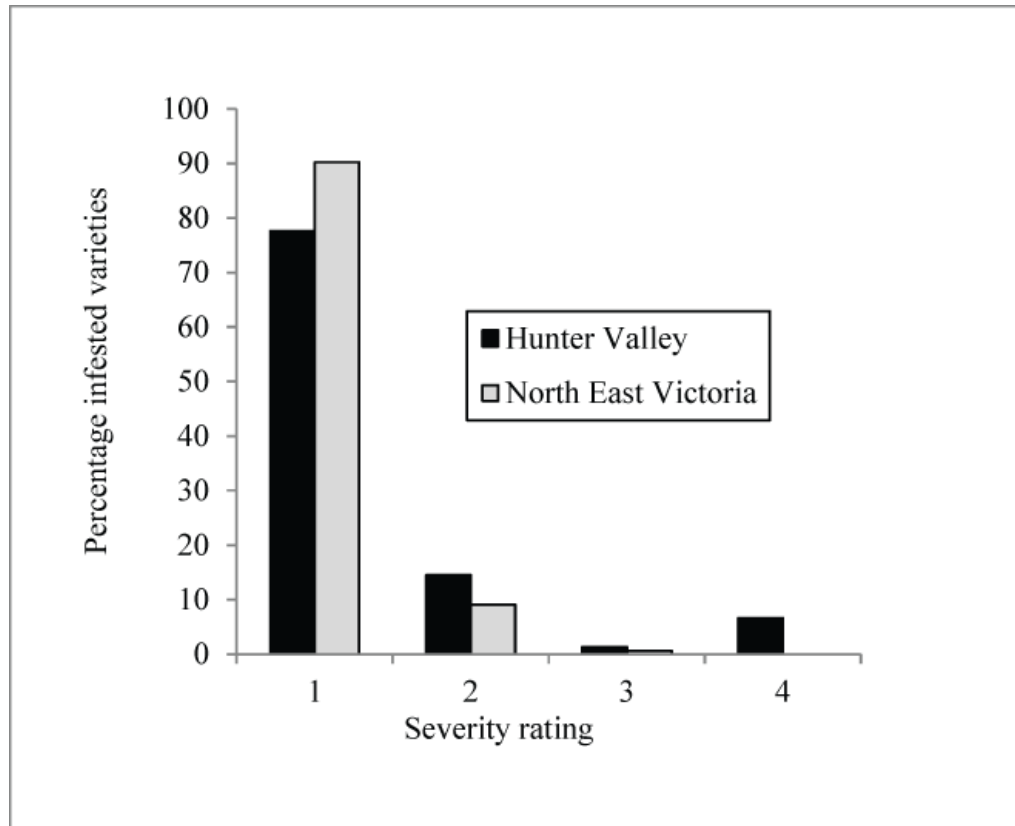
**Table 2. Grape varieties infested with Grape scales as reported in a grape grower survey in North east Victoria and the Hunter Valley wine zones**

| Wine grape-zone           | Variety                 | Severity of Grape scale infestation |             |               |               | Total |
|---------------------------|-------------------------|-------------------------------------|-------------|---------------|---------------|-------|
|                           |                         | 1<br>(No infestation)               | 2<br>(<25%) | 3<br>(25-50%) | 4<br>(51-75%) |       |
| <b>Hunter Valley</b>      | <b>Red wine grape</b>   |                                     |             |               |               |       |
|                           | Barbera                 | -                                   | 1           | -             | -             | 1     |
|                           | Shiraz                  | 11                                  | -           | -             | 1             | 12    |
|                           | <b>White wine grape</b> |                                     |             |               |               |       |
|                           | Chardonnay              | 2                                   | 6           | -             | 2             | 10    |
|                           | Pinot Gris              | -                                   | 1           | -             | -             | 1     |
|                           | Riesling                | 2                                   | 1           | -             | -             | 3     |
|                           | Semillon                | 8                                   |             | 1             | 1             | 10    |
|                           | Traminer                | 2                                   | 1           |               |               | 3     |
|                           | Verdelho                | 2                                   |             |               | 1             | 3     |
| Viogner                   | -                       | 1                                   | -           | -             | 1             |       |
| <b>Northeast Victoria</b> | <b>Red wine grape</b>   |                                     |             |               |               |       |
|                           | Cabernet Sauvignon      | 11                                  | 1           | -             | -             | 12    |
|                           | Pinot Noir              | 3                                   | 3           | -             | -             | 6     |
|                           | <b>White wine grape</b> |                                     |             |               |               |       |
|                           | Chardonnay              | 11                                  | 4           | -             | -             | 15    |
|                           | Cienna                  | -                                   | 1           | -             | -             | 1     |
|                           | Crouchen                | -                                   | 1           | -             | -             | 1     |
|                           | Flora                   | -                                   | 1           | -             | -             | 1     |
|                           | Orange muscat           | 3                                   | -           | 1             | -             | 4     |
|                           | Riesling                | 5                                   | 2           | -             | -             | 7     |
| Sauvignon Blanc           | 3                       | 1                                   | -           | -             | 4             |       |
| Semillon                  | 2                       | 1                                   | -           | -             | 3             |       |

*Values are the number of times Grape scale was recorded as a pest at that rating for a particular variety. No incidence of Level 5 (> 75% plants) was reported.*

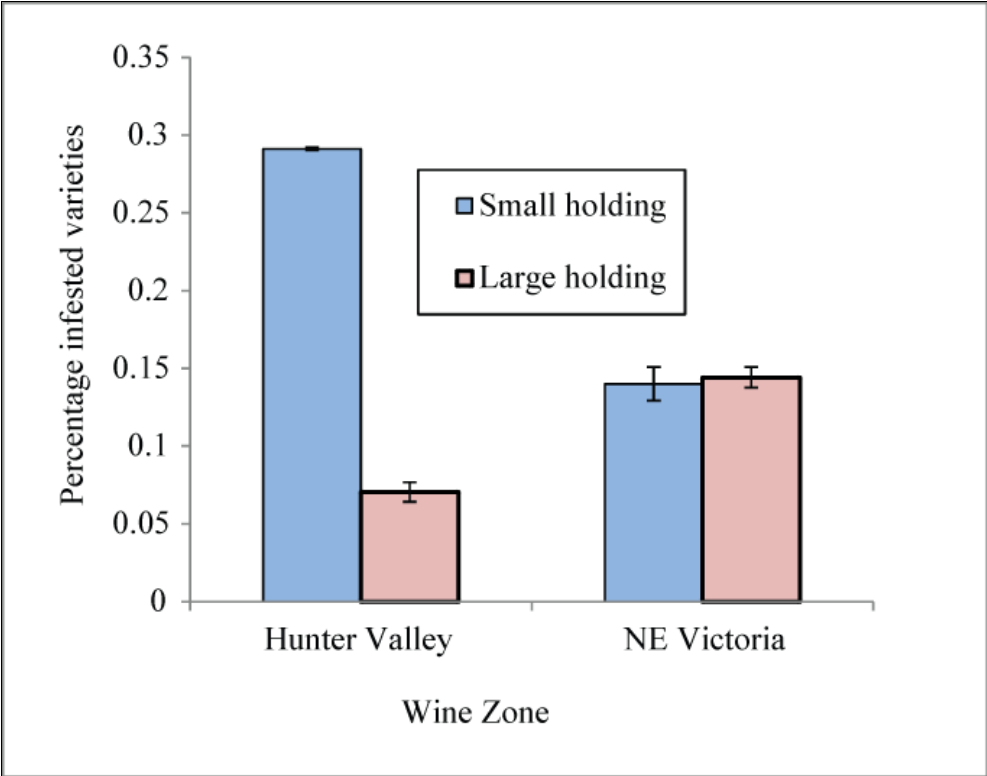


**Figure 1. Comparison of percentage of red and white wine grape varieties infested with Grape scale. Error bar represent the standard error of mean percentage of grapevine varieties infested with Grape scale**

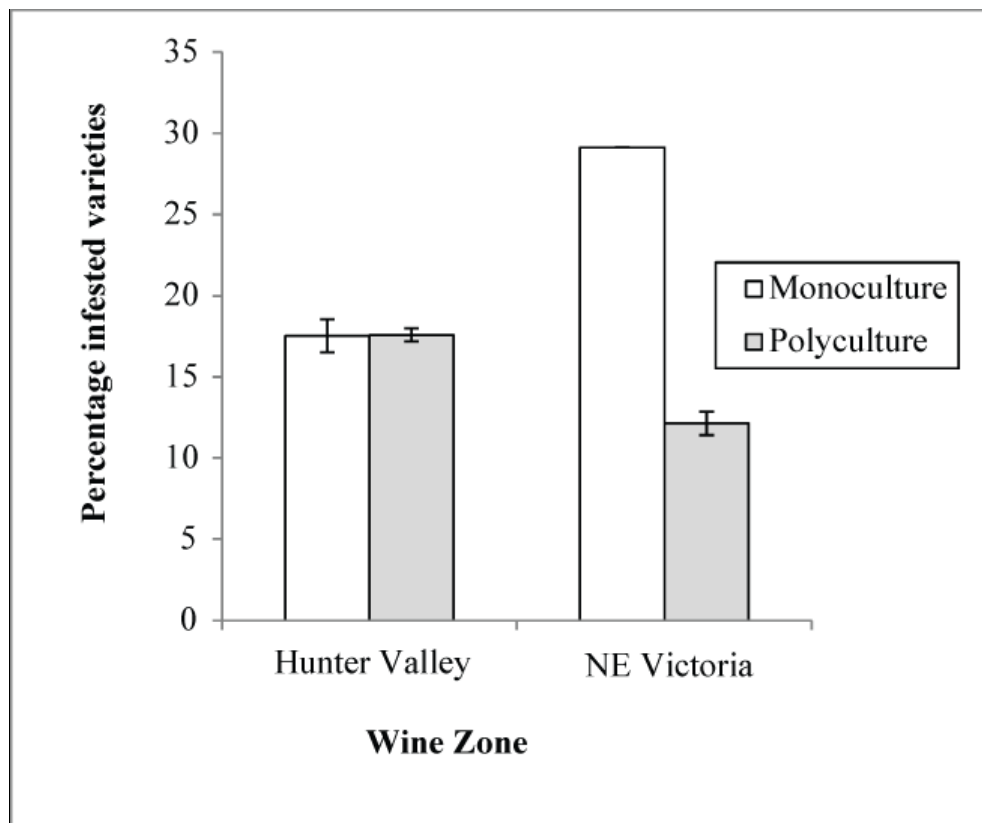


**Figure 2. Severity rating of *Vitis vinifera* varieties infested with Grape scale in the two wine zones.**





**Figure 3. Mean percentage *Vitis vinifera* varieties infested with Grape scales between vineyard holding sizes across two wine zones, the Hunter Valley and North East Victoria. Error bar represent the standard error of mean percentage of grapevine varieties infested with Grape scale**



**Figure 4. Mean percentage of *Vitis vinifera* varieties infested with Grape scales between cropping system across the two wine zones, the Hunter Valley and North East Victoria. Error bar represent the standard error of mean percentage of grapevine varieties infested with Grape scale**

## Appendix 1. Descriptions of life cycle and body features of *P. persicae* and *P. prunosum*



## Appendix 2. Morphology of life stages of *P. pruinatum*

### *Description of first instar*

**Mounted material examined n = 27;**

Canberra, Riesling variety, n = 6

Canberra, Chardonnay Variety, n = 6

Canberra, Sauvignon Blanc, n = 5

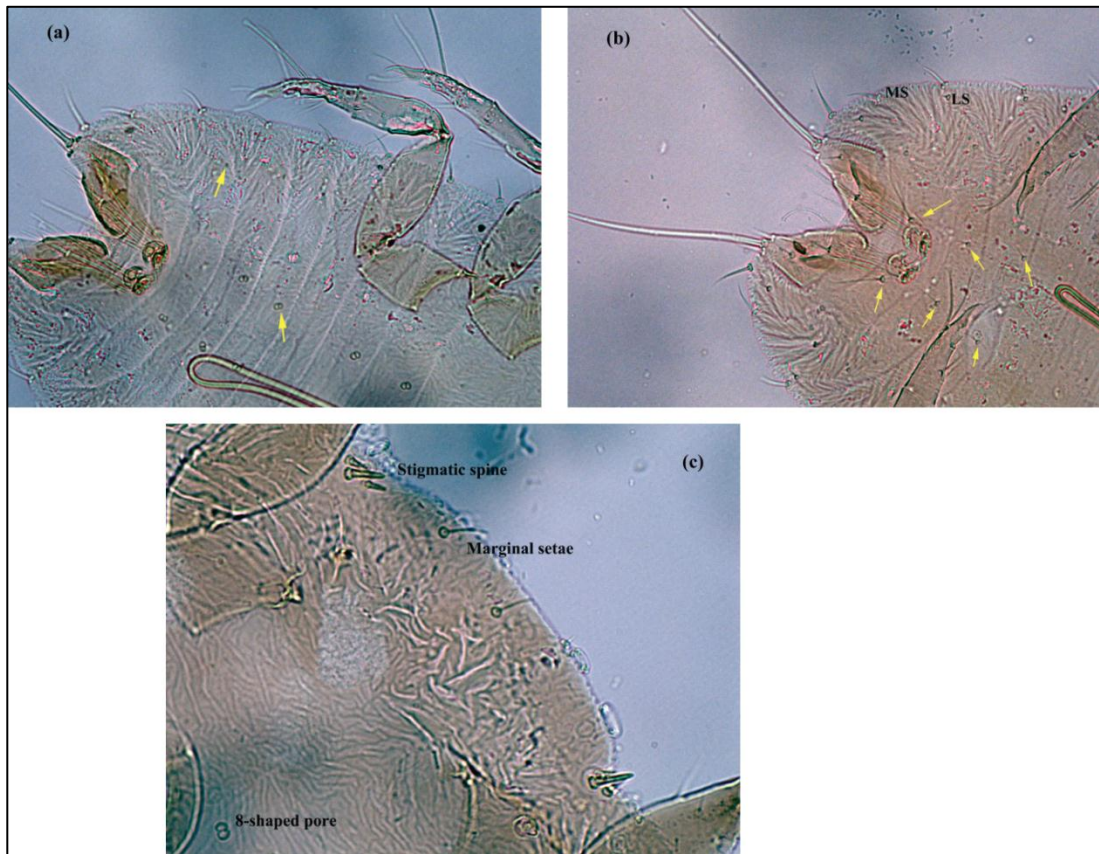
Canberra, Pinot Noir , n = 5

Body oval; body length, 0.32 – 0.44 (0.39)  $\mu\text{m}$ , width 0.18 – 0.24 (0.22)  $\mu\text{m}$  (Chapter 2).

Triangular anal plates, not forged, no longer than the body with three apical setae per anal cleft, middle setae longer than the two lateral setae, and also included one mesal setae.

### Dorsum

One pair of trilocular pores situated on the head apex above the antennae. Two longitudinal rows of rows of 8-shaped pores present, with 9 – 10 pairs located sub-medially, nine lateral to the margin on each side (Figure 1a). The sub-medial pores are larger than the lateral pores. Four pairs of sub-medial setae, one pair above the thorax region, one pair each on the prothorax, mesothorax, and metathorax.



**Figure 1. Digital image of body feature of first instar of *P. pruinosum* (a) Two longitudinal rows of 8-shaped pores, arrow shows the arrangement of pores (b) Arrangement of marginal and ventral setae, LS = lateral setae, MS = marginal setae, arrows show the 3 pairs of setae above ano-genital cleft (c) Two marginal setae between anterior and posterior stigmatic spinal setae.**

### Margin

Marginal setae setose, thirty two setae located around the body margin; eight were located below the posterior stigmatic spine, two between anterior stigmatic spine and posterior stigmatic spine (Figure 1c), and two between anterior stigmatic spine and eye spot on each side, and then eight were located between the two eye spots. Stigmatic spine setae were bluntly spinose, 3 setae per cleft with middle setae slightly longer than the lateral setae.

### Venter

Antennae six-segmented with an average length of 116.6  $\mu\text{m}$ ; sixth segment longer than the third segment, the sixth segment 35.5  $\mu\text{m}$ , fifth segment 14.7  $\mu\text{m}$ , fourth segment 9.6  $\mu\text{m}$ , third segment 29.3  $\mu\text{m}$ , second segment 10.7  $\mu\text{m}$  and first segment 17  $\mu\text{m}$ . Spiracles close to the margin with two quinquelocular pores (or five-locular pores) between the spiracle and stigmatic spine. Venter with two longitudinal rows of setae on each side, one row is submarginal and the other sub-medial. Setae are located on each segment of insect body. There are three pairs of long setae above the anal-genital complex (Figure 1b), and one pair of this located between the antennae. In the leg region, tibia is longer than tarsus with length of tibia 29 – 41 (mean 36.5)  $\mu\text{m}$  and length of tarsus 27 – 30.4 (mean 28.4)  $\mu\text{m}$ .

### *Description of second instar*

**Mounted material examined n = 32**

Canberra, Riesling variety, n = 10

Canberra, Chardonnay variety, n = 12

Canberra, Sauvignon Blanc variety, n = 7

Canberra, Pinot Noir variety, n = 3

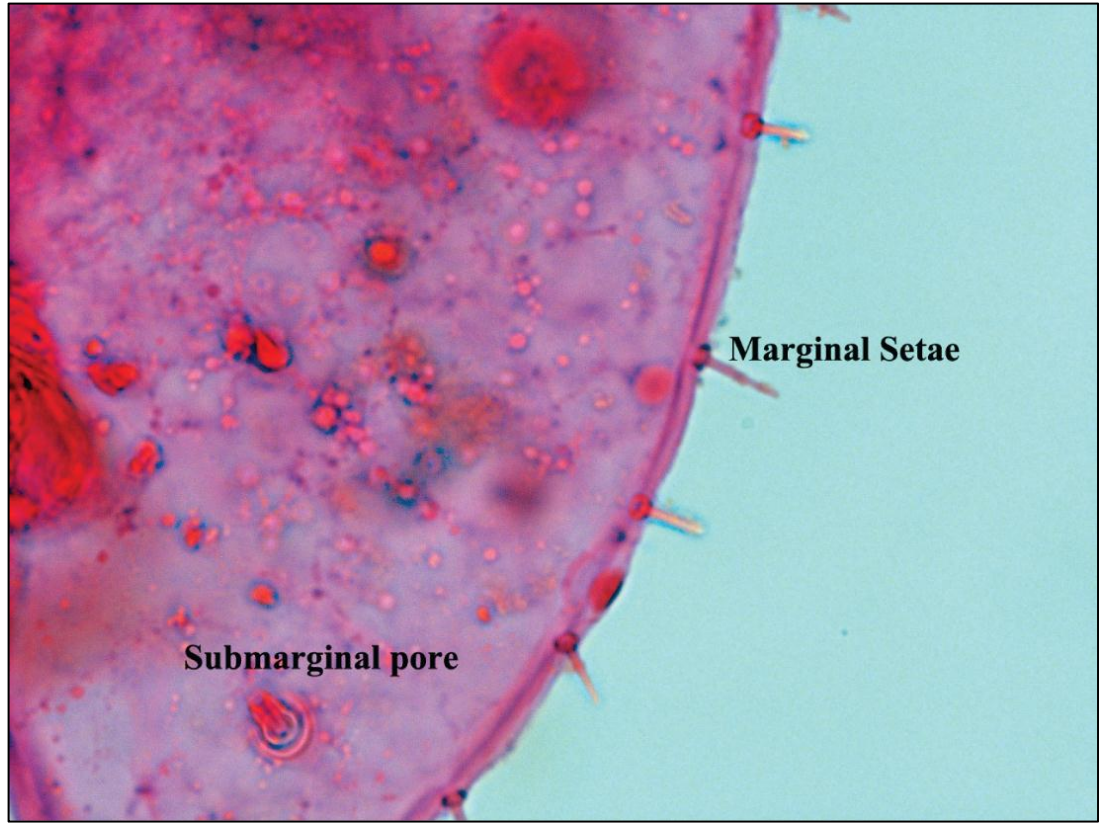
Body elongate oval; length 0.5 – 1.1 (mean 0.92)  $\mu\text{m}$ , width 0.23 – 0.77 (mean 0.49)  $\mu\text{m}$  (Chapter 2). Short anal cleft and three apical setae with middle setae longer than the two lateral setae on each cleft.

### Dorsum

There are ten sub-marginal tubercles, five on each side; two located behind the posterior stigmatic spine, one between posterior and anterior stigmatic spine and two above the anterior stigmatic spine.

### Margin

Marginal setae are straight blunt spinose (Figure 2). There are three setae per stigmatic cleft, setae blunt spinose, middle is longer than the two lateral setae. There are 96 – 102 marginal setae per body, eighteen between eye spots, eight between eye spot and anterior stigmatic spine on each side, seven to nine between the anterior and posterior stigmatic



**Figure 2. Blunt spinose marginal setae and sub-marginal tubercle of second instar of *P. prunosum***



spine on each side, and twenty two to twenty four behind the posterior stigmatic spine and anal cleft on each side.

### Venter

Antennae is six-segmented with mean length of 184  $\mu\text{m}$ , third segment larger than the sixth segment; third segment mean 52  $\mu\text{m}$ , sixth segment mean 42  $\mu\text{m}$ , fifth segment 20 $\mu\text{m}$ , fourth segment 20  $\mu\text{m}$ , second segment 24  $\mu\text{m}$  and first segment 26  $\mu\text{m}$ . Body setae short and bristle arrange in two longitudinal rows on each side, the sub-lateral row extended around the body including the head region. The sub-medial row extending over the meta, mesa and prothoracic area. There are two pairs of large inter-antennal setae and three pairs of large setae on the abdomen above the anal-genital complex. A row of quinquelocular pores extending between the spiracle and spiracular stigmatic cleft. In the leg region, tibia is longer than the tarsus, with length of tibia 64 – 71 (mean 67.4)  $\mu\text{m}$  and length of tarsus 52 – 58 (mean 55.4)  $\mu\text{m}$  (2d).

### *Description of adult stage*

#### **Mounted material examined n = 42**

Canberra, Riesling variety, n = 15

Canberra, Chardonnay variety, n = 18

Canberra, Sauvignon Blanc variety, n = 9

Body elongate and oval; length 1.2 – 7.3 (mean 3.61)  $\mu\text{m}$ , width 0.65 – 4.9 (mean 2.41)  $\mu\text{m}$  (Chapter 2). Short anal cleft with three apical setae.

### Dorsum

There are 12 – 19 submarginal tubercles. In young adult with body length of 1.2 – 2 mm consist of 12 submarginal tubercles, six on each side; three behind posterior stigmatic spine, one between anterior stigmatic spine and two above the anterior stigmatic spine on each side. In mature adult with body length 2.5 – 6 mm, 14-19 submarginal pores were present, 7 – 9 on one side, 4 - 6 behind posterior stigmatic spine, 1 between anterior and posterior stigmatic spine and 2 -3 above the anterior spine.

### Margin

Marginal setae blunt spinose and three setae per stigmatic cleft, middle setae slightly longer than the two lateral setae.

### Venter

Antennae is six-segmented with an average length of 245.7  $\mu\text{m}$ , third segment longer than the sixth segment; third segment 85.1  $\mu\text{m}$ , sixth segment 49.9  $\mu\text{m}$ , 5<sup>th</sup> segment 20  $\mu\text{m}$ , fourth segment 17.7  $\mu\text{m}$ , second segment 38  $\mu\text{m}$  and first segment 35  $\mu\text{m}$ . There are two longitudinal rows of setae on each side, the sub-lateral setae extended from the anal cleft region to the head, the sub-medial setae extend between abdominal and thoracic area. There

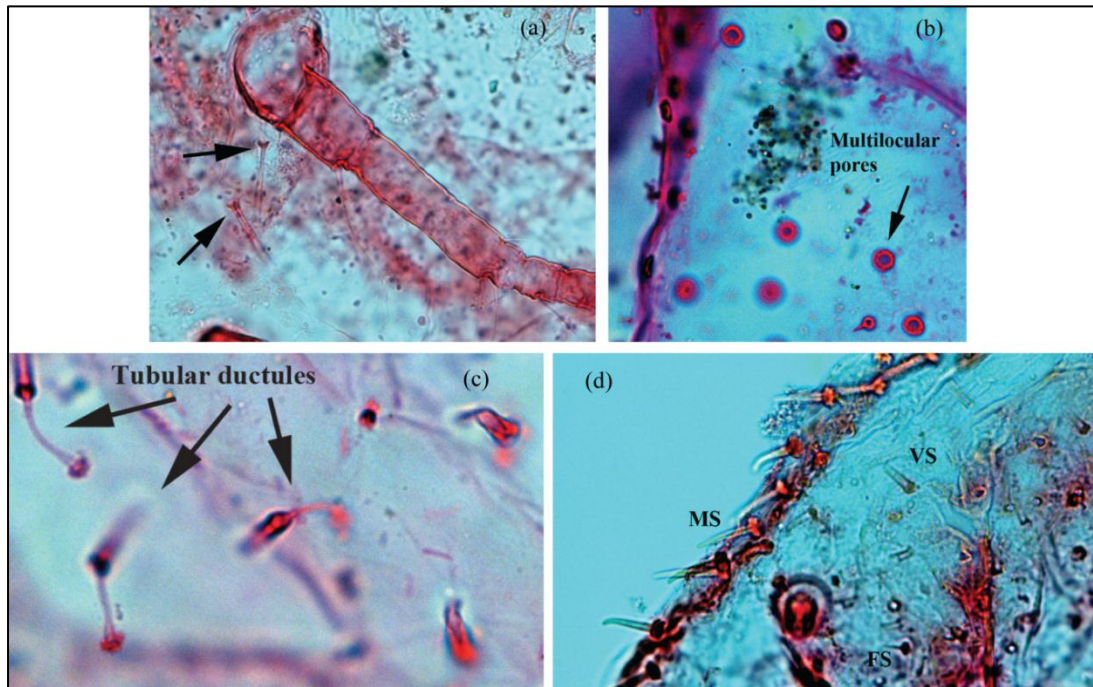
are two pairs of inter-antennal setae, the medial being larger than the lateral (Figure 3a), and three pairs of setae above anal-genital complex. Ventral setae blunt spinose scattered above thoracic region, and scattered fine sharp setae (Figure 3d). Quinquelocular pores (5-locular pores) between spiracle and stigmatic cleft are present. Multilocular pores are present on the abdomen and anal-genital areas and sparingly scattered over the ventral body (Figure 3b). Numerous tubular ducts are scattered over the body. Tubular duct has deep-cup shaped invagination, thin inner ductule and large terminal gland (Figure 3d). In the leg region, tibia is longer than tarsus with length of tibia 105 – 120 (mean 115)  $\mu\text{m}$  and length of tarsus 76 – 85 (mean 79)  $\mu\text{m}$ .

#### ***General descriptions on morphology***

First instars of *P. persicae* and *P. pruinorum* can be separated in mixed population by body length, arrangement and number of 8-shaped pores on the dorsum and leg morphology. *P. pruinorum* has large body length, mean  $0.39 \pm 0.003$  mm than *P. persicae* with body length of  $0.31 \pm 0.004$  mm (Chapter 2). *P. pruinorum* has two longitudinal rows of 8-shaped pores, 9 -10 pairs located sub-medially and 9 pairs lateral to the margin compared with three rows of longitudinal 8-shapes pores, 11 pairs sub-medially, variable numbers on submarginal and lateral rows in *P. persicae* (Boratynski, 1970, Brittin, 1940). In the leg region, tibia is longer than tarsus for *P. pruinorum* while tarsus is either equal or longer than tibia described for *P. persicae*.

1

2



3

4

5 **Figure 3. Digital image of body feature of adult stage of *P. prunosum* (a) Arrows**  
6 **showing single pair of inter-antennal setae (b) Ventral multilocular pores above the**  
7 **anal genital region (c) Ventral tubular ductules (d) Marginal setae, blunt spinose, MS**  
8 **= marginal setae, VS = Ventral blunt spinose setae, FS = Ventral fine sharp setae**

In second instar, the described species of *P. persicae* has eight submarginal tubercles, four on each side of body which consisted of one above the anterior stigmatic spine, one between the anterior and posterior stigmatic spine and two behind the posterior stigmatic spine (Boratynski, 1970, Brittin, 1940). In *P. pruinorum*, there are ten submarginal tubercles, five on each side of body which consisted of two tubercles above the anterior stigmatic spine, one between the anterior and posterior stigmatic spines and two behind the posterior stigmatic spine. Marginal setae in *P. pruinorum* is straight bluntly spinose whereas in *P. persicae* is bent at the tip and blunt spinose (flagellate).

In mature adults, average body length of *P. persicae* 4.3 – 7.9 (mean 6.4) mm is smaller than *P. pruinorum* 4.3 – 6.1 (mean 5.2) mm (Chapter 2). Described species of *P. persicae* has 24 – 36 submarginal tubercles (Boratynski, 1970) but *P. pruinorum* has 12 -19 submarginal tubercles. Marginal setae in *P. pruinorum* is straight bluntly spinose while in *P. persicae* is bent and blunt spinose. Adult of *P. pruinorum* consistently has one submarginal tubercles between anterior and posterior stigmatic spines whereas in describe species of *P. persicae* (Boratynski, 1970) has two to three submarginal tubercles between anterior and posterior stigmatic spines.

*P. pruinorum* consistently has six-segmented antennae in first, second and adult life stages. Described species of *P. persicae* has six segmented antennae in first and second instars and seven segmented antennae in third instar and adult stage (Boratynski, 1970, Brittin, 1940).

There may exist a third instar stage in *P. pruinorum* (Chapter 2) which can be difficult to separate from the third instar stage of *P. persicae* when in mixed infestation. However,

separation can be made using the shape of marginal setae and arrangement of submarginal tubercles. *P. persicae* third instar has six submarginal tubercles between the two anterior stigmatic spines in the head region whereas four to five in *P. pruinorum* third instar stage (1.2 – 2.5 mm). Also third instar of *P. persicae* has seven segmented antennae whereas *P. pruinorum* with six segmented antennae.

The mature adults can be separated by antennae segment, shape of marginal setae and arrangement of sub-marginal tubercles. In *P. persicae*, antennae is seven segmented, marginal setae bent and blunt spinose and two to three submarginal tubercles between anterior and posterior stigmatic spines. *P. pruinorum* has six segmented antennae, marginal setae straight and blunt spinose, and one marginal tubercle between anterior and posterior stigmatic spines.

Young adults of *P. persicae* and *P. pruinorum* can be separated in the field using visual characters (Figure 4). *P. persicae* has retiform (net-like) patterns on the dorsum and mature adults purplish brown. *P. pruinorum* has black stripes between body margins, covering the entire body and eventually covered with wax resembling a frosty appearance.

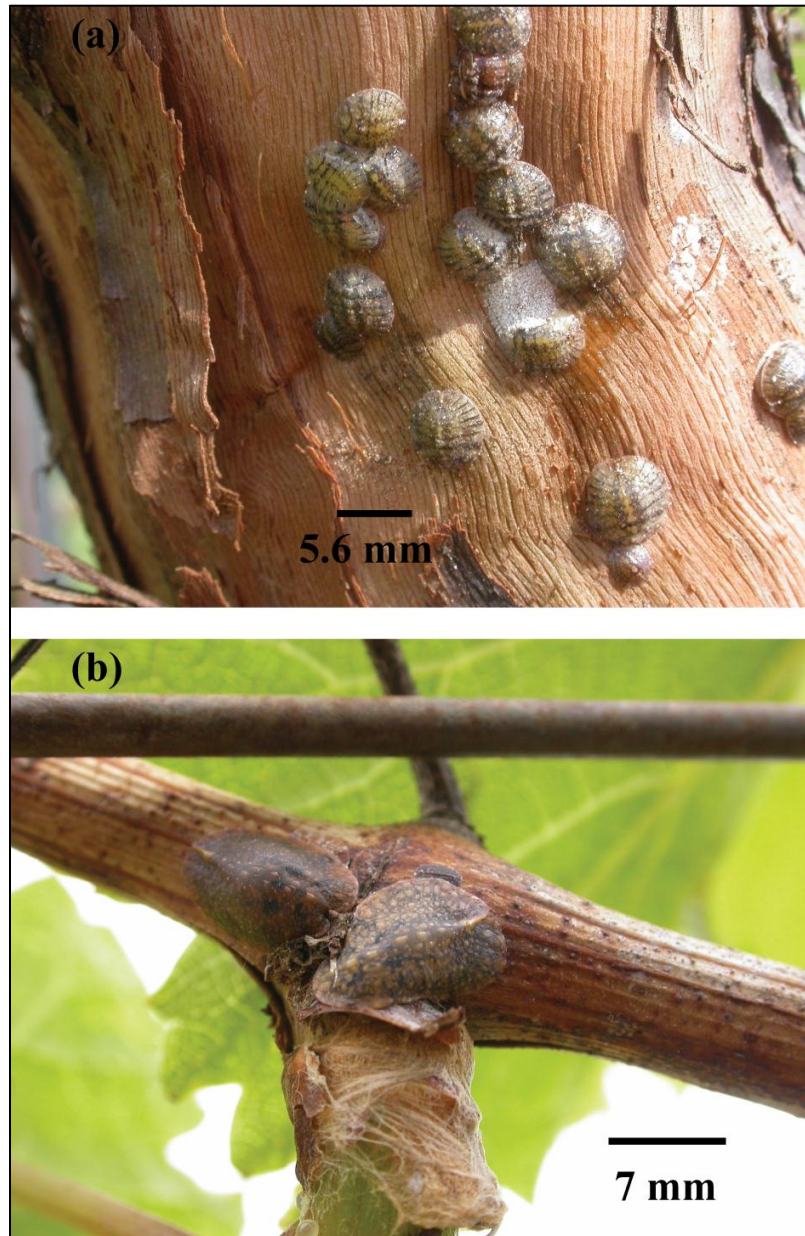


Figure 4. Visual body feature of adults of *P. pruinosum* and *P. persicae* (a) *P. pruinosum* on *V. vinifera* Chardonnay vine trunk (b) *P. persicae* on *V. vinifera* Chardonnay vine cordon.

## CHAPTER 5

**Host Suitability and Population Dynamics of  
Grapevine Scale *Parthenolecanium persicae* (Fabricius)  
and Frosted Scale *Parthenolecanium pruinosum*  
(Cocquillet) (Hemiptera: Coccidae) on Grapevines  
*Vitis vinifera* L in the Canberra Wine Region**

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## Summary

Grapevine scale (*Parthenolecanium persicae*) and Frosted scale (*P. pruinosum*) have been reported on grapevines (*Vitis vinifera*) in Australia. However, whether their population abundance varies across grapevine varieties is unknown. Population changes may be influenced by density-dependent and/or density-independent factors in vineyards and on grapevines. Numbers of scales present on grapevines infested with both grapevine and frosted scales were higher than grapevines infested with single scale species. In mixed infested grapevines, numbers of grapevine scale were higher than frosted scale on *V. vinifera* cv Riesling while on *V. vinifera* cv Chardonnay, frosted scale numbers were higher than grapevine scale. This difference implies that these scale insects may be competitive with increased population density or that each species has different preferences in grapevines. Riesling and Chardonnay varieties were more susceptible to frosted scale than *V. vinifera* Sauvignon Blanc and Pinot Noir varieties and may suggest a density-independent population regulation. Further study is required to verify the importance of Sauvignon Blanc and Pinot Noir as resistant varieties and their use as a pest management option for grapevine and frosted scales.

Keywords: Frosted scale, grapevine scale, grapevine variety, population dynamics, *Vitis vinifera*

## 1.0 Introduction

Grapevine scale (*Parthenolecanium persicae* (Fabricius); also called European Peach scale or Big Brown scale) and frosted scale (*P. pruinosum* (Cocquillet)) are significant economic pests of deciduous plants in the temperate and subtropical regions of the world. The two species are collectively referred to as grape scale. Both scales effectively colonise and feed on a number of plant species including European grapevines *Vitis vinifera* L (Hely et al. 1982, Buchanan and Amos 1992, Pellizzari 1997, Rakimov 2010, Rakimov et al. 2013). Scale induced damage to grapevines is caused through sustained feeding within the plant vascular tissue and extraction of plant nutrients all year round, although feeding during winter months may be reduced. Secondary damage may arise from sooty mould fungus forming on honeydew excreted by the scales and also through insect transmission of plant viruses during feeding (Belli et al. 1994, Pellizzari 1997, Douglas and Kruger 2008). Extraction of plant nutrients in grapevines may result in the loss of plant photosynthesis (Zvereva et al. 2010, Zvereva et al. 2012). Although observable damage of grapevines by both scale insects can be occasionally invisible (latent), infestation builds up overtime to cause an epidemic or outbreak if control measures are lacking and/or when suitable (highly preferred) grapevine varieties are cultivated. Grape scales (defined here as combination of grapevine scale and frosted scale) feed on both red and white grapevine varieties, yet there is limited information on the population response of these scale species associated with grapevine varieties. There is evidence that grapevine varieties can influence scale insect population dynamics (Martinson and Dennehy 1995, Moreau et al. 2006, Moreau et al. 2007, Sharon 2009, Duso et al. 2012). Fundamental knowledge on varietal suitability/susceptibility to grape scales and subsequent impacts on their population dynamics is needed to develop effective management strategies.

Suitable host plants increase the probability of insect fitness and population outbreaks (Haukioja et al. 1987, Wallner 1987, Awmack and Leather 2002). If individual plants vary in their suitability (quality and quantity), natural selection will favour insects with the ability to recognise appropriate host plants (Mayhew 1997, West and Cunningham 2002, Barrett and Heil 2012). Adaptations for recognising plants may include avoiding certain plant metabolites, the presence of predatory insects and adverse climatic conditions (Bernays 1998, Bernays 2001). Scale populations can increase on preferred host plants as a result of the avoidance of conditions that would limit population growth (Gullan and Kosztarab 1997, Glynn and Herms 2004, Spitzer 2006). Both grapevine scale and frosted scale have univoltine (monocyclic) biology in temperate regions. Grapevine scale may also have a second partial generation in warmer regions and has been reported to have four development stages, three larval and the adult stages (Pellizzari 1997, Rakimov 2010). In contrast Frosted scale may have only three developmental stages (Simbiken, unpublished). Both insect species will remain sedentary on feeding sites throughout their development (Greathead 1997). Survival is associated with grapevine phenology, and plant chemical changes associated with grapevine growth, fruit set, berry expansion and ripening and leaf senescence (Hely et al. 1982, Pellizzari 1997, Rakimov 2010). The most critical period for scale insects is the survival of juvenile stages; where mortality is reportedly high in summer and winter months (Loch and Zaluki 1997, Loch and Zalucki 1998). Low mortality during this period will increase survival of larvae, resulting in a greater abundance of the adult stage.

Life stages of insects are usually fixed but the performance (i.e. survival rate) of each life stage reaching the next stage may depend on the availability of suitable plant

nutrients (Hutchinson et al. 1997, Boggs 2009). Studies that document increases in resource intake in juvenile stages have shown improved adult performance and reproduction (Bauerfeind 2005, Boggs and Freeman 2005, Hogendorp et al. 2006, Boggs 2009, Dmitriew and Rowe 2011, Fisher et al. 2011). Photosynthesis in grapevines increases just after budbreak in Austral spring (September) during the period when leaves are just appearing. Post-anthesis in October, leaf photosynthesis declines with leaf maturity. Therefore the nutritional quality of leaf nutrients may decline over time (Wermelinger and Koblet 1990, Mullins et al. 1992). However, the carbohydrate reserve is progressively stored in the branches, trunks and roots after flowering and reaches a peak post-harvest (Lebon et al. 2008, Holzapfel et al. 2010, Zufferey et al. 2012), within the parenchyma cells of the roots and branches (Lebon et al. 2008, Zufferey et al. 2012). Thus, feeding on parenchymal cells may provide nutrients necessary for maintaining growth of scale insects during this period.

In this study the population dynamics of grapevine and frosted scale insects feeding on different grapevine varieties in vineyards in the Canberra wine region were examined. Grape scales were monitored in the field during the 2010/2011 growing season to determine how population varied over the season. The relative abundance of various life stages of both scale species was recorded to determine how different varietal host plants influenced population growth and development of life stages.

## **2.0 Materials and Methods**

### **2.1 Site Details**

The cool climate wine region of Canberra, Australia has an average growing season temperature ranging between 13.4°C–19.9°C (Hall et al. 2010). Three vineyards were selected and were separated by distances of 5-7kms; vineyard A, S35°13'38.6", E149°23'42.6", 852 metres above sea level (masl), vineyard B, S35°11'19", E149°21'28", 768 masl and vineyard C, S35°10'16.1", E149°19'38.6", 775 masl. Grapevines in vineyards A and C were spur-pruned in August and September respectively. Mixed cane and spur pruning of grapevines were undertaken in vineyard B in September. All vineyards were irrigated with a Regulated Deficit Irrigation (RDI) system and with trellising of grapevines at 0.7–1.5 metres above ground. The grapevines were planted at a spacing of 0.8m x 3m within rows and between rows respectively, consisting of approximate plant density of 4167 plants per hectare across the three vineyards. Temperature data of Canberra wine region was obtained from the Canberra Airport weather station, 149.20°E, 35.31°S, located 10 kilometres from the vineyards.

## **2.2 Insect Monitoring**

In a pilot survey, conducted in 2010 Grapevine scale and Frosted scale were the only scales present on grapevines in the three vineyards. Frosted scale was found on *V. vinifera* cv Chardonnay grapevines in vineyard A and on *V. vinifera* cv Riesling, Sauvignon Blanc and Pinot Noir in vineyard C. A mixed population of grapevine scale and frosted scale was found on Chardonnay and Riesling in vineyard B. All scales were identified using published morphological descriptions (Brittin 1940, Boratynski 1970, Gill 1988). A descriptive characteristic of scales was the production of wax on the bodies, which helped with aging and separating the two species. Young adults of frosted

scale usually develop wax over the dorsum and the wax covering may be reduced over time until the adult is fully matured. The mature Frosted scale adult is characterised by a deeply convex dorsum without wax material. Minimal wax is produced on the dorsum of grapevine scale however dry conditions may trigger production of wax. The dorsum of adults of grapevine scale is flat and slightly convex. This coccid is the larger of the two grape scales. Microstructures and body features observed in another study on *P. pruinsoum* (Simbiken in preparation) was used to aid in recognition.

In total six plots were studied; one in vineyard A, three in vineyard B and two in vineyard C. Plot sizes were Chardonnay plot 24m x 50m in vineyard A, Riesling plot 20m x 60m, Sauvignon Blanc plot 36m x 55m and Pinot Noir plot 18m x 70m in vineyard B and Chardonnay plot 36m x 44m and Riesling plot 24m x 48m in vineyard C. Two separate studies were undertaken to determine (i) host suitability and (ii) population growth, with data collected monthly. All insect counts and measurements were done non-destructively. Counts of scales and numbers of each life-stage were made on the branch and leaves derived from the same branch using digital images taken using a Nikon 4500 Coolpix digital camera. Digital images of branches, insects and a ruler consisting of 0.5 mm scale interval were captured at the beginning of each sampling date. Both studies were conducted between October 2010 and October 2011.

### **2.3 Host Plant Suitability**

In the host suitability study, six grapevines within each of the six plots were randomly selected representing 36 data points per sample date. A single growing branch with leaves on each grapevine was selected for insect count. All insects were counted and

location noted whether on a branch or a leaf. Both mixed populations of the two scale species and single species presence were recorded along with grapevine variety.

## **2.4 Population Growth**

The population growth study consisted of repeated sampling monthly of six grapevines that were originally randomly selected from each vine plot (36 grapevine branches or six grapevines x six plots). The sample grapevines used were selected within each plot from a total of 14 to 18 rows. The six grapevines consisted of individual scale species whilst grapevines with mixed infestations of both scales were excluded.

Insect measurements were also verified in the laboratory by collection of an insect subsample on each sampling date. Insects were removed from the branches with a brush into Eppendorf tubes (1.5 mL) and taken to laboratory in a cooler box. Insect on leaves were measured from the random leaf samples. Insect size was determined by length as measured using a dissecting microscope after calibration with a 1 mm ( $\pm 0.001$  mm) calibration slide. Developmental growth of the grape scales was determined from the insect measurements during each sampling date.

## **2.5 Statistical Analysis**

The reliability of the digital imaging method was determined by regressing the count made from the digital images and the direct insect count at the beginning of the study in October 2010. A simple linear regression was performed with physical count as the response variate (y) and the digital count as the explanatory variate (x). The physical count was positively correlated to the digital count and explained most of the variability

in the physical count ( $y = 0.02 + 0.99x$ ,  $F = 467.8$ ,  $df = 1, 34$ ,  $p < 0.001$ ,  $R^2 = 0.97$ ). The insect size was determined using ImageJ® 1.47 program and the reference ruler.

Host suitability was determined by analysing the count data using the repeated measure correlation model ANOVA. Count data was used as the response variate and the fixed variables (or explanatory variates) were sampling date, vineyard and grapevine variety. The subjects were randomly selected plants within the vine plot. The response data was logarithm transformed ( $x+1$ ) to conform to the properties of normal distribution and for ensuring homogeneity of variance. ANOVA was carried out using Genstat® version 16 software. In this analysis, insect count included both grapevine and frosted scales found in mixed populations on the selected grapevines. Ante-dependence order 1 reduced variance more than the other models and was used as the correlation model with subjects across equally spaced time points. To determine whether grape scale populations did not vary across sample date within each season, General Linear Model (GLM) with Poisson distribution and log-linked function ANOVA was used with count data as response variate and the fixed factor were site and variety.

Individual grapevines infested with mixed populations of grapevine scale and frosted scale were observed and to determine the mean ( $\pm$  SEM) of mix infestation of grapevine and frosted scale and individual infestation between grapevines, a one-way ANOVA was used on logarithm transformed ( $x+1$ ) count data, with infestation type (single scale species infested vine and mix scale infested grapevines) as treatment factors.

To determine the differences between the relative proportion of individual scale insects and whether this is influenced by grapevine variety, a GLM logit-linked function of the binomial distribution was used. Count data for individual insect species was used as the



response variate and the total insect count as binomial totals while the fixed factors were Austral temperate season and grapevine variety. Seasons under consideration were Autumn, Winter and Spring. Between April and November, life stages of grapevine and frosted scales could be determined using digital images. Most summer populations of both insect species were in the first and second instar and often these life-stages were difficult to separate using digital imagery and thus required microscopic examination. A two sample T-test with two sided probability was used to determine the population differences between grapevine and frosted scales within each season.

Phenology of life stages of grapevine scale and frosted scale found in each sample date in the cropping season was determined by measuring the insect body length and using a frequency plot of length to determine numbers of groups present.

GLM logit-linked function of the binomial distribution was used to determine the number of grape scales present on grapevine leaf and branch between the four seasons.

## **3.0 Results**

### **3.1 Host Plant Suitability**

Populations were relatively low in winter and spring and high in the summer and autumn across vineyards and grape varieties (Figs. 1 and 2). The population density of grape scales varied between the three vineyards and across four varieties (Table 1). There was a significant interaction between vineyard and sampling date ( $p < 0.001$ ) and between grapevine variety and sampling date ( $p < 0.003$ ) (Table. 1). When populations

were analysed within a season, significant interactions for vineyard x date were found for spring and winter; while interactions between variety and date were found only for spring (Table 2). In the summer and autumn, relatively high grape scale infestation occurred in vineyard C compared to vineyards A and B (Fig. 1). Numbers of scale insects on Riesling and Chardonnay was relatively high, moderate on Sauvignon Blanc and low on Pinot Noir (Fig. 2).

Within individual grapevines, where both scale species were present, numbers of grapevine scale on Riesling were higher than numbers of frosted scale ( $F = 72.29$ ,  $df = 1, 64$ ,  $p < 0.001$ ) (Fig. 3). Frosted scale population numbers were higher on Chardonnay than grapevine scale numbers. There was no interaction between season and variety ( $F = 1.59$ ,  $df = 2, 64$ ,  $p < 0.213$ ).

On grapevine varieties infested only with frosted scale, the highest density of frosted scale was observed on Chardonnay and Riesling, a moderate density on Sauvignon Blanc and relatively low density on Pinot Noir ( $F = 37.96$ ,  $df = 3, 257$ ,  $p < 0.001$ ) (Fig. 4).

Individual grapevines with mixed infested scale had significantly higher infestation than grapevines with single scale species infestation ( $F = 264.52$ ,  $df = 1, 430$ ,  $p < 0.001$ ).

### **3.2 Population Age Structure**

Development of both grapevine scale and frosted scale life stages followed a seasonal trend. First and second instar stages for both species were found mostly in the summer months between December and February (Figs. 5 and 6). In the autumn and winter

months (March-Aug), third instar of grapevine scale and second instar of frosted scale were the dominant life stages. Both scale species reached adulthood in the spring between September and November. The location on plants of the various instars differed. First and second instars were mostly found on the leaves in the summer, but in autumn to spring, the scales were present on the branches and cordons ( $F = 84.47$ ,  $df = 3, 113$ ,  $p < 0.001$ , Fig. 7). Life stages found in autumn and winter months were third and second instar stages for grapevine and frosted scale respectively.

#### **4.0 Discussion**

Numbers of grape scales varied significantly among vineyards and grapevine varieties. Grapevine scale numbers were higher on Chardonnay and Riesling grapevines than the Sauvignon Blanc and Pinot Noir varieties. Frosted scale was the dominant species in the three vineyards. In only one of the three vineyards and two grapevine varieties Chardonnay and Riesling in that vineyard, were mixed populations of grapevine and frosted scale observed. In these two varieties with mixed populations on individual grapevines, frosted scale density was higher than the grapevine scale on Chardonnay. In contrast on Riesling, the density of grapevine scale was higher than frosted scale. Mixed infestation on individual grapevine has previously been reported in Australian vineyards (Rakimov et al. 2013). In our study when mixed infestation of grapevine and frosted scale were present on individual grapevines, population density was comparably higher than grapevines infested with single scale infestation. Development of frosted scale may be reduced on Pinot Noir and Sauvignon Blanc compared with Riesling and Chardonnay.

Both Grapevine and Frosted scales appear to prefer Riesling and Chardonnay grapevines in this study. Although a varietal association has not previously been reported for these scale species, host-plant species effects (*Vitis labrusca* Bailey vs *V. vinifera* L.) do occur for other sap-sucking insects as reported in *Erythroneura* leafhoppers (*Erythroneura comes* (Say) (Martinson and Dennehy 1995). Varietal effects on the population dynamics of other grapevine insect pests have also been reported (Moreau et al. 2007, Moreau et al. 2006, Sharon et al. 2009).

Differences in numbers of scale insects on the different varieties do suggest that host plant resistance to these insects may be present. Grapevine resistance may arise from antibiosis (plant defence chemicals), antixenosis (leaf morphological properties) and/or general plant responses, such as infested leaf drop. Effects of plant defence chemicals on plant sap-feeding insects are more pronounced than leaf structural properties (Guerrieri and Digilio 2008). Susceptible varieties may have weak defence chemicals and/or the insect may have developed detoxifying enzymes to manipulate plant defence chemicals (Barrett and Heil 2012). Scale insects tend to be grouped in colonies as a result of its characteristic dispersal, genetic and reproductive system (parthenogenesis) can allow adaptation to plant chemical properties and then rapid spread through a population (Gullan and Kosztarab 1997, Ross et al. 2010, Ross et al. 2013). This group of insects have high affinity for local adaptation to maternal than in noval host plants (Boyero et al. 2007, Glynn and Herms 2004, Spitzer 2006).

Grapevine scale is widely distributed in vineyards in Australia. More recently frosted scale was recorded on grapevines in several wine regions of South Australia, Victoria and New South Wales (Rakimov et al. 2013). Frosted scale may become the more important pest of grapevines as these insects can develop more rapidly as they have one

fewer juvenile stage. Rakimov et al. (2013) suggested that frosted scale is an important pest due to its widespread infestation on grapevines and ornamental plants. Control of Frosted scale may incorporate both density-dependent and density-independent effects. The numbers of frosted scale on Pinot Noir indicated that incorporation of that variety into a mix cropping may act as a resistance species in a density-independent manner. However, the difference in numbers of frosted scale compared with grapevine scale on Riesling and Chardonnay varieties implies that either invasion onto plants or density-dependent responses to the congeneric species is important for regulation of scales. Feeding response of Frosted scale on Sauvignon Blanc and Pinot Noir may need further clarification.

Both Grapevine scale and Frosted scale have an univoltine life cycle in the Canberra wine region (cool climate region). Development of life stages for both scale insects is structured into seasonal changes of climatic and host plant phenology. The population of both grape scales in spring is lower than in summer and autumn. First and second instars of both scales were confined to summer. Populations found in autumn and winter were attached to the feeding site under the bark crevices and concealed places of the vine branches and cordons. In our study population decline in winter months may be attributable to winter temperatures of below  $-5^{\circ}\text{C}$  recorded in July 2011, which may have increased mortality of grape scales on exposed vine surfaces. Feeding likely resumes around the time nutrient flows in the vine occur during bud-break and shoot development. This was evident in body size expansion into adult stages after the winter diapause. The overwintering life stages were different for the two species, being the third instar for grapevine scale and the second instar for frosted scale. Such overwintering strategies are consistent with the biology of both grape scales in the

temperate regions (Michelbacher and Hitchcock 1956, Pellizzari 1997, Pfeiffer 1997, Swirski et al. 1997).

The population age structure of grapevine and frosted scales can be used for timing of pest control measures. Spraying is common in most vineyards and is done both before fruit development and after harvesting. Several biological control agents have been released in Australia against the grapevine scale and other endemic bio-agents may be used against these grape scales (Rakimov 2010). The timing of these control methods is best when the first and second instar stages are present because their small size and soft cuticle may be more susceptible to the contact insecticides, and also attack by parasitic wasps. Once the overwintering and adult stages are reached, however, such techniques may be relatively ineffective, as the cuticle of overwintering and adult stages is hydrophobic and highly resistant to external application of insecticides and the seasonal conditions may not be appropriate for wasp activity.

In our study variation of grapevine varieties within the vineyard affected the distribution pattern of grape scales. In general, both grapevine and frosted scales were more abundant on Chardonnay and Riesling varieties whereas Sauvignon Blanc and Pinot Noir were only moderately attractive. This was apparent with the population increase in summer without a shift in varietal preference. Distribution of grape scale numbers within grapevine architecture across seasons reflects life stage preference of these structures for feeding. In summer grape scale numbers were mainly located on grapevine leaves whereas in autumn, winter and spring on grapevine branches and cordons. The consistent preference of grape scale for Chardonnay and Riesling varieties was apparent between vineyards which indicates that this trait including preference for

feeding sites can be used as a basis for the development of an efficient pest monitoring and targeted management systems in vineyards with mixed grapevine varieties.

## **5.0 Conclusion**

Our study showed that grape scale infestation was spatially and temporarily variable between vineyards. Relatively high population growth of grape scale was observed on *V. vinifera* Chardonnay and Riesling varieties than on Sauvignon Blanc and Pinot Noir varieties. Higher population density occurred on individual grapevines with mixed grapevine and frosted scales compared to single scale species infested grapevines. A marginal varietal effect was observed on frosted scale populations, with higher population growth on Riesling and Chardonnay than Sauvignon Blanc and Pinot Noir. Chardonnay and Riesling are widely cultivated in the Australian viticulture industry (Gunning-Trant et al. 2012) and their widespread use of these varieties has important consequences for the spread and incidence of insect pests (Hendry et al. 2011, Wallner, 1987). This information is useful for developing monitoring and management strategies for grapevine and frosted scales in vineyards with mixed varieties.

## **6.0 Acknowledgements**

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## 7.0 References

- Awmack CS and Leather SR. 2002. Host plant quality and fecundity in herbivores insects. *Annual Review of Entomology* **47**, 817-844.
- Barrett LG and Heil M. 2012. Unifying concepts and mechanisms in the specificity of plant–enemy interactions. *Trends in Plant Science* **17**, 282-292.
- Bauerfeind SS, Fischer, K and Larsson S. 2005. Effects of food stress and density in different life stages on reproduction in a butterfly. *Oikos* **111**, 514-524.
- Belli G, Fortusini A, Casati P, Belli L, Bianco P and Prati S. 1994. Transmission of a grapevine leafroll associated closterovirus by the scale insect *Pulvinaria vitis* L. *Rivista di Patologia Vegetale* **4**, 105-108.
- Bernal JS, Luck RF, Morse JG and Drury MS. 2001. Seasonal and scale size relationships between Citricola Scale (Homoptera: Coccidae) and its parasitoid complex (Hymenoptera: Chalcidoidea) on San Joaquin Valley citrus. *Biological Control* **20**, 210-221.
- Bernays EA. 1998. Evolution of feeding behavior in insect herbivores. *BioScience* **48**, 35-44.
- Bernays EA. 2001. Neural limitations in phytophagous insects: Implications for diet breadth and evolution of host affiliation. *Annual Review of Entomology* **46**, 703-727.



- Boyero JR, Ruiz-López R, Rodríguez N, Vela JM, Moreno R and Pascual F. 2007. Varietal influence of orange trees on armoured scale insect fecundity (Hemiptera: Diaspididae). *International Journal of Pest Management* **53**, 217-225.
- Boggs C and Freeman K. 2005. Larval food limitation in butterflies: effects on adult resource allocation and fitness. *Oecologia* **144**, 353-361.
- Boggs CL. 2009. Understanding insect life histories and senescence through a resource allocation lens. *Functional Ecology* **23**, 27-37.
- Boratynski, K. 1970. On some species of 'Lecanium' (Homoptera, Coccidae) in the collection of the Naturhistorisches Museum in Vienna; with description and illustration of the immature stages of *Parthenolecanium persicae*. *Analen des Naturhistorischen Museums Wien* **74**, 63-76.
- Bostanian N, Thistlewood H, Hardman J and Racette G. 2009. Toxicity of six novel fungicides and sulphur to *Galendromus occidentalis* (Acari: Phytoseiidae). *Experimental and Applied Acarology* **47**, 63-69.
- Brittin G. 1940. The life history of *Lecanium (Eulecanium) persicae* (Fabricius) and descriptions of the different instars. *Transactions and Proceedings of the Royal Society of New Zealand* **69**, 413-421.

- Buchanan G, and Amos T. 1992. Grape pests. pp. 209-231. In: *Viticulture Volume 2 - Practices* (eds B. Coombe and P. Drys) Winetitles, Adelaide, Australia.
- Dmitriew C and Rowe L. 2011. The effects of larval nutrition on reproductive performance in a food-limited adult environment. *PLoS ONE* **6**, e17399.
- Douglas N and Krüger K. 2008. Transmission efficiency of Grapevine leafroll-associated virus 3 (GLRaV-3) by the mealybugs *Planococcus ficus* and *Pseudococcus longispinus* (Hemiptera: Pseudococcidae). *European Journal of Plant Pathology* **122**, 207-212.
- Duso C, Pozzebon A, Kreiter S, Tixier MS and Candolfi M. 2012. Management of phytophagous mites in European vineyards. In: *Arthropod Management in Vineyards* (eds NJ Bostanian, C Vincent and R Isaacs) pp. 191-217. Springer, Netherlands.
- Fischer B, Taborsky B and Kokko H. 2011. How to balance the offspring quality–quantity tradeoff when environmental cues are unreliable. *Oikos* **120**, 258-270.
- Gill R. 1988. The scale insects of California, Part 1: The soft scales (Homoptera: Coccoidea: Coccidae). California Department of Food and Agriculture. Technical Series in Agricultural Biosystematics and Plant Pathology **1**.
- Glynn C and Herms DA. 2004. Local adaptation in Pine Needle Scale (*Chionaspis pinifoliae*): Natal and novel host quality as tests for specialization within and among Red and Scots Pine. *Environmental Entomology* **33**, 748-755.

- Greathead DJ. 1997. Crawler behaviour and dispersal. In: *World Crop Pests. Soft Scale Insects: Their Biology, Natural Enemies and control. Vol 7A.* (eds Y Ben-Dov and CJ Hodgson) pp. 339-342. Elsevier, Amsterdam
- Guerrieri E and Digilio MC. 2008. Aphid-plant interactions: a review. *Journal of Plant Interactions* **3**, 223-232.
- Gullan PJ and Kosztarab M. 1997. Adaptations in scale insects. *Annual Review of Entomology* **42**, 23-50.
- Haukioja E, Neuvonen S, Barbosa P and Schultz J. 1987. Insect population dynamics and induction of plant resistance: the testing of hypotheses. In: *Insect Outbreaks* (eds P Barbosa and JC Schultz) pp. 411-432.
- Hely PC, Pasfield G and Gellatley JG. 1982. *Insect Pests of Fruit and Vegetables in NSW.* Inkata Press.
- Hogendorp BK, Cloyd RA and Swiader JM. 2006. Effect of nitrogen fertility on reproduction and development of citrus mealybug, *Planococcus citri* Risso (Homoptera: Pseudococcidae), feeding on two colors of Coleus, *Solenostemon scutellarioides* L. Codd. *Environmental Entomology* **35**, 201-211.
- Holzapfel BP, Smith JP, Field SK and Hardie WJ. 2010. Dynamics of Carbohydrate Reserves in Cultivated Grapevines. *Horticultural Reviews* **37**. John Wiley and Sons, Inc.

- Hutchinson JMC, Mcnamara JM, Houston AI and Vollrath F. 1997. Dyar's rule and the investment principle: optimal moulting strategies if feeding rate is size-dependent and growth is discontinuous. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **352**, 113-138.
- Lebon G, Wojnarowicz G, Holzapfel B, Fontaine F, Vaillant-Gaveau N and Clément C. 2008. Sugars and flowering in the grapevine (*Vitis vinifera* L.). *Journal of Experimental Botany* **59**, 2565-2578.
- Loch AD and Zalucki MP. 1998. Outbreaks of pink wax scale, *Ceroplastes rubens* Maskell (Hemiptera: Coccidae), on umbrella trees in south eastern Queensland: Patterns of parasitisation. *Australian Journal of Entomology* **37**, 328-334.
- Loch, AD and Zalucki, MP. 1997. Variation in length, fecundity and survival of pink wax scale, *Ceroplastes rubens* Maskell (Hemiptera: Coccidae), on umbrella trees. *Australian Journal of Zoology* **45**, 399-407.
- Martinson TE and Dennehy TJ. 1995. Varietal preferences of *Erythroneura* leafhoppers (Homoptera: Cicadellidae) feeding on grapes in New York. *Environmental Entomology* **24**, 550-558.
- Mayhew PJ. 1997. Adaptive patterns of host-plant selection by phytophagous insects. *Oikos* **79**, 417-428.

- Michelbacher A and Hitchcock S. 1956. Frosted scale on walnuts. *California Agriculture* **10**, 11-14.
- Michelbacher A and Swift JE. 1954. Parasites of the frosted scale. *California Agriculture* **8**, 9 -10.
- Moreaua J, Benreya B and Thiérya D. 2006. Grape variety affects larval performance and also female reproductive performance of the European grapevine moth *Lobesia botrana* (Lepidoptera: Tortricidae). *Bulletin of Entomological Research* **96**, 205-212.
- Moreaua J, Thiery D, Troussard JP and Benrey B. 2007. Grape variety affects female but also male reproductive success in wild European grapevine moths. *Ecological Entomology* **32**, 747-753.
- Mullins MG, Bouquet A and Williams LE. 1992. *Biology of the Grapevine*. UK, Cambridge University Press.
- Pellizzari G. 1997. Grapevine. In: *World Crop Pests. Soft Scale Insects: Their Biology, Natural Enemies and control. Vol 7B*. (eds Y Ben-Dov and CJ Hodgson) pp. 323-331. Elsevier, Amsterdam.
- Pfeiffer, DG. 1997. Deciduous fruit trees. In: *World Crop Pests. Soft Scale Insects: Their Biology, Natural Enemies and control. Vol 7B*. (eds Y Ben-Dov and CJ Hodgson) pp. 293-322. Elsevier, Amsterdam

- Rakimov, A. 2010. Aspects of the biology, ecology and biological control of soft scale insects (Coccidae) in Australian vineyards. PhD Thesis, University of Melbourne, Dept. of Zoology. Melbourne, Australia
- Rakimov A, Ben-Dov Y, White V and Hoffmann AA. 2013. Soft scale insects (Hemiptera: Coccoidea: Coccidae) on grapevines in Australia. *Australian Journal of Entomology* **52**, 371-378.
- Ross L, Hardy NB, Okusu A and Normark BB. 2013. Large population size predicts the distribution of asexuality in scale insects. *Evolution* **67**, 196-206.
- Ross L, Pen I and Shuker DM. 2010. Genomic conflict in scale insects: the causes and consequences of bizarre genetic systems. *Biological Reviews* **85**, 807-828.
- Sharon R, Zahavi T, Soroker V and Harari AR. 2009. The effect of grapevine cultivars on *Lobesia botrana* (Lepidoptera: Tortricidae) population levels. *Journal of Pest Science* **82**, 187-193.
- Spitzer B. 2006. Local maladaptation in the soft scale insect *Saissetia coffea* (Hemiptera: Coccidae). *Evolution* **60**, 1859-1867.
- Swirski E, Ben-Dov Y and Wysoki M. 1997. Persimmon. In: *World Crop Pests. Soft Scale Insects: Their Biology, Natural Enemies and control. Vol 7B*. (eds Y Ben-Dov and CJ Hodgson) pp. 265-270. Elsevier, Amsterdam

- Tena A, Soto A and Garcia-Marí F. 2008. Parasitoid complex of black scale *Saissetia oleae* on citrus and olives: parasitoid species composition and seasonal trend. *BioControl* **53**, 473-487.
- Wakgari WM. 2001. The current status of the biocontrol of *Ceroplastes destructor* Newstead (Hemiptera: Coccidae) on Citrus and Syzygium in South Africa. *Biocontrol Science and Technology* **11**, 339-352.
- Wallner W. 1987. Factors affecting insect population dynamics: differences between outbreak and non-outbreak species. *Annual Review of Entomology* **32**, 317-340.
- Wermelinger B and Koblet W. 1990. Seasonal growth and nitrogen distribution in grapevine leaves, shoots and grapes. *Vitis* **29**, 15-26.
- West SA and Cunningham, PJ. 2002. A general model for host plant selection in phytophagous insects. *Journal of Theoretical Biology* **214**, 499-513.
- Zufferey V, Murisier F, Vivin P, Belcher S, Lorenzini F, Spring JL *et al.* 2012. Carbohydrate reserves in grapevine (*Vitis vinifera* L.'Chasselas'): the influence of the leaf to fruit ratio. *Vitis* **51**, 103-110.
- Zvereva EL, Lanta V. and Kozlov MV. 2010. Effects of sap-feeding insect herbivores on growth and reproduction of woody plants: a meta-analysis of experimental studies. *Oecologia* **163**, 949-960.

Zvereva EL, Zverev V and Kozlov MV. 2012. Little strokes fell great oaks: minor but chronic herbivory substantially reduces birch growth. *Oikos* **121**, 2036-2043.



**Table 1. Repeated measure ANOVA of grape scale population within each season (Spring, Summer, Autumn and Winter) across sample date, vineyards and grapevine varieties in 2010/2011 growing season**

| <b>Fixed factors</b> | <b>Wald statistic</b> | <b>df</b> | <b>Wald/df</b> | <b>F-Probability</b> |
|----------------------|-----------------------|-----------|----------------|----------------------|
| Date                 | 206.03                | 10        | 73.8           | <0.001               |
| Vineyard             | 93.06                 | 2         | 52.6           | <0.001               |
| Variety              | 35.52                 | 3         | 51.3           | <0.001               |
| Vineyard x date      | 293.03                | 20        | 106.3          | <0.001               |
| Variety x date       | 68.03                 | 30        | 125.4          | <0.003               |

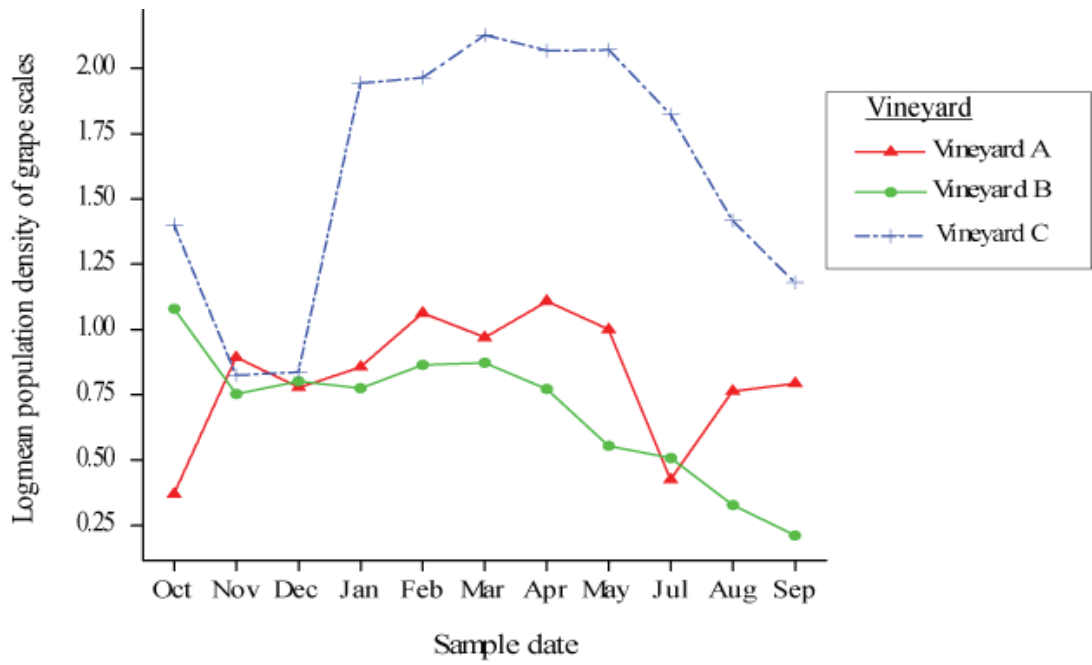
**Table 2. General Linear Model ANOVA of grape scale population across sample date, vineyards and grapevine varieties in the 2010/2011 growing season**

| <b>Fixed factors</b> | <b>Wald statistic</b> | <b>df</b> | <b>Wald/df</b> | <b>F-Probability</b> |
|----------------------|-----------------------|-----------|----------------|----------------------|
| <b>Spring</b>        |                       |           |                |                      |
| Date                 | 57.35                 | 2         | 32.3           | <0.001               |
| Vineyard             | 5.17                  | 2         | 31.3           | 0.091                |
| Variety              | 13.0                  | 3         | 29.9           | 0.012                |
| Vineyard x date      | 50.73                 | 4         | 36.6           | <0.001               |
| Variety x date       | 27.16                 | 6         | 37.8           | 0.002                |
| <b>Summer</b>        |                       |           |                |                      |
| Date                 | 9.61                  | 2         | 34.7           | 0.016                |
| Vineyard             | 179.84                | 2         | 28.9           | <0.001               |
| Variety              | 31.56                 | 3         | 28.9           | <0.001               |
| Vineyard x date      | 4.81                  | 4         | 39.2           | 0.340                |
| Variety x date       | 3.95                  | 6         | 41.6           | 0.699                |
| <b>Winter</b>        |                       |           |                |                      |
| Date                 | 12.43                 | 1         | 30             | 0.001                |
| Vineyard             | 168.72                | 2         | 30             | <0.001               |
| Variety              | 13.44                 | 3         | 30             | 0.010                |
| Vineyard x date      | 3.40                  | 2         | 30             | 0.200                |
| Variety x date       | 6.69                  | 3         | 30             | 0.105                |
| <b>Winter</b>        |                       |           |                |                      |
| Date                 | 17.57                 | 1         | 30             | <0.001               |
| Vineyard             | 165.51                | 2         | 30             | <0.001               |
| Variety              | 18                    | 3         | 30             | 0.002                |
| Vineyard x date      | 16.89                 | 2         | 30             | 0.001                |
| Variety x date       | 7.38                  | 3         | 30             | 0.082                |

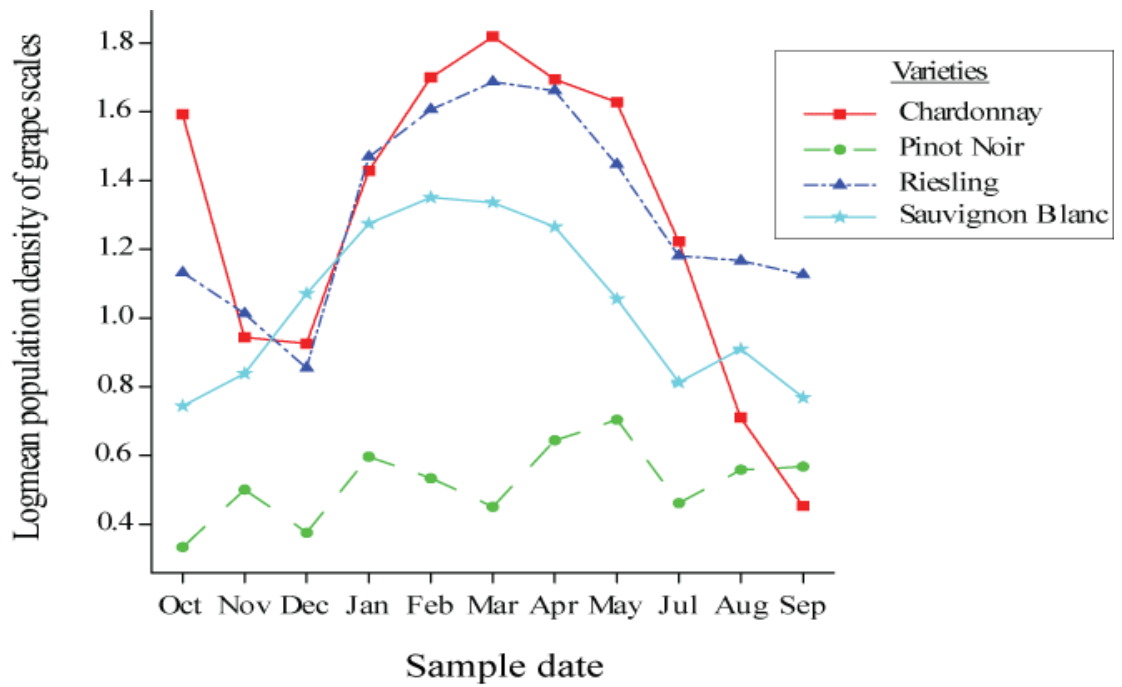
**Table 3. Population of grapevine and frosted scales within each season Autumn, Winter and Summer.**

| <b>Season</b>            | <b>Autumn (mean <math>\pm</math> SEM)*</b> | <b>Winter (mean <math>\pm</math> SEM)*</b> | <b>Spring (mean <math>\pm</math> SEM)*</b> |
|--------------------------|--|--|--|
| <b>Grapevine scale</b>   | 184.5 $\pm$ 66.7                           | 101.5 $\pm$ 44.4                           | 37.1 $\pm$ 14.1                            |
| <b>Frosted scale</b>     | 173.8 $\pm$ 41.1                           | 42.2 $\pm$ 18.3                            | 8.1 $\pm$ 2.7                              |
| <b>T-test value</b>      | 0.14                                       | 1.24                                       | 2.02                                       |
| <b>df</b>                | 38.24                                      | 30.63                                      | 24.64                                      |
| <b>Probability value</b> | 0.891                                      | 0.226                                      | 0.055                                      |

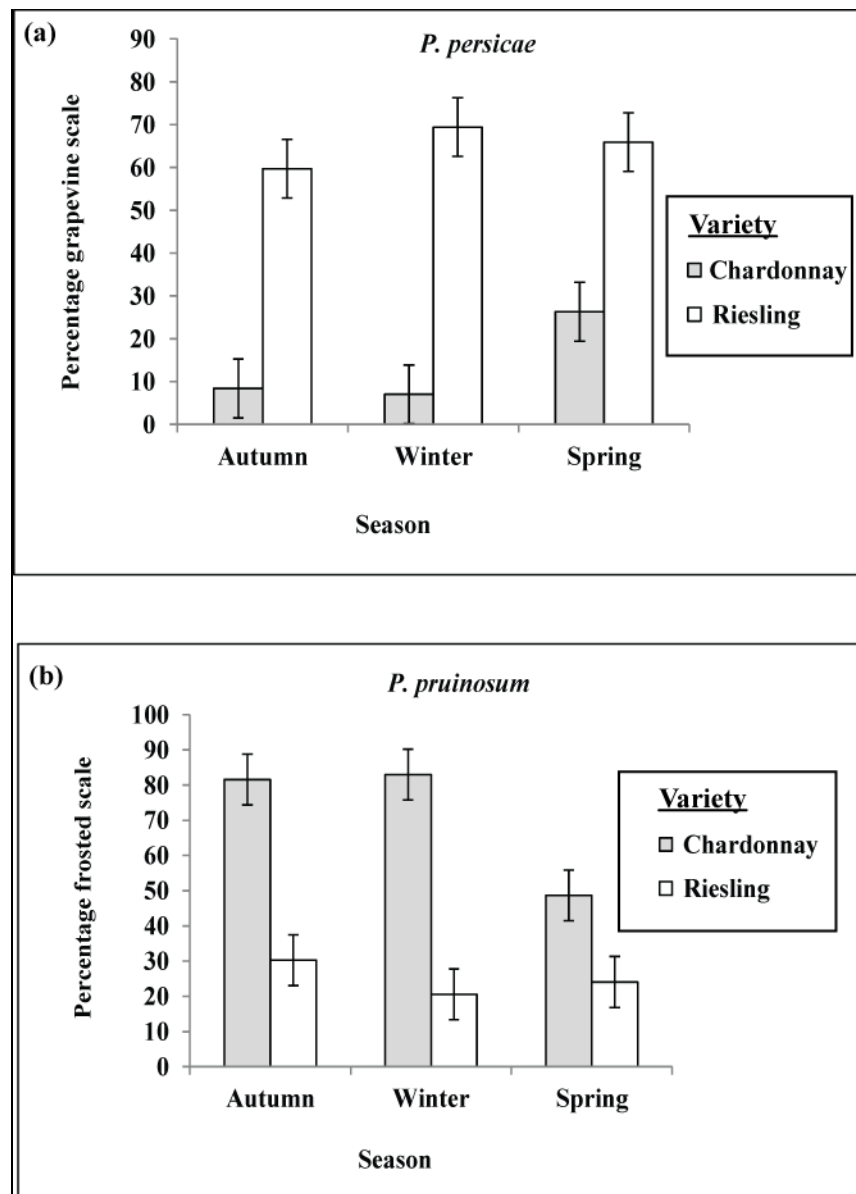
\* where n = 24



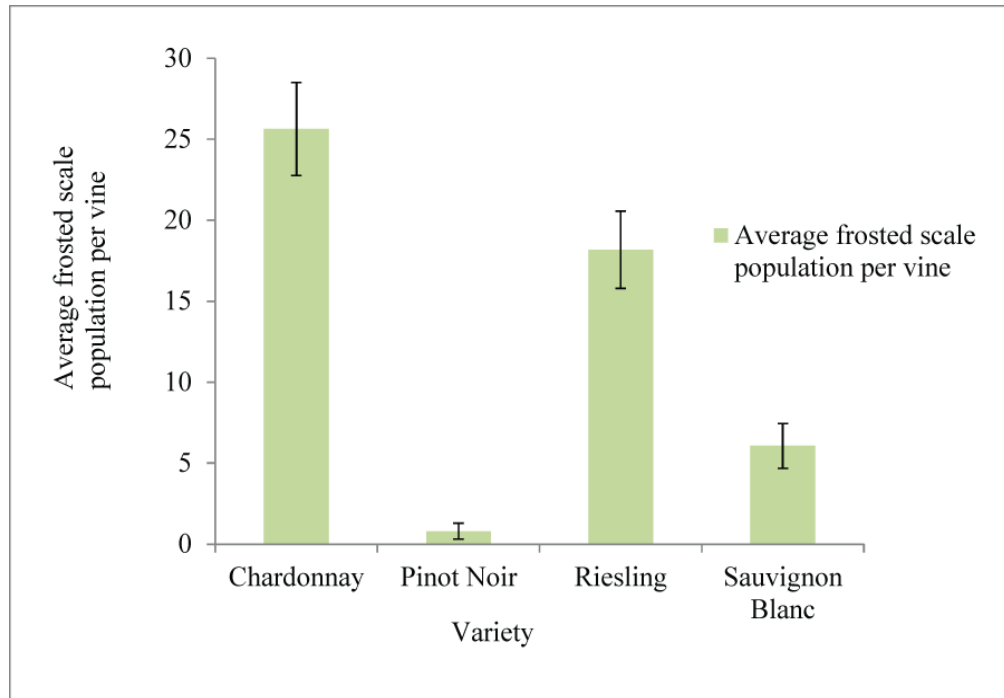
**Fig. 1. Grape scale (grapevine and frosted scales) population dynamics across the three vineyards in the Canberra winegrape region over a single season.**



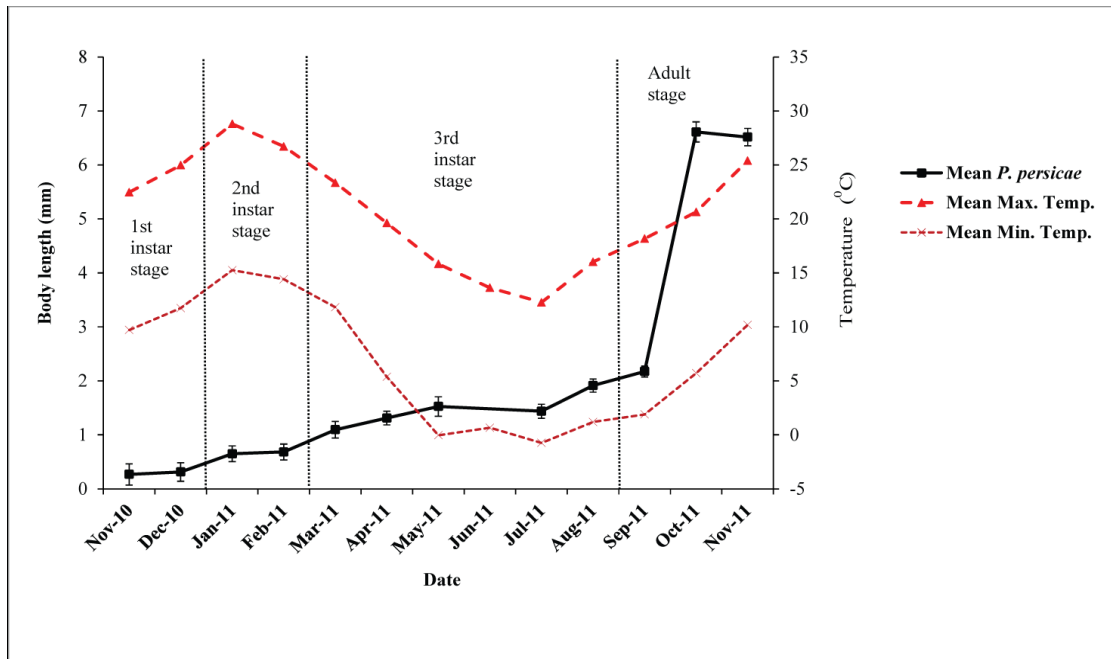
**Fig. 2. Grape scales (grapevine and frosted scales) population dynamics on four grapevine varieties over a single season.**



**Fig. 3. Population density of (a) grapevine scale and (b) frosted scale on individual grapevines of Chardonnay and Riesling varieties across each season where mixed scale populations were present. Error bars indicate standard error of mean**

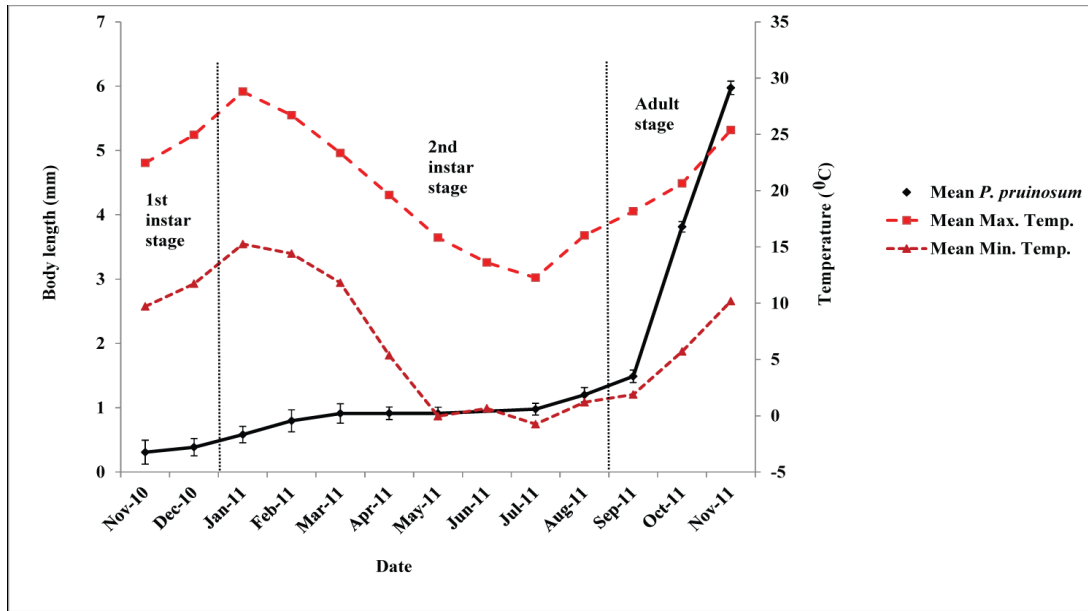


**Fig. 4. Population density of frosted scale on grapevine varieties without grapevine scale. Error bars indicate standard error of mean**



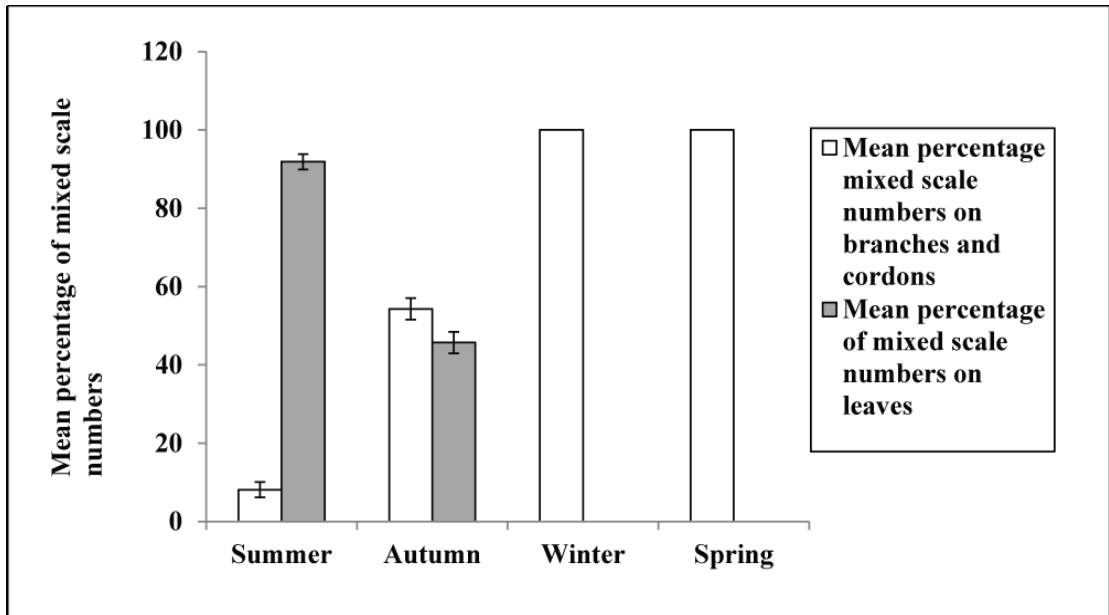
**Fig. 5. Phenology of life stages of grapevine scale over the 2010-2011 growing season. Error bars indicate standard error of mean**





**Fig. 6. Phenology of life stages of frosted scale over the 2010-2011 growing season.**

**Error bars indicate standard error of mean**



**Fig. 7. Mean percentage of mixed scale numbers on grapevine leaf and branch across each season. Error bars indicate standard error of mean**

# CHAPTER 6

## **Development and feeding effect of frosted scale *Parthenolecanium pruinosum* Cocquillet. (Hemiptera: Coccidae) on selected *Vitis vinifera* L varieties**

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## Summary

Frosted scale is a sap-sucking insect pest that feeds on a number of commercial European grapevine *Vitis vinifera* varieties across several wine regions of Australia. The ability to develop and the impact of its feeding activity on grapevines have not been documented. To address this knowledge gap, a glasshouse study examined the development and feeding effect of Frosted scale on three *V. vinifera* varieties Pinot Noir, Riesling and Sauvignon Blanc. A replicated glasshouse experiment was established by allocating the potted rootlings of the three grapevine varieties into two treatment regimes, control (uninfested) and treatment (infested with Frosted scale) grapevines. The six treatment and controls were allocated to nine replicated blocks, six replicates per block. The investigation was carried out in synchrony with grapevine and Frosted scale development in the summer months between November 2011 and March 2012. Frosted scale population abundance was relatively high on Riesling, moderate on Pinot Noir and least on Sauvignon Blanc. The presence of Frosted scale resulted in a significant reduction in leaf chlorophyll content and the number of internodes per grapevine branch. Although chlorophyll content varied between the varieties and differed between controls and infested plants, increasing numbers of scales did not significantly affect the chlorophyll content. Increasing numbers of Frosted scale on the different varieties significantly decreased the number of internodes per grape. Proportion of dropped leaves was higher in all varieties exposed to frosted scale than corresponding control plants. Riesling dropped more leaves than Pinot Noir or Sauvignon Blanc. An increase in first and second instar populations of Frosted scale feeding mainly on grapevine leaves was observed. This feeding may reduce leaf chlorophyll and the number of internodes per grapevine branch. Pinot Noir, Riesling and Sauvignon Blanc varieties were all susceptible to Frosted scale feeding under

glasshouse conditions, but the performance response in various parameters of each variety differed with the presence of the insects. Grape growers could expect Frosted scale population and loss of vine vigour to increase on highly favoured or susceptible *V. vinifera* varieties such as Riesling, Pinot Noir and Sauvignon Blanc varieties may show mild tolerance but further studies examining how Frosted scale density affects grapevine growth parameters such as photosynthesis and total biomass are needed to verify whether resistance/tolerance traits are present.

Keywords: Frosted Scale, grapevine vigour, insect population, *Parthenolecanium pruinosum*.

## 1.0 Introduction

*Parthenolecanium pruinatum* Coc. (Hemiptera: Coccidae) is a conspicuous pest of deciduous fruit trees (Hely et al. 1982, Michelbacher and Hitchcock 1956, Pfeiffer, 1997) in temperate and sub-tropical regions. In Australia, this scale is found on several ornamental plants, fruit trees and more recently on European grapevine *Vitis vinifera* L. (Hely et al. 1982, Rakimov 2010, Rakimov et al. 2013). Injury to *V. vinifera* may arise directly through insect feeding and/or indirectly through secondary disease infection. Furthermore, grapevine damage can be accentuated by concurrent infestation with grapevine scale *Parthenolecanium persicae* Fabricius (Rakimov et al. 2013). Although Frosted scale can develop on *V. vinifera*, there is very little biological information on the effect of scale development and its feeding activity on grapevine performance traits.

*P. pruinatum* originated in Nearctic regions and was first reported in Australia, in 1928 (Hely et al. 1982; Rakimov 2010). The species has an univoltine life cycle (monocyclic) in both Northern (Pfeiffer 1997) and Southern Hemispheres (Simbiken et al. in prep.), with adults emerging in spring. Young and mature adults will produce a frosty wax secretion over the sclerotised dorsal surface depending on the climatic conditions (Gill 1988, Michelbacher and Hitchcock 1956, Rakimov 2010). Reproduction is mainly parthenogenetic and ovipositioning occurs towards the end of spring and early summer (Simbiken et al. submitted). First instars (or crawlers) disperse and search out feeding sites on soft succulent branches and leaves (Greathead 1997). Before leaf fall, juveniles migrate back onto the woody branch for diapause (Simbiken et al. in press). Pfeiffer (1997) suggested Frosted scales have two to three juvenile life stages before adult stage. Recently three life stages, first and second juvenile stages and an adult stage have been uncovered for population breeding on *V. vinifera* in Australia and that the second instar

stage occurred predominantly in autumn and winter months (Simbiken unpublished). Feeding resumed in spring and second instars developed into adults. Leaves and lignified branches of grapevines play a critical role in the growth, survival and reproduction of Frosted scale. Nutrients are drawn internally from the phloem cells by inserting the stylet and feeding through a salivary tube (Foldi, 1997). First instars feed mainly on the abaxial side of the leaf away from direct sunlight (Simbiken pers. observ.). On grapevine branches and woody cordons, juveniles inhabit crevices present on the sheltered side of grapevines. Feeding by scale insects can occur on different plant parts, and the reduction of photosynthesis and plant biomass is typical symptoms of host damage caused by plant sap-feeders (Zvereva et al. 2010). On grapevines, sap-feeding insects can cause serious impact on vine performance and wine quality traits, as both nutrients and water are withdrawn from the plant. Grape phylloxera (*Daktulosphaira vitifoliae* Fitch) infested grapevines showed reduced chlorophyll content in Shiraz, Carbenet sauvignon and Pinot Noir varieties (Blanchfield et al. 2006). A reduction of chlorophyll content can also occur in water-deficient grapevines (Blanchfield et al. 2006). Grapevines infested with potato leafhopper (*Empoasca fabae* Harris) show reduced plant biomass (Lenz et al. 2009) and reduced photosynthesis in leaves (Lenz et al. 2012). Feeding by mealybugs (*Pseudococcus* sp.) caused significant reduction in phenolics, anthocyanins and tannins in Chardonnay and Carme´ne`re varieties and the reduction can be severe with increased insect infestation levels (Bordeu et al. 2012). *V. vinifera* is a cultured perennial shrub that depends upon the carbohydrate stored in the stems and roots in the preceding season for growth of new shoots, flower and berries. Carbohydrates (non-soluble sugars) are usually stored in the parenchymal cells of grapevine roots and branches (Lebon et al. 2008, Zufferey et al. 2012). These reserves are needed to ensure grapevine performance, yield and wine quality in the subsequent growing season. Grapevine leaves emerging early have high photosynthetic rates that

progressively decline with age. Photosynthesis in the leaves occurring after bud break is considered critical for flowering and fruit set (Holzapfel et al. 2010, Lebon et al. 2008, Mullins et al. 1992, Zufferey et al. 2012). High photosynthetic rate enables the developing shoots to rapidly accumulate biomass between 9 and 48 days after budbreak (DAB) (Borghazan et al. 2012, Greer et al. 2009). Flower development and root regrowth occur at 40 DAB when sufficient energy has been acquired as a result of photosynthesis (Mullins et al. 1992). Fruit bunch development is a high sink source, in terms of plant energy requirement. Fruit biomass increases gradually through to harvest (approximately 200 DAB) (Greer and Sicard 2009, Howell 2001). Howell (2001) showed fruit development accounted for 40% of grapevine biomass. Feeding from parenchyma and phloem cells by sap-feeding insects may be an additional sink, thus inhibiting vine growth and fruit set.

The aim of this study was to determine Frost scale development and the effect of its feeding on three *V. vinifera* varieties: Riesling, Sauvignon Blanc and Pinot Noir. Differences in Frosted scale survival on these varieties has previously been observed (Simbiken et al. in prep) in commercial vineyards near Canberra, with a high infestation on Riesling, moderate infestation on Sauvignon Blanc and low infestation on Pinot Noir. These survival differences may be attributed to various biotic and abiotic factors. To minimise the influence of abiotic factors, a glasshouse study comparing Frosted scale development on each grapevine variety was undertaken. The three grapevine varieties were monitored with or without Frosted scale. The differences between infested and non-infested plants indicated the effect of Frosted scale on plant performance, while any changes in population of the scales on the three varieties could indicate how the scales responded to host plant differences.



## **2.0 Materials and Methods**

### **2.1 Study Vines**

The study was conducted under glasshouse conditions at DEPI Rutherglen, Northeast Victoria. One-year old rootlings (plants with well-developed root system) of ungrafted *V. vinifera* cv. Sauvignon Blanc (clone H5V10) and cv. Pinot Noir (clone 667) were sourced from Kingara Nurseries (Irymple, Victoria) and rootlings of cv. Riesling (clone GM198) were obtained from Glenavon Nurseries Pty Ltd (Langhorne Creek, South Australia). Varieties were selected as they were previously observed to vary in their susceptibility to Frosted scale in the Canberra Wine region (Simbiken et al. in prep).

### **2.2 Soil Treatment**

Individual rootlings were potted in a soil mix consisting of 1:1:4 Bunnings® Perlite, Vermiculite and Bunnings® Peat Moss. Potting mix was sterilised at 70<sup>0</sup>C for 24 hours before use. Rootlings were raised in standard pots 20cm (diameter) x 20cm (height). A total of 25 Riesling, 26 Pinot Noir and 26 Sauvignon Blanc rootlings were potted for the trial. Potted rootlings were fertilised with Rustec®; a slow release broad spectrum liquid fertiliser. Fertiliser was initially diluted by mixing 100 ml in 10 litres of water. From this solution, 100 ml was applied to each potted grapevine. Grapevines were established in the glasshouse under the 8 hr Light:16 hr Dark photoperiod, day and night temperatures of approximately 24<sup>0</sup>C and 17<sup>0</sup>C respectively, and 70% soil humidity by irrigation. The glasshouse room was illuminated by two Philips Son-T Agro 400 growth lights (Philips, North Ryde, NSW). Each potted plant received approximately 250 ml water per day

using an automated drip irrigation system. Each potted rootling had a metal cone trellis cage with base diameter 45 cm, top diameter 25 cm and height 140 cm for grapevine support during the trial period (Figure 1a).

Rootlings of uniform age were used in the experiment. The rootlings were potted in October and allowed to grow for a month before artificial infestation with Frosted scale. Eighteen potted rootlings of each variety were used, with the extra rootlings raised in a separate shade house. The experiment consisted of three varieties exposed to one of two treatment regimes; uninfested control grapevines and grapevines infested with Frosted scale (refer to experimental set up below).

### **2.3 Insect Material**

Grapevines that were to be infested with Frosted scale received ten gravid adult females. Female adults were bunched and placed on each infested treatment vine by using thin cotton wool supported by cellotape (Figure1b.). Frosted scale insects were collected from *V. vinifera* cv Riesling in a commercial vineyard in the Canberra wine region, the day before placement onto the vines and identified by morphological characteristics (Boratynski, 1970, Brittin, 1940) . Data for Frosted scale development and grapevine performance parameters were collected monthly for four months, December to March. The length of study period allowed the development of first and second instar stages of Frosted scale following egg-laying by gravid adult females.

### **2.4 Experiment Set up**

The three grapevine varieties and two treatments (control and infested plants) were organised in a randomised complete-block design (RCBD) with nine block/replications. There were nine replicate blocks with each block consisting of six benches. Six rootlings were placed on the six benches consisting of three grapevine varieties and two treatments grapevines for each variety, which was randomly arranged in two rows of three grapevines (Figure 1c). The potted grapevines were separated by 20 cm space to avoid grapevines touching during the experiment while the bench/blocks were separated by 70 cm to allow working space.

## **2.5 Data Parameters**

Frosted scale development on the three grapevine varieties and the two treatments (uninfested and infested) were monitored monthly. Censuses were made on the grapevine leaves and branch that was initially used for the release of gravid female adults. A hand-held lens (10x magnification) was used for monthly frosted scale counts between December and February. In March, the infested branch including its leaf was detached from the grapevine for a final scale count using a dissecting stereo-microscope (40x). The effect of scale feeding on plant growth parameters was monitored monthly and also at the end of the study period. Grapevine performance parameters monitored monthly were total leaf number, number of dry leaves dropped, total internode per branch and leaf chlorophyll content. Leaf chlorophyll, mean leaf area and total plant dry and fresh matter were collected for each plant at the end of the trial. Leaf chlorophyll content was measured using a SPAD-502 chlorophyll meter (SPAD, soil and plant analyzer development) (Konica-Minolta, Osaka, Japan). SPAD units are values defined by the manufacturer which indicate the relative amount of chlorophyll present in plant leaves (Chang and Robison 2003). Leaf area was measured with a Paton Electronic

Planimeter® by taking the average of three leaves obtained in the middle of the grapevine branch of individual potted rootling. Grapevine biomass was taken by measuring both fresh and dry mass of foliage and root of grapevines. Foliage and root contents were fresh weighed and then oven-dried at 70°C for 24 hours to determine moisture content and dry mass of the plants. The roots were rinsed clean of soil with tap water prior to obtaining fresh weight.

## **2.6 Data Analysis**

Statistical analysis of the number of Frosted scales was determined by complete randomised analysis of variance (ANOVA) with designed factors being treatment (infested or control), grapevine variety (Riesling, Pinot Noir and Sauvignon Blanc) and sampling date and bench/block included as a random factor. Insect count (x) on each grapevine plant was log transformed ( $\log_{10} x + 1$ ) prior to analyses.

Two time points, December and March, were used to determine the differences between number of scales on branches and leaves. These months were associated with the settling and commencement of feeding for first instar and second instars respectively. A two-tailed T-test was used to determine whether a difference existed between the settling sites of two instars.

Grapevine growth parameters for each variety (average leaf number per grapevine, average internode per grapevine branch, leaf chlorophyll content, leaf area, moisture content and grapevine dry mass) were compared between treatments (infested vs control) and between grapevine varieties with the block/replicate included as a random factor. The parameters were analysed as a randomised complete block design (RCBD)

using either ANOVA or restricted maximum likelihood (REML) analysis within the Generalised Linear Mixed Model (GLMM) procedure of Genstat version 16. The number of dead leaves dropped relative to total leaves at the beginning were analysed using GLM with a binomial distribution and logit transformation, with treatment and variety as factors. Significant difference by Wald statistics was used to determine if the grapevine parameters differed between the treatments for each variety.

Simple linear regression ANOVA was used to analyse whether the increase in Frosted scale population affected the grapevine parameters within a variety.

### **3.0 Results**

#### **3.1 Scale Numbers with Variety and Time**

Frosted scale numbers were significantly higher on *V. vinifera* cv Riesling than Pinot Noir which had significantly more scales than Sauvignon Blanc ( $F = 32.52$ ,  $df = 2$ , 190,  $p < 0.001$ ) (Table 1 and Figure 2). Scale populations on infested grapevines were significantly higher than control grapevines across the three varieties ( $F = 762.5$ ,  $df = 1$ , 190,  $p < 0.001$ ). However, a significant interaction term between varieties and treatment factors was present ( $F = 30.34$ ,  $df = 2$ , 190,  $p < 0.001$ ) as several control grapevines from Pinot Noir and Riesling were infested with first and second instars. Scale presence on control vines was limited to certain blocks and varieties. Control Pinot Noir grapevines were infested, albeit on small abundance on three replicate/blocks, while on Riesling control grapevines only a single grapevine one replicate/block had first instars present. No scales were found on control Sauvignon Blanc grapevines (Figure 2).

Frosted scale numbers did not vary with sample date ( $F = 1.61$ ,  $df = 3$ ,  $190$ ,  $p < 0.188$ ). There was no significant interactions between sample date and variety ( $F = 0.88$ ,  $df = 6$ ,  $190$ ,  $p < 0.509$ ) and between sample date and treatment ( $F = 1.40$ ,  $df = 3$ ,  $190$ ,  $p < 0.243$ ). Significantly more first instars in December were found on leaves than on branches ( $16.2 \pm 4.76$  vs  $1.4 \pm 0.37$ ,  $n = 54$ ) (T-test =  $-3.12$ ,  $df = 53.65$ ,  $p < 0.003$ ). The same pattern occurred in March with the second instars when leaves had more insects than branches ( $32.7 \pm 9.20$  vs  $5.5 \pm 1.51$ ,  $n = 54$ ) (T-test =  $-2.92$ ,  $df = 55.58$ ,  $p < 0.005$ ).

### **3.2 Changes with Grapevine Development in the Presence of Scales**

The presence of Frosted scale resulted in a significant reduction in leaf chlorophyll content (Wald statistic =  $5.29$ ,  $p < 0.027$ ) and the number of internodes per grapevine branch (Wald statistic =  $6.45$ ,  $p < 0.015$ ) compared to non-infested grapevines across the three grapevine varieties (Table 2). In infested grapevines leaf chlorophyll content was reduced by an average 10% across the three varieties, and the number of internodes was reduced by an average 15.5%. There was no significant interaction between variety and treatment in the grapevine growth parameters measured; number of leaf per grapevine, leaf area, number of internodes per grapevine branch, leaf chlorophyll content, grapevine moisture content and total grapevine dry matter (biomass) (Table 3), although varietal differences were present for all these parameters except internodes per branch.

Increasing numbers of Frosted scale on the different varieties significantly decreased the number of internodes per grapevine ( $F = 6.83$ ,  $df = 1$ ,  $48$ ,  $p < 0.012$ , Figure 3). However, no significant difference was found across varieties in their response to

increasing number of scales ( $F = 2.42$ ,  $df = 2, 48$ ,  $p < 0.1$ ). Although chlorophyll content varied between the varieties (Table 2) and differed between controls and infested plants, increasing numbers of scales did not significantly affect the chlorophyll content ( $F = 0.9$ ,  $df = 1, 48$ ,  $p < 0.35$ ) (Figure 4).

Part of the reason that no scale effects were observed between varieties for many of the measured parameters was that the varieties responded differently to the presence of scales (Table 2). Total grapevine dry mass was reduced in Riesling and Pinot Noir in infested grapevines by 15.6% and 3.5% respectively, but in Sauvignon Blanc infested grapevines had increased in dry mass by 8.8% (Table 2). The same pattern is shown in moisture content. Both Sauvignon Blanc and Pinot Noir increased the number of leaves, while Riesling had a decrease in leaves per branch. Mean leaf area was reduced in all plants, but by only a small percentage (1.6% Riesling, 7.9% Pinot noir and 3.2% Sauvignon Blanc). Treatment had a significant effect on the number of leaves dropped, with the proportion of dropped leaves was higher in all varieties exposed to scales than the corresponding control plants (Table 4). The number of dropped leaves was significantly different among the varieties, with Riesling dropping more leaves than either Pinot Noir or Sauvignon Blanc, which did not differ significantly (Table 4). However, no interaction of the treatment with variety was present.

#### **4.0 Discussion**

This study investigated population changes of Frosted scale on three *V. vinifera* varieties and the effect of the insect on selected grapevine performance traits. The study showed that grapevine variety had a significant impact on Frosted scale abundance. The largest Frosted scale population was present on Riesling grapevines, while numbers

were reduced on Pinot Noir grapevines and the fewest numbers occurred on Sauvignon Blanc. Varietal differences between *Vitis vinifera* and *V. lambrusca* in development, survivorship and fecundity of the mealybug, *Dysmicoccus brevipes*, was also recently reported and was suggested as a potential component of an IPM for grapevine management (Bertin et al. 2013), although how the varieties caused the mealybug response was not reported.

The presence of Frosted scale caused a significant reduction in leaf chlorophyll content and numbers of internodes per branch for all three varieties. As the numbers of scales increased, the number of internodes per branch decreased, but the increase in scales observed did not have any further effect on chlorophyll content. Grapevine biomass, leaf area, moisture content and numbers of leaf were not significantly affected by insect feeding, although varieties did differ in their response to scale numbers, but the response was inconsistent. The proportion of dry dropped leaves increased when scales were present, with a higher proportion dropped by Riesling. The number of leaves dropped may affect overall plant performance, resulting in the reduced growth of this variety. Dropped leaf number in Pinot Noir and Sauvignon Blanc did not differ, but there were other differences in these plants, suggesting that other varietal performance parameters may vary. The timing of leaf drop was not measured, but if leaves are dropped early in insect settlement, some control of Frosted scale population growth would be possible. Certainly the reduced numbers of Frosted scale on the Sauvignon Blanc could result from early leaf drop.

The study showed that Pinot Noir, Riesling and Sauvignon Blanc varieties might have different thresholds of Frosted scale activity which induce damage symptoms. This threshold density may be higher on Pinot Noir than on Riesling and Sauvignon Blanc.



Lenz *et al.*, (2009) showed that grapevine biomass was reduced only when the potato leafhopper *Empoasca fabae* Harris reached a threshold density of three nymphs per leaf and below this level grapevines can recover with little or no damage effects. Recovery effort and/or compensatory growth might be possible due to the fact that nutrients stored in stems and roots of grapevines could be utilised to reduce the effect of the scale feeding. A reduction in leaf chlorophyll and internode density across the three grapevine varieties observed in this study may affect the overall performance of the host plants and grape yield. Further work is needed to relate the leaf chlorophyll and internode reduction to grape quality to assess how Frosted scale populations may be related to subsequent economic loss.

Increasing Frosted scale abundance might cause increased stress which impacts on grapevine growth (density dependent effect). Frosted scale fed mostly on grapevine foliage rather than on branches across the three varieties. Due to the study duration, the effect of Frosted scale feeding could be detected by changes in the chlorophyll content and branch length (internode density/grapevine branch), but a density dependent effect was only apparent in the numbers of internodes per branch. Decreased plant chlorophyll content has been detected following sap-feeding by insects previously (Blanchfield *et al.* 2006, Cocco *et al.* 2013, Girma *et al.* 1998b, Koch *et al.* 2014, Lenz *et al.* 2009, Nagaraj *et al.* 2002ab). A reduction in chlorophyll content may reduce photosynthetic rate which can affect plant biomass (Lenz *et al.* 2012, Nagaraj *et al.* 2002ab), and that pattern is consistent with that observed in Riesling. Although the sap-feeding potato leafhoppers caused reduced photosynthesis and a reduction in plant biomass depending on insect density (Lenz *et al.* 2012), the different grapevine varieties can respond differently. Although chlorophyll content and leaf decreased in all grapevine varieties, the dry mass and moisture content only decreased in Riesling and both of those

parameters increased in Sauvignon Blanc. The differences in grapevine response to the presence of Frosted scale indicates that the plants vary in either their ability to reduce scale population growth and establishment (e.g. Sauvignon Blanc) or to tolerate the increase numbers of scale without obvious effects on plant growth and health (e.g. Pinot Noir). As this study was using rootlings, there is a possibility for delayed effect and/or compensatory growth by the grapevines depending on the scale population density and duration of feeding activity in which this study did not consider. Only Riesling appeared to be susceptible to the presence of Frosted scale and responded as though no resistance mechanism was present in those grapevines to minimise the extraction of nutrients by the insect.

The observed infestation of the control grapevines indicate that dispersal by first instar stages occurred from the infested plants. Aerial dispersal may have been facilitated by the cooling fans as the replicate/blocks were controls were established were in the direction of the glasshouse cooling fans (Figure 1c.). However, as this instar is mobile, movement from one plant to another along the wire table may also have occurred. Although both Riesling and Pinot Noir control grapevines appear to have been infested as a result of dispersal, the lack of scales on the Sauvignon Blanc grapevines in the same region suggests that scales could not readily establish on that variety. Currently it is not known what is different about Sauvignon Blanc that may limit establishment and population increase of Frosted scale on this grapevine variety compared to other varieties.

The relatively large increase in Frosted scale abundance on Riesling suggests that this grapevine variety is a preferred host for Frosted scale insect and that it can both establish and disperse on this variety. This result is consistent with field observations

(Simbiken et al. in prep) that indicated a higher density of Frosted scales on Riesling than on Pinot Noir and Sauvignon Blanc. However, in this glasshouse study, the Pinot Noir was more susceptible to scales having both a higher population and having scales establish on control grapevines than Sauvignon Blanc. In the field studies, it appeared that Pinot Noir was more resistant to scale than Sauvignon Blanc however that might be a result of differing environmental conditions in field studies.

The differences in grapevine performance under insect infested conditions may vary depending on variety as was observed on *V. vinifera* Shiraz, Cabernet Sauvignon and Pinot Noir varieties when infested with grape phylloxera (*Daktulosphaira Vitifoliae* Fitch) (Blanchfield et al. 2006). Blanchfield et al. (2006) observed that leaf chlorophyll content in Shiraz grapevines on both infested and non-infested grapevines were comparably lower than Cabernet Sauvignon under glasshouse conditions whilst under field conditions Cabernet sauvignon infested and non-infested grapevines had lower leaf chlorophyll content than Pinot Noir. Our glasshouse study showed similar results with chlorophyll content and grapevine performance affected by scale presence. The development and feeding impact of Frosted scale on *V. vinifera* shown here reflects the potential influence of this insect on wine grape production. Significant effects of grapevine stress and subsequent wine quality flavours have been attributed to insect pests and diseases (Bordeu et al. 2012, Cabaleiro et al. 1999, Cretazzo et al. 2010, Ocete et al. 2008). Bordeu et al. (2012) showed that infestation of mealy bug (*Pseudococcus* sp.) is likely to reduce compounds such as phenolics, anthocyanins and tannins that are critical in wine production for influencing the flavours associated with certain wines. Further work is needed to identify whether such changes occur in the various varieties and how the varieties may differ in the fruit response to the presence of Frosted scales. The potential of varieties to act as barriers to scale dispersal within vineyards also needs

to be assessed as a part of biocontrol methods, as interspersal of varieties that are resistant to scale settlement may limit the need for spraying.

## **5.0 Conclusion**

In the glasshouse, the Frosted scale population was relatively high on Riesling, moderate on Pinot Noir and least on Sauvignon Blanc. But the response of these varieties to insect feeding differed, with variation in some of the plant parameters measured. Overall both chlorophyll content and numbers of internodes were reduced in the presence of Frosted scale, but increasing scale numbers only affected internode numbers. The number of dry fallen leaves also increased in scale-infested plants, which could limit the numbers of scales that develop if leaf drop occurs soon after scale infestation and could also affect photosynthetic activity of the host plant.

## **6.0 Acknowledgements**

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## 7.0 References

- Blanchfield AL, Robinson SA, Renzullo LJ and Powell KS. 2006. Phylloxera-infested grapevines have reduced chlorophyll and increased photoprotective pigment content — can leaf pigment composition aid pest detection? *Functional Plant Biology* **33**, 507-514.
- Boratynski K. (1970). On some species of 'Lecanium' (Homoptera, Coccidae) in the collection of the Naturhistorisches Museum in Vienna; with description and illustration of the immature stages of *Parthenolecanium persicae*. *Analen des Naturhistorischen Museums Wien* **74**, 63-76.
- Bordeu E, Troncoso DO and Zaviezo T. 2012. Influence of mealybug (*Pseudococcus* spp.)-infested bunches on wine quality in Carmenere and Chardonnay grapes. *International Journal of Food Science & Technology* **47**, 232-239.
- Borghezan M, Gavioli O, Vieira HJ and Silva ALD. 2012. Shoot growth of merlot and cabernet sauvignon grapevine varieties. *Pesquisa Agropecuaria Brasileira* **47**, 200-207.
- Cabaleiro C, Segura A and García-Berrios JJ. 1999. Effects of grapevine leafroll-associated virus 3 on the physiology and must of *Vitis vinifera* L. cv. Albariño

following contamination in the field. *American Journal of Enology and Viticulture* **50**, 40-44.

Chang SX and Robison DJ. 2003. Nondestructive and rapid estimation of hardwood foliar nitrogen status using the SPAD-502 chlorophyll meter. *Forest Ecology and Management* **181**, 331-338.

Cocco A, Marras P, Nuvoli T, Mameli M and Lentini A. 2013. Preliminary results on the influence of nitrogen fertilization on the development of *Planococcus citri* and *Planococcus ficus* on grapevine. *IOBC-WPRS Bulletin* **85**, 19-24.

Cretazzo E, Padilla C, Carambula C, Hita I, Salmerón E and Cifre J. 2010. Comparison of the effects of different virus infections on performance of three major grapevine cultivars in field conditions. *Annals of Applied Biology* **156**, 1-12.

Gill RJ. 1988. The scale insects of California, Part 1: the soft scales (Homoptera: Coccoidea: Coccidae). In: *Analysis and Identification Branch, California Dept. of Food and Agriculture*. Sacramento, California, USA.

Girma M, Kofoed KD and Reese JC. 1998. Sorghum germplasm tolerant to greenbug (Homoptera: Aphididae) feeding damage as measured by reduced chlorophyll loss. *Journal of the Kansas Entomological Society* **71**, 108-115.

Greathead DJ. 1997. Crawler behaviour and dispersal. In: *World Crop Pests. Soft Scale Insects: Their Biology, Natural Enemies and control. Vol 7A*. (eds Y Ben-Dov and CJ Hodgson). pp. 339-342. Elsevier. Amsterdam, Netherlands..

- Greer DH and Sicard SM. 2009. The net carbon balance in relation to growth and biomass accumulation of grapevines (*Vitis vinifera* cv. Semillon) grown in a controlled environment. *Functional Plant Biology* **36**, 645-653.
- Hely PC, Pasfield G and Gellatley JG. 1982. *Insect Pests of Fruit and Vegetables in NSW*, Inkata Press.
- Holzapfel BP, Smith JP, Field SK and Hardie WJ. 2010. Dynamics of carbohydrate reserves in cultivated grapevines. *Horticultural Reviews* **37**. John Wiley and Sons, Inc.
- Howell GS. (2001) sustainable grape productivity and the growth-yield relationship: A review. *American Journal of Enology and Viticulture* **52**, 165-174.
- Koch K, Bradshaw J, Heng-Moss T and Sarath G. 2014. categories of resistance to greenbug and yellow sugarcane aphid (Hemiptera: Aphididae) in three tetraploid switchgrass populations. *BioEnergy Research* **7**, 1-10.
- Lebon G, Wojnarowicz G, Holzapfel B, Fontaine F, Vaillant-Gaveau N and Clément C. 2008. Sugars and flowering in the grapevine (*Vitis vinifera* L.). *Journal of Experimental Botany* **59**, 2565-2578.
- Lenz MS, Isaacs R, Flore JA and Howell GS. 2009. Vegetative growth responses of pinot gris (*Vitis vinifera* L.) grapevines to infestation by potato leafhoppers

(*Empoasca fabae* Harris). American Journal of Enology and Viticulture **60**, 130-137.

Lenz MS, Isaacs R, Flore JA and Howell GS. 2012. Photosynthetic performance of pinot gris (*Vitis vinifera* L.) grapevine leaves in response to potato leafhopper (*Empoasca fabae* Harris) Infestation. American Journal of Enology and Viticulture **63**, 357-366.

Michelbacher A and Hitchcock S. 1956. Frosted scale on walnuts. California Agriculture **10**, 11-14.

Mullins MG, Bouquet A and Williams LE. 1992. Biology of the grapevine. UK, Cambridge University Press.

Nagaraj N, Reese JC, Kirkham MB, Kofoid K, Campbell LR and Loughin TM. 2002. Relationship between chlorophyll loss and photosynthetic rate in greenbug (Homoptera: Aphididae) Damaged Sorghum. Journal of the Kansas Entomological Society **75**, 101-109.

Nagaraj NJ, Reese JC, Kirkham M, Kofoid K, Campbell LR and Loughin T. 2002. Effect of greenbug, *Schizaphis graminum* (Rondani)(Homoptera: Aphididae), biotype K on chlorophyll content and photosynthetic rate of tolerant and susceptible sorghum hybrids. Journal of the Kansas Entomological Society **75**, 299-307.



- Ocete R, Lara M, Maistrello L, Gallardo A and López MA. 2008. Effect of *Xylotrechus arvicola* (Olivier) (Coleoptera, Cerambycidae) Infestations on Flowering and Harvest in Spanish Vineyards. *American Journal of Enology and Viticulture* **59**, 88-91.
- Pfeiffer DG. 1997. Deciduous fruit trees. In: *World Crop Pests. Soft scale insects: their biology, natural enemies and control. Vol. 7B.* (eds Y. Ben-Dov and CJ Hodgson). pp. 293-322. Elsevier Science, Amsterdam, Netherlands.
- Rakimov A. 2010. Aspects of the biology, ecology and biological control of soft scale insects (Coccidae) in Australian vineyards. PhD Thesis, University of Melbourne, Dept. of Zoology.
- Rakimov A, Ben-Dov Y, White V and Hoffmann AA. 2013. Soft scale insects (Hemiptera: Coccoidea: Coccidae) on grapevines in Australia. *Australian Journal of Entomology* **52**, 371-378.
- Zufferey V, Murisier F, Vivin P, Belcher S, Lorenzini F, Spring JL and Viret O. 2012. Carbohydrate reserves in grapevine (*Vitis vinifera* L.'Chasselas'): the influence of the leaf to fruit ratio. *Vitis* **51**, 103-110.
- Zvereva E, Lanta V and Kozlov M. 2010. Effects of sap-feeding insect herbivores on growth and reproduction of woody plants: a meta-analysis of experimental studies. *Oecologia* **163**, 949-960.

**Table 1. ANOVA for the numbers of frosted scale per grapevine branch across three grapevine varieties**

| <b>Source</b>              | <b>df</b> | <b>F-value</b> | <b>Probability</b> |
|----------------------------|-----------|----------------|--------------------|
| <b>Variety</b>             | 8         | 32.52          | <0.001             |
| <b>Treatment</b>           | 2         | 762.48         | <0.001             |
| <b>Date</b>                | 1         | 1.61           | 0.188              |
| <b>Variety x treatment</b> | 2         | 30.34          | <0.001             |
| <b>Variety x date</b>      | 6         | 0.88           | 0.509              |
| <b>Treatment x date</b>    | 3         | 1.40           | 0.243              |

**Table 2. Average grapevine growth parameters ( $\pm$  SEM) per grapevine exposed to frosted scale feeding across the three *V. vinifera* varieties**

| Variety                | Leaf density/per grapevine |                | Internode density/branch |                |                | Leaf Area (mm <sup>2</sup> ) |                |                | Leaf chlorophyll content (SPAD units) |                |                | Moisture content (g) |                  | Grapevine dry matter (g) |                  |                |                |                |
|------------------------|----------------------------|----------------|--------------------------|----------------|----------------|------------------------------|----------------|----------------|---------------------------------------|----------------|----------------|----------------------|------------------|--------------------------|------------------|----------------|----------------|----------------|
|                        | Control                    | Treated        | Total                    | Control        | Treated        | Total                        | Control        | Treated        | Total                                 | Control        | Treated        | Total                | Control          | Treated                  | Total            | Control        | Treated        | Total          |
| <b>Pinot Noir</b>      | 40.0 $\pm$ 3.7             | 43.1 $\pm$ 2.7 | 41.5 $\pm$ 3.4           | 39.0 $\pm$ 2.7 | 31.8 $\pm$ 3.6 | 35.4 $\pm$ 1.9               | 6527 $\pm$ 342 | 6013 $\pm$ 530 | 6272 $\pm$ 237                        | 20.9 $\pm$ 1.5 | 19.7 $\pm$ 1.1 | 20.3 $\pm$ 0.7       | 301.8 $\pm$ 40.8 | 280.5 $\pm$ 37.6         | 291 $\pm$ 30.5   | 49.5 $\pm$ 2.4 | 47.7 $\pm$ 2.1 | 48.6 $\pm$ 2.4 |
| <b>Riesling</b>        | 75.1 $\pm$ 5.1             | 69.4 $\pm$ 2.6 | 72.8 $\pm$ 3.5           | 32.9 $\pm$ 1.8 | 26.3 $\pm$ 4.0 | 30.2 $\pm$ 1.9               | 6442 $\pm$ 290 | 6339 $\pm$ 305 | 6408 $\pm$ 242                        | 17.0 $\pm$ 1.1 | 14.7 $\pm$ 0.8 | 16.1 $\pm$ 0.7       | 326.3 $\pm$ 72.0 | 278.6 $\pm$ 46.7         | 307.2 $\pm$ 31.2 | 66.3 $\pm$ 5.9 | 55.9 $\pm$ 2.9 | 62 $\pm$ 2.5   |
| <b>Sauvignon Blanc</b> | 64.2 $\pm$ 7.9             | 75.1 $\pm$ 4.9 | 71.2 $\pm$ 3.6           | 32.1 $\pm$ 2.3 | 29.8 $\pm$ 1.1 | 30.5 $\pm$ 2.0               | 4921 $\pm$ 189 | 4765 $\pm$ 260 | 4808 $\pm$ 250                        | 13.1 $\pm$ 0.9 | 11.6 $\pm$ 0.8 | 12.2 $\pm$ 0.8       | 147.4 $\pm$ 15.3 | 159.2 $\pm$ 21.7         | 156.8 $\pm$ 32.2 | 43.8 $\pm$ 2.7 | 47.9 $\pm$ 3.4 | 45.5 $\pm$ 2.6 |

**Table 3.** ANOVA of grapevine performance parameters exposed to frosted scale across the three *V. vinifera* varieties, Pinot Noir, Riesling and Sauvignon Blanc. *df* = degrees of freedom, *Wald statistic* is similar to the *F* statistic for the stated degrees of freedom, *p* is the probability with *p*<0.05 accepted as a significant relation. *p* values reported are exact, except when *p*<0.001.

| Source              | Leaf             |       |        | Internode/branch |      |               | Leaf Area/Grapevine |       |        | Chlorophyll content |       |               | Moisture              |      |        | Grapevine dry        |      |        |
|---------------------|------------------|-------|--------|------------------|------|---------------|---------------------|-------|--------|---------------------|-------|---------------|-----------------------|------|--------|----------------------|------|--------|
|                     | number/Grapevine |       |        |                  |      |               | (mm <sup>2</sup> )  |       |        | (SPAD Units)        |       |               | content/Grapevine (g) |      |        | matter/Grapevine (g) |      |        |
|                     | df               | Wald  | p      | df               | Wald | p             | df                  | Wald  | p      | df                  | Wald  | p             | df                    | Wald | p      | df                   | Wald | p      |
|                     | statistic        |       |        | statistic        |      |               | statistic           |       |        | statistic           |       |               | statistic             |      |        | statistic            |      |        |
| Varieties           | 2                | 47.15 | <0.001 | 2                | 5.45 | 0.078         | 2                   | 12.06 | <0.001 | 2                   | 77.80 | <0.001        | 2                     | 27.1 | <0.001 | 2                    | 23.2 | <0.001 |
| Treatment           | 1                | 0.47  | 0.497  | 1                | 6.45 | <b>0.015*</b> | 1                   | 0.81  | 0.73   | 1                   | 5.29  | <b>0.027*</b> | 1                     | 0.54 | 0.468  | 1                    | 0.90 | 0.348  |
| Variety x Treatment | 2                | 2.79  | 0.260  | 2                | 1.05 | 0.596         | 2                   | 0.20  | 0.817  | 2                   | 0.41  | 0.816         | 2                     | 0.87 | 0.650  | 2                    | 4.72 | 0.108  |

*\*Bold numbers indicate significant reduction in the infested grapevines*

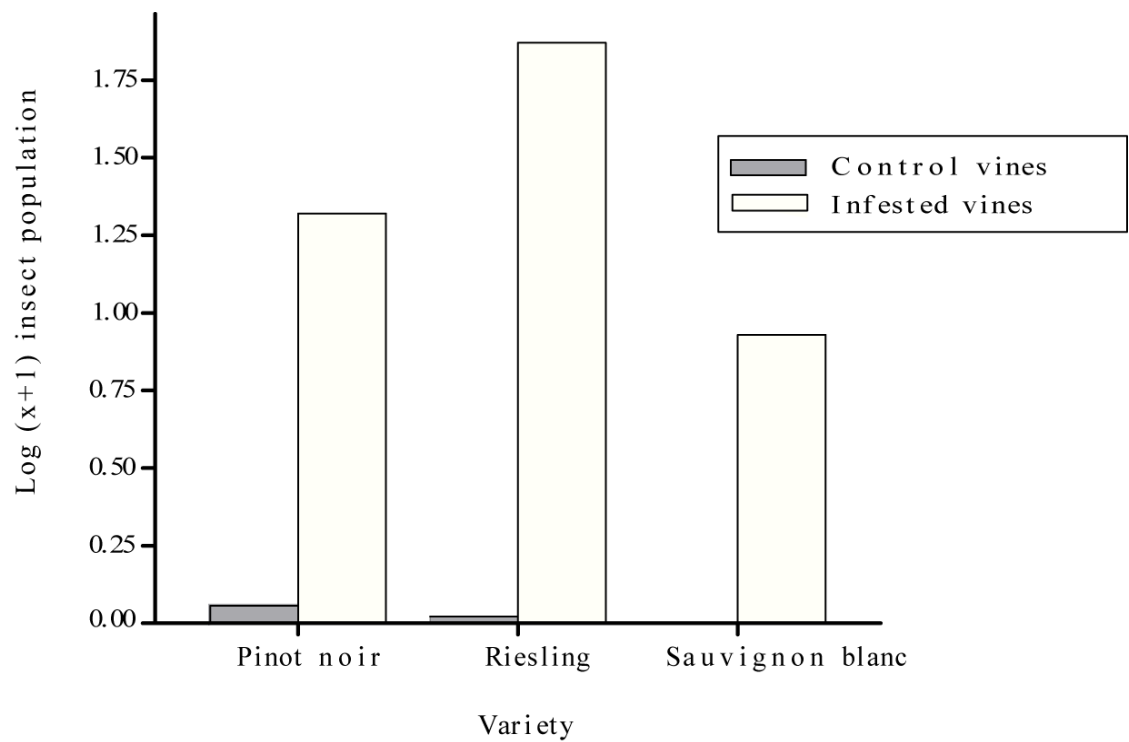
**Table 4. Values for regression analysis of total dropped dry grapevine leaves relative to total number of leaves at beginning of glasshouse trial using a binomial distribution and a logit link function.**

| Parameter             | Estimate | Standard error | t value | p                |
|-----------------------|----------|----------------|---------|------------------|
| Constant (Pinot noir) | -2.67    | 0.235          | -11.4   | <b>&lt;0.001</b> |
| Treatment (infested)  | 0.4      | 0.19           | 2.09    | <b>0.042</b>     |
| Variety - Riesling    | 1.10     | 0.26           | 4.25    | <b>&lt;0.001</b> |
| Variety - Sauvignon   | 0.34     | 0.25           | 1.35    | 0.184            |
| Blanc                 |          |                |         |                  |

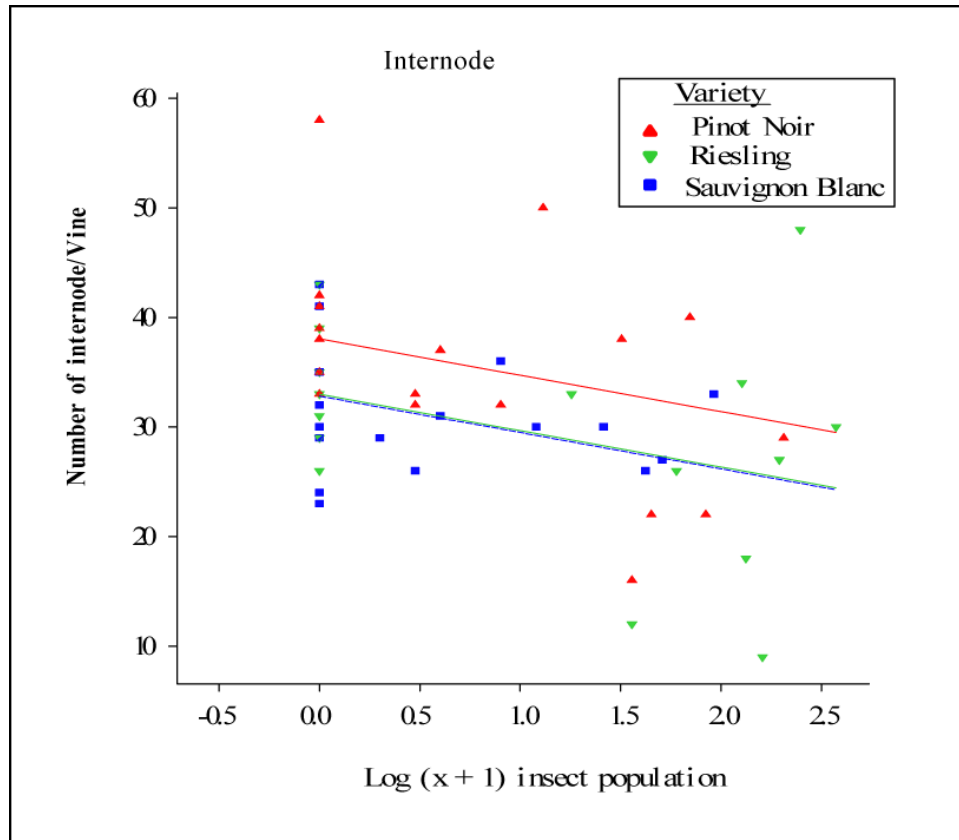
*The interaction between treatment (infested versus control) and variety (Pinot Noir, Riesling and Sauvignon Blanc) was dropped from the final statistical model as it was not significant. df for model = 50*



**Figure 1. Experimental layout (a) Treatment grapevines raised on metal bench with a cone trellis for support, (b) Frosted scale release method on grapevines (arrow) and (c) floor plan and experimental layout**

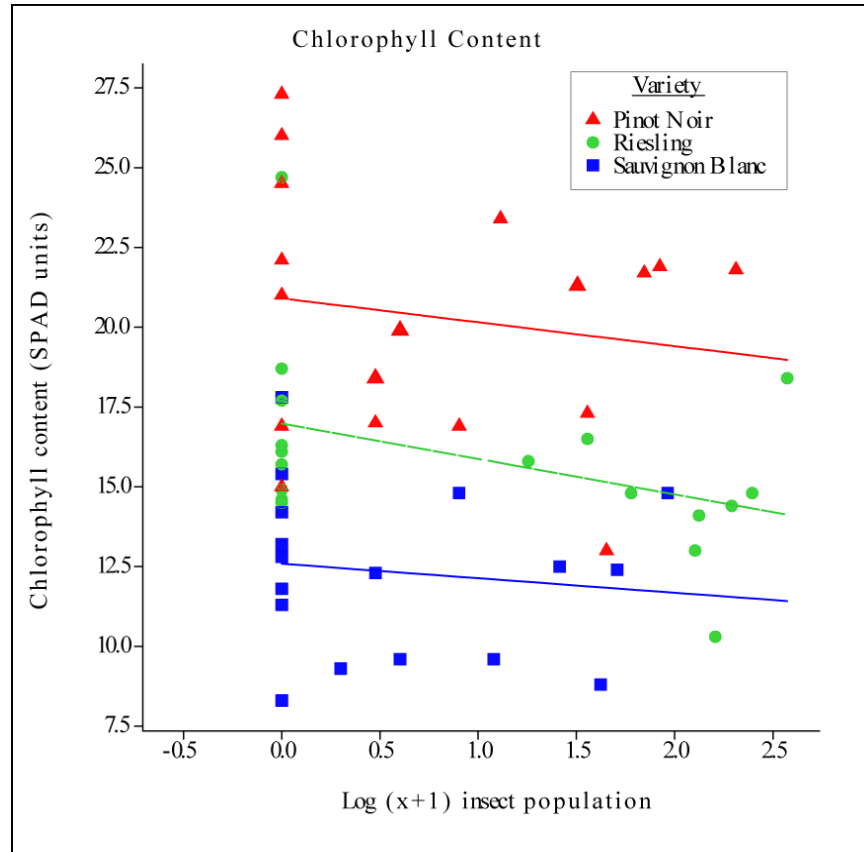


**Figure 2. Differences in numbers of frosted scale (log (number +1)) on *V. vinifera*, showing differences in scale abundance between treatment (infested vs uninfested) and grapevine variety.**



**Figure 3. Relationship between the average number of internodes per grapevine branch of three *V. vinifera* varieties and scale abundance (Riesling,  $y = 32.9 - 3.32 (\log x + 1)$ , Pinot Noir,  $y = 38.0 - 3.32 (\log x + 1)$ , Sauvignon Blanc,  $y = 32.8 - 3.32 (\log x + 1)$ ).**





# **CHAPTER 7**

## **Summary of Major Results, Implications and Future Research Directions**

## 1.0 Introduction

Grapevine scales *P. persicae* and *P. pruinosum* are significant pest of perennial crops including grapevines in the temperate regions of Northern and Southern Hemispheres. Both scale insects are exotic and widely distributed in the temperate and sub-tropical regions of Australia. Although the scale insects are occasional pests, they have the ability to increase their population rapidly to outbreak levels if control measures are ineffective.

Using both the laboratory and field studies several questions on the life stage development, population growth, host plant preference, population dynamics and feeding effect on grapevine performance were investigated in this thesis. In this chapter, I will first summarise main results from each chapter, then discuss the implications of the work and conclude with future research directions.

## 2.0 Summary of Major Results

*P. persicae* and *P. pruinosum* typically have monocyclic (univoltine) life cycle on grapevines in Canberra Wine region. The number of life stages may differ between these scale insects with three juvenile instars and an adult stage in *P. persicae* and in *P. pruinosum*, two juvenile instars and an adult stage (Chapter 2). There is a possible third instar stage in *P. pruinosum* that can be found after the second instar towards the end of Winter and early Spring. The number of life stages may be caused by seasonality (abiotic), quality of environment (biotic) and abundant and availability of host plants (Hutchinson et al. 1997, Kivelä et al. 2013, Pöykkö et al. 2012). The third instar stage of *P. persicae* and

second instar stage of *P. pruinosum* are the important overwintering life stages. These juvenile life stages of both species develop into adults when environmental conditions are suitable in spring.

The length and width of stylet bundle increased with instar in *P. pruinosum*. Length of stylet bundle in first instar was two times longer than the body length (0.39 mm vs 0.7 mm), equal to body length in second instars (0.91 mm vs 0.9 mm) and shorter than body length in adults (3.6 mm vs 1 mm). Stylet length to the body length ratio decreases with instar, which suggests differences in the feeding location.

Insects reproducing asexually (parthenogenesis) have been considered to be serious pests in agricultural systems (Hoffmann et al. 2008) as a result of high intrinsic growth rate across this group (Ross et al. 2013, Silvertown et al. 2002). Gravid females of grapevine and frosted scales have high fecundity, produce a high percentage of viable offspring and thus have a high intrinsic growth rate (Chapter 3). Gravid females of frosted scale had reduced fecundity when present on Sauvignon Blanc compared with individuals present on Chardonnay and Riesling grapevines. The differences in fecundity were not a result of reduced body size, although smaller individuals were present on Sauvignon Blanc and Riesling. Gravid females of grapevine scale have a higher reproductive output compared with frosted scales (Chapter 3), despite general body size of frosted scale females being typically larger than grapevine scale females (Chapter 2). The reason for this difference is unclear, but suggests differences in their capacity for producing eggs and growth patterns, as frosted scale eggs, first instar and second instar stages are larger than grapevine scale eggs, first instar and second instar stages.

Pest status of grape scale complex across the wine regions is not associated with climate, although vineyard size and intra-varietal cropping system can result in differences in scale numbers (Chapter 4). Differences between white wine compared with red wine varieties does appear to indicate differences in the presence of scale though. In individual vineyards, pest status can be associated with certain grapevine varieties and several varieties can be favoured by the presence of scales more than others even amongst the white wine varieties.

Effects of plant defence chemicals on plant sap-feeding insects are more pronounced than the leaf structural properties (Guerrieri *et al.*, 2008). Susceptible varieties may have weak defence chemicals and/or the insect may have developed detoxifying enzymes to manipulate plant defence chemicals (Barrett *et al.*, 2012). Chapter five demonstrated that there are varietal effects on population dynamics. The populations of grapevine and frosted scales were higher on Riesling and Chardonnay (white wine varieties) than on Sauvignon Blanc (white wine variety) and Pinot Noir (red wine variety).

In instances where grapevine and frosted scales are both present on the same grapevines, infestation tends to be higher than it does with individual species infestation. However, in grapevines infested only with frosted scales, numbers of scales were higher on Chardonnay and Riesling compared with Pinot Noir and Sauvignon Blanc (Chapter 5)

First and second instars for both scale species were present in the summer months (December to February) (Chapter 5). In the autumn and winter months (March-Aug), third instars of grapevine scale and second instars of frosted scale were the most common life

stages. Both scale species reached adulthood in the spring between September and November. First and second instars were present on the leaves in the summer, but in autumn to spring, the scales were present on the branches and cordons.

In Chapter 6, a glasshouse experiment considered the population growth and development of first and second instars of *P. pruinosum* on three varieties of grapevine (Riesling, Pinot Noir and Sauvignon Blanc) and how their feeding affected grapevine growth. Frosted scale population was relatively high on Riesling, moderate on Pinot Noir and lowest on Sauvignon Blanc. Feeding activity reduced chlorophyll content and branch internode density in the three grapevines. In other parameters, the responses of the varieties differed, indicating that the plants respond differently in the presence of scales. Differences in plant response suggest that some varieties are tolerant of infestation, and other varieties may have resistance traits that are sensitive to the presence of scale.

### **3.0 Implications**

As each chapter had its own discussion, this section will only discuss those aspects that may extend across more than one chapter. The areas that will be examined are: 1) the location of feeding and the role this might play among instars and across species; 2) trade-offs between body size and egg size and how this might differ with species and plant variety; and 3) tolerance versus resistance characteristics of grapevine varieties and how that might vary with climate and cropping within vineyards.

Stylet structure and feeding sites changed with development in the two scale species. The long and narrow stylet in crawler stages of *P. prunosum* can penetrate and access plant tissues, even vascular bundles (Foldi 1997), but the process of feeding once the later instars move onto the woody material is less clear. Presumably the access to nutrients on the leaves may require less force, as the first instars may not be able to use the anchoring to the substrate to help with generating the forces necessary to penetrate woody plant material. The ability to generate force for stylet penetration may be associated with the capacity of the different instars to adhere to the surface. The first and second instars are still relatively mobile, and their leg contact points limit the downward force for penetration. As the scales age, the mobility is reduced and the substrate adherence may increase so that penetration force can be greater. The lower populations observed on Pinot Noir and Sauvignon Blanc in the field (as well as the difference between reds and whites; Chapter 4) may be caused by the force needed to penetrate cell walls, as the presence of phenolics can affect cell wall toughness (Santiago et al. 2013). Differences in cell wall toughness can confer resistance to feeding by herbivorous chewing insects (Clissold 2008), but such a relationship has not been established for piercing mouthparts. Although nutritional differences can also play a role in the variation observed in insect numbers on the plants, if the early instars cannot penetrate the leaves, then population numbers would be reduced as a direct result of starvation.

Adults grew rapidly in both species prior to oviposition commencing. However, body size in scale insects is variable (Chapter 2), and the eggs of the two species differed in size. *P. persicae* had the higher fecundity, although mortality of eggs may be higher as a result of parasitism (Rakimov, 2010), while *P. prunosum* has fewer eggs, but no egg mortality

(Rakimov 2010). Although the fecundity studies were done on the largest and gravid *P. persicae* (Chapter 3), *P. pruinosum* was larger than *P. persicae* in all instars as well as adult (Chapter 2). The difference in numbers of scales on Chardonnay and Riesling when both species were present (Chapter 5) indicated that either the nutritional quality of the two varieties favoured one species over the other in terms of development, or that adult fecundity or survivorship of early instars was enhanced differentially. These observations suggest that trade-offs between adult body size and fecundity or survivorship may permit one species to do better than the other on these plants. Further work is necessary to determine whether species reach larger body sizes depending upon the variety that they colonise and thus influence the number of offspring produced.

When scales are present, varieties had different responses for the various parameters measured (Chapter 6). Clearly, the greenhouse study demonstrated that the response of the varieties during early growth could differ, although a reduction in photosynthesis and number of internodes seemed to be a common response. However the number of scales present on Pinot Noir was higher in the greenhouse compared with Sauvignon Blanc, but just the opposite effect was observed in the field study (Chapter 5). Two components differed in these studies, 1) age of the vines and 2) environment to which the scales and plants were exposed. In both conditions, the highest number of scales was found on Riesling. These results suggest that varieties have at least three different methods for dealing with the scales. The grapevines can tolerate the presence of the scales, even at relatively high infestations, such as may occur with Chardonnay and Riesling. Alternatively, some type of resistance mechanism may be present, either a constitutive resistance such as cell wall composition as suggested previously, or an inducible defence



that is only present when the scales are feeding. Examples of the inducible defences can be leaf drop when first and second instars are present, such as may have occurred with Sauvignon Blanc in the greenhouse, or a change in nutrient levels that could explain the lower levels of scales on Pinot Noir. Again, the mechanisms that could cause these changes are currently unknown and further examination of potential resistance mechanisms is needed.

## **4.0 Future Research Directions**

Future Research directions relating to study areas feeding behaviour, biology and ecology of grapevine and frosted scales covered by this thesis are highlighted below.

### **4.1 Insect Development and Feeding Location**

To obtain sufficient nutrition for growth and reproduction, grapevine and frosted scales were assumed to feed within vascular bundles. Measurements of the stylet bundle showed that that the stylet length changed with changes in feeding location and instar. Further studies using electrical penetration graph (EPG) and histological studies may improve our understanding on nutrient acquisition and the role of nutrients on life history structure and population.

As the scales develop from first instars to adults, leg length decreases, so that contact with the substrate increases. As previously suggested, force generation for stylet penetration

may be improved as more contact occurs. Two questions that need to be addressed with regards to feeding are: 1. Does feeding location within grapevines vary with life stage? 2. What is the role of stylet bundle and tentorial muscle structures for penetration of plant substrate and does anchorage in scale insects aid in force production?

As second instars of *P. pruinosum* can move and re-establish feeding between leaves and woody stems (and can even move to other locations on woody stems if temperatures are high enough during Winter) (Simbiken pers. obs.), studies using electrical penetration graph (EPG) would permit characterisation of feeding locations within the vines.

How substrate contact and leaf toughness interact to determine location of feeding and potentially contribute to differences in grapevine variety resistance to scale infestation is more difficult, but such work is necessary to determine if these parameters are involved in choice of feeding site. Typically, the stylet sheath and the pressure generated by the insertion of the stylet are thought to anchor the insect, but further work would clarify this aspect of feeding biology in these insects.

How might differences in plant nutrition among grape varieties affect the body size of scales, and could this regulate scale population by influencing their fecundity? Although nutritive differences may exist, either as a response to feeding location or resource availability, differences in scale body sizes could be affected by climate or other environmental variables as well, so a detailed study of microclimates would need to be included to control for that level of difference between grapevines. Previous studies on scale insects showed that mothers might determine the number and size of eggs and

therefore offspring performance depending on the quality of environment (Speight 1994, Spitzer 2004, Tanga et al. 2013). Some suggestion of resistance to population growth occurring by this method was suggested as Sauvignon Blanc did have more of the smaller sized insects at maturity (Chapter 3).

Chapters 5 and 6 showed that considerable variation in grape scale numbers can be found on different grapevine varieties. These differences may be associated with differential response of grapevine varieties to insect feeding. Grapevine varieties may have different phenolic and tannin compounds that can affect their nutrient composition. Comparison of the salivary secretions with honeydew of scales on different grapevine varieties might indicate what materials are different among the varieties that scales are ingesting. Alternatively, direct measurement of proteins and other chemicals from the digestive system could characterise how ingesta differs among varieties.

## **4.2 Management of Grapevine and Frosted Scales**

Temperature and availability of resources are important factors controlling development of insects (Kim et al. 2008, Tanga et al. 2013). Movement of second instars of *P. pruinosum* in the winter months was observed, suggesting that diapause could be facultative, although development to the second instar stage was within a certain time interval (Chapter 5). *P. persicae* under prolonged warm temperatures can develop a partial second generation (Rakimov, 2010). Adult growth and reproduction are restricted to the spring and early summer. Some phenotypic variation in the number of generations is present within the *Parthenolecanium* genus, but the consequences on body size development, reproductive

output and population growth is unknown. Further study on the effect of temperature on the association between numbers of generation and body size is needed to understand the climate effects of population growth, and whether this may be important under climate change scenarios, so that scales may become a larger problem for grapevines.

Application of insecticide is implemented in the off-season, especially in the winter months. Such practise avoids detrimental effects on biological control agents, such as parasitoids. Chemical application during warmer periods in winter when instars are active could be an effective control method. Further work is required to identify the temperatures than can induce movements of second or third instars in winter.

Future studies are required to investigate the effect of mixed cultivation of Pinot Noir, Sauvignon Blanc, Riesling and Chardonnay on the distribution, population growth and pest severity in vineyards. The current study showed increase grape scale population in vineyards planted with suitable varieties. However, mixed cultivation of grapevine varieties tends to reduce pest populations. Alternate management strategy utilising a resistant variety, such as Pinot Noir, as a barrier crop against grapevine and frosted scales may enhance integrated pest management strategy in vineyards.

## **5.0 General Conclusions**

Grapevine and frosted scales can cause significant economic damage to grapevines. Currently sporadic outbreaks can occur due to extensive use of pesticides in the viticulture industry. However, crop losses due to insect feeding activity and population growth can be

localised resulting in vineyard and region specific problems. Factors correlating outbreaks are scarcely known, although availability and abundant host plant and reduction in the effectiveness of natural enemy population have significant influence on pest outbreak. Rigorous studies relating these factors to the pest status of grapevine and frosted scales have not been considered. It is unclear under what basis current control measures are implemented. This thesis reports on a number of advances in our knowledge of grapevine and frosted scales feeding behaviour, biology and ecology that significantly influence insect-plant interactions and pest outbreaks. The continued research in the feeding behaviour, nutrition and ecology of grapevine and frosted scales is essential for the improvement in pest management against these pest insects.

## 6.0 References

Bertin, A., Bortoli, L.C., Botton, M. and Parra, J.R.P. (2013) Host plant effects on the development, survival, and reproduction of *Dysmicoccus brevipes* (Hemiptera: Pseudococcidae) on grapevines. *Annals of the Entomological Society of America* **106**, 604-609.

Barrett LG and Heil M. 2012. Unifying concepts and mechanisms in the specificity of plant–enemy interactions. *Trends in Plant Science* **17**, 282-292.

Clissold FJ. 2007. The biomechanics of chewing and plant fracture: Mechanisms and implications. In: *Advances in Insect Physiology*. (eds J Casas and CJ Simpson). pp. 317 -372. Academic Press.

Foldi I. 1997. Internal anatomy of the adult female. In: *World Crop Pests. Soft Scale Insects: Their Biology, Natural Enemies and control. Vol 7A*. (eds Y Ben-Dov and CJ Hodgson) pp. 73-90. Elsevier Science, Amsterdam, Netherlands.

Guerrieri E and Digilio MC. 2008. Aphid-plant interactions: a review. *Journal of Plant Interactions* **3**, 223-232.

Hoffmann AA, Tracy Reynolds K, Nash MA and Weeks AR. 2008. A high incidence of parthenogenesis in agricultural pests. *Proceedings of the Royal Society Series B: Biological Sciences* **275**, 2473-2481.

- Hutchinson JMC, Mcnamara JM, Houston AI and Vollerath F. 1997. Dyar's rule and the investment principle: optimal moulting strategies if feeding rate is size-dependent and growth is discontinuous. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **352**, 113-138.
- Kim SC, Song JH and Kim DS. 2008. Effect of temperature on the development and fecundity of the Cryptic Mealybug, *Pseudococcus cryptus*, in the laboratory. *Journal of Asia-Pacific Entomology* **11**, 149-153.
- Kivelä SM, Välimäki P and Gotthard K. 2013. Seasonality maintains alternate life-history phenotypes. *Evolution* **67**, 3145-3160.
- Pöykkö H and Hyvärinen M. 2012. To grow fast or to grow big? Time-limited larvae of *Eilema depressum* speed up their growth and reduce number of instars. *Entomologia Experimentalis et Applicata* **142**, 145-152.
- Rakimov A. 2010. Aspects of the biology, ecology and biological control of soft scale insects (Coccidae) in Australian vineyards. PhD Thesis, University of Melbourne, Dept. of Zoology. Melbourne, Australia
- Ross L, Hardy NB, Okusu A and Normark BB. 2013. Large population size predicts the distribution of asexuality in scale insects. *Evolution* **67**, 196-206.

- Santiago R, Barros-Rios J and Malvar RA. 2013. Impact of cell wall composition on maize resistance to pests and diseases. *International Journal of Molecular Sciences* **14**, 6960-6980.
- Silvertown J, Mcconway KJ, Hughes Z, Biss P, Macnair M and Lutman. 2002. Ecological and genetic correlates of long-term population trends in the park grass experiment. *The American Naturalist* **160**, 409-420.
- Speight MR. 1994. Reproductive capacity of the horse chestnut scale insect, *Pulvinaria regalis* Canard (Hom., Coccidae). *Journal of Applied Entomology* **118**, 59-67.
- Spitzer BW. 2004. Maternal effects in the soft scale insect *Saisetia coffeae* (Hemiptera: Coccidae). *Evolution* **58**, 2452-2461.
- Tanga CM, Ekesi S, Govender P and Mohamed SA. 2013. Effect of six host plant species on the life history and population growth parameters of *Rastrococcus iceryoides* (Hemiptera: Pseudococcidae). *Florida Entomology* **96**, 1030-1041.