The impact of silicon fertilisation on the chemical ecology of grapevine, *Vitis vinifera*; constitutive and induced chemical defences against arthropod pests and their natural enemies

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Certificate of Authorship

"I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it does not incorporate any material previously published or submitted for a degree or diploma in any university, except where due acknowledgement is made in the thesis. Any contribution made to the research by colleagues with whom I have worked at Charles Sturt University or elsewhere during my candidature is fully acknowledged.

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Editorial Note

This thesis has been formatted in accordance to Charles Sturt University's Academic Manual (Part H1, Section 4, "*The rule for the presentation of print thesis, other examinable print works and the written component of examinable non-print work*").

The referencing style used in this thesis is APA (6th), as recommended by the Faculty of Science, School of Agriculture and Wine Sciences. Please note the specific and sometimes confusing requirement of this referencing style (http://www.csu.edu.au/division/studserv/my-studies/learning/pdfs/apa.pdf).

Publications associated with this thesis

Simpson, M., Connick, V.J., Guisard, Y., Reynolds, O.L., Saliba, A., and Gurr, G.M. (In Press). Chapter 6: Chemical ecology providing novel strategies against vineyard pests in Australia. In Bostanian, N., Vincent, C., and Issacs, R. (Eds) *Arthropod Management in Vineyards.* Springer Verlag, Germany.

A copy of this book chapter is attached in the Appendix. The book chapter provides a concise review of current research on novel integrated pest management (IPM) strategies within a vineyard context. The strategies reviewed included the use of synthetic herbivore-induced plant volatiles (HIPVs) and habitat manipulation to promote beneficial arthropod populations, and effects of silicon fertilisation of grapevine defences. My contribution to the book chapter includes Section 6.6; *Silicon's role in plant defences against pests*, Section 6.7.2; *Use of silicon to enhance plant defence mechanisms*, and a collaboration with Marja Simpson in Section 6.3; *Vineyard pests occurring in Australia and their natural enemies*, and Table 6.1; *Major grapevine pests, crop damage caused and their natural enemies in Australia*.

Abbreviations used in this thesis

Al	aluminium
ASM	acibenzolar-S-methyl
C	carbon
Ca	calcium
Cd	cadmium
DMAPP	dimethylallyl diphosphate
Fe	iron
GC-MS	gas chromatography-mass spectrometry
GLV	green leaf volatiles
HIPV	herbivore-induced plant volatile
ICP-AES	inductively coupled plasma-atomic emission spectrometer
IPM	integrated pest management
IPP	isopentenyl diphosphate
JA	jasmonic acid
K	potassium
L.	Linnaeus
MEP	methylrythritol pathway
Mg	magnesium
MVA	mevalonate pathway
N	nitrogen
P	phosphorous
RH	relative humidity
S	sulphur
SA	salicylic acid
SAR	systemic acquired resistance
SARDI	South Australian Research & Development Institute
Si	silicon
Si+	silicon treated
Si-	silicon untreated (control)
SPME	solid phase micro extraction
VOC	volatile organic compound

Abstract

There is a large body of literature reporting the benefits of silicon (Si) fertilisation to agricultural crops, however, much of this research has focused on high Si-accumulating crops such as sugarcane, *Saccharum officinarum* (L.), rice, *Oryza* spp., and wheat, *Triticum aestivum* (L.), and medium Si-accumulators such as cucumber, *Cucumis sativus* (L.). This research project has investigated the role of Si in a low Si-accumulating crop, grapevine, *Vitis vinifera* (L.). Silicon fertilisation is known to improve the physical strength of plants through the deposition of amorphous silicate in the epidermal cells of the stems, leaves and roots of the plant. Numerous studies have shown that the improved mechanical strengthening of the plant as a result of Si fertilisation directly improves plant constitutive defences against arthropod pests, including folivores, borers, phloem and xylem feeders. Studies have also shown that Si fertilisation directly reduces pest damage by enhancing the induced chemical defences of plants following insect herbivore attack. Defensive enzymes, released upon arthropod pest attack, are believed to contain deterrent, toxic and anti-nutritional properties. Silicon fertilisation may also indirectly affect plant pests via induced chemical defences by altering and enhancing the production of herbivore-induced plant volatiles (HIPVs). HIPVs are released by the plant upon herbivore damage or oviposition, and can result in increased

attractiveness of the plant to the pest's natural enemies; predatory and parasitic (beneficial) arthropods.

There has been very little research on the effects of Si fertilisation on grapevines, and only two studies have been conducted investigating the tri-trophic effects of Si fertilisation on beneficial insects. Grapevines are an important agricultural crop in Australia and there are considerable benefits to be gained by improving the plant's own constitutive and induced defences against arthropod pests through Si fertilisation. This study investigated the effects of Si-treatment of grapevines on important native Australian insects; two insect pest species, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) and *Phalaenoides glycinae* (Lewin) (Lepidoptera: Noctuidae), and two beneficial insect species, *Dicranolaius bellulus* (Guérin-Méneville) (Coleoptera: Melyridae) and *Mallada signata* (Schneider) (Neuroptera: Chrysopidae). Laboratory-based experiments included studies of the effects of Si fertilisation of grapevines on 1) the feeding damage and larval growth of *E. postvittana*, 2) the tri-trophic effects of a) *E. postvittana*-infested grapevines on *D. bellulus*, and b) *P. glycinae*-infested grapevines on *M. signata,* and 3) plant volatile production. This research project also investigated the response of *M. signata* to synthetic grapevine HIPVs.

The first experiment observed the effects of Si fertilisation of grapevines on feeding damage and larval weight of the pest, light brown apple moth, *E.*

postvittana. The area of excised Si+ (silicon treated) and Si- (control) grapevine leaves consumed by *E. postvittana* third-fourth instar larvae during a 24 hour period was measured using Delta-T Scan® image analysis software. Larvae were weighed immediately prior to and on completion of the trial to ascertain weight gain/loss. *Epiphyas postvittana* consumed significantly greater quantities of leaf material from Si+ plants compared to Si-, and larvae fed on Si+ leaves lost significantly less weight than larvae fed on Si- leaves. The area of grapevine leaf consumed and *E. postvittana* larval weight were positively correlated with the Si content of the leaves. This finding is consistent with reduced digestion efficiency, caused by enhanced levels of defensive compounds produced in the plant, leading to increased consumption; an effect that was promoted by the exogenous application of Si.

The second experiment observed the effect of Si fertilisation of grapevines on induced plant defences, investigating the attraction the predatory beetle, *D. bellulus* to grapevine plants infested with *E. postvittana* larvae. The response of *D. bellulus* to Si+ and Si- grapevines infested with *E. postvittana* third-fourth instar larvae was measured using a Y-tube olfactometer. Analysis of grapevine leaf tissue samples found the mean total Si content of Si+ plants (0.315% Si, range = 0.188-0.559% Si) was greater than for Si- plants (0.177% Si, range = 0.065-0.253% Si). *Dicranolaius bellulus* exhibited a significant positive response to pest-infested grapevines

with the highest leaf-tissue Si-content, suggesting that the Si status of grapevines affected HIPV production, increasing the attractiveness of that plant to the arthropod predator.

The third experiment also observed the effect of Si fertilisation of grapevines on induced plant defences. This study investigated the direct effect of Si fertilisation using solid-phase micro-extraction (SPME)/gas chromatography-mass spectrometry (GC-MS) analysis to identify and quantify the volatiles emitted by Si+ and Si- grapevines infested with the Australian grapevine moth, *P. glycinae*. The indirect, tri-trophic effects of Si fertilisation and *P. glycinae*-infestation was determined using a Y-tube olfactometer bioassay to observe the response of the predatory green lacewing, *M. signata* to the pest-infested plants. Analysis of grapevine leaf tissue samples found the mean total Si content of Si+ plants (0.217% Si, range = 0.20-0.23% Si) was greater than for Si- plants (0.132% Si, range = 0.13-0.14% Si). GC-MS analysis identified seven volatile compounds emitted from *P. glycinae*-infested grapevines. Importantly, of these seven compounds, six have not previously been reported being emitted from grapevines. Within this volatile blend, *n*-heptadecane was produced only by Si+ plants, and *cis*-thio rose oxide was produced in greater quantities by Si- plants compared to Si+ plants. Despite the effect of Si on grapevine volatiles, with the production of one compound apparently suppressed and

that of another triggered, Y-tube olfactometer bioassays found *M. signata* was attracted to pest-infested grapevines from both Si+ and Si- treatments.

The fourth experiment observed the response of *M. signata* to synthetic HIPVs. The use of synthetic HIPVs to attract natural enemies to crops, including grapevines, is receiving increasing attention as a novel pest management strategy. Making this approach viable demands a knowledge of which compounds effectively attract which species. The green lacewing, *Mallada signata* is a generalist predator which feeds on a range of insect pest species and is of particular importance because it is the only green lacewing species cultured commercially for inundative biological control in Australia. No information is available on the response of *M. signata* to HIPVs. The present study tested the response of *M. signata* to volatile compounds, reported in the literature as being emitted by grapevines following arthropod feeding. Y-tube olfactometer bioassays were conducted using *β*-caryophyllene (terpenoid), (*Z*)-3-hexenyl acetate (green leaf volatile), and methyl salicylate (aromatic compound) at the rates of 0.2, 2, 20 and 200 μg/arena using a hexane control. Significantly greater numbers of *M. signata* chose *β*-caryophyllene at 0.2 and 20 μg, and methyl salicylate at 0.2 and 200 μg compared to the control, however, no attraction was found to (*Z*)-3-hexenyl acetate at any of the rates tested. Knowledge of Chrysopidae responses to the tested HIPVs under laboratory conditions is

limited, and this paper provides a crucial early-step in our understanding of the attractiveness of individual HIPVs to a key predator, *M. signata*.

Overall, this thesis demonstrates that Si had little effect on constitutive defences in grapevines. As grapevines are low Si-accumulators, they restrict Si entering plant xylem from the roots, resulting in a lower level of accumulation of polymerised Si in the epidermal tissue; a defence mechanism found in high and intermediate Si-accumulators which makes plant tissue more abrasive and difficult to penetrate and chew by arthropod pests. Significantly, however, this thesis shows that Si fertilisation affected induced chemical defences in grapevines. Induced defences are triggered by silicic acid in the transpiration stream of the plant, not polymerised Si, and this study indicated that even the low level of Si uptake in grapevines was sufficient to elicit an induced chemical response to pest attack.

Chemical ecology plays an important role in induced plant defence mechanisms activated by herbivore feeding or oviposition. HIPVs produced by plants' can be employed in biological control to recruit arthropod predators and parasitoids (beneficial arthropods) of pests. Treating soils with Si compounds has many known benefits for a range of plant species, but this thesis presents evidence that Si application to plant roots can also alter the HIPV profile and increase the attraction of beneficial arthropods into crops. The use of novel pest management compounds such as Si fertilisation or synthetic HIPVs is an emerging branch of ecology, yet there

is still much to be discovered about the complex nature of plant−pest−predator interactions. This thesis provides a concise series of studies examining how Si-fertilisation affects the chemical ecology of grapevines, testing for effects on both the constitutive and induced chemical defences, and considers the utility of these novel treatments to major vineyard pest problems. The emphasis in this study is on vineyard protection in Australia, as wine and table grapes are a substantial and valuable industry, but many of the outcomes apply generally to other agricultural crops.

Chapter 1

Literature Review

1.1 Introduction

The main aim of this thesis was to test how the application of silicon (Si) to soil growing grapevines, *Vitis vinifera* (Linnaeus) alters the chemical ecology of the plant–pest–predator interactions. This study also explores the scope for development of a novel pest management strategy based on enhanced biological insect pest control in vineyards.

Ecology is defined as how an environment and the organisms within it relate to one another, and a sound understanding of the ecology within vineyards is essential in order to effectively manage grapevine pests and disease. Chemical ecology is, therefore, viewing ecological interactions from a chemical perspective (Jones, 1988). Dicke and Takken (2005) describe chemical ecology as the ecology of body odour; plants and arthropods (and other animals) emit chemicals to interact with the organisms around them. Plant emissions include chemical cues which attract beneficial arthropods to the plant during times of pest-infestation (Dicke & Takken, 2005). Integrated pest management (IPM) is a pest-control strategy employed by many agricultural industries, and it uses a variety of complementary and environmentally-sensitive pest-control practices (Eilenberg, Hajek, & Lomer, 2001; Gurr, Scarratt, Wratten, Berndt, & Irvin, 2004). These practices include biological pest control, which relies on ecological interactions within the cropping environment by using predation, parasitism, and other mechanisms (i.e., entomopathogenic bacteria, sterile arthropods

technique, and host plant resistance) to suppress pest populations (Eilenberg, et al., 2001; Gurr, et al., 2004). Another IPM strategy is to ensure plants have adequate nutrition to maintain vigorous and healthy growth and enhance their own resistance to arthropod pests (Altieri & Nicholls, 2003; Zehnder, Gurr, Kühner, Wade, Wratten, & Wyss, 2007). Silicon is not listed as an essential nutrient for plants, however, there is increasing evidence for its requirement for healthy growth and for enhancing the plants defence systems against arthropod pests (Epstein, 1994).

The role of Si in plant biology is still not fully elucidated and it currently does not meet the definition of essentiality as determined by Arnon and Stout (1939) (Epstein, 1994; Liang et al., 2006). However, many studies suggest it performs an important, if not essential, role for a variety of plant species (Hammerschmidt, 2005; Kvedaras, Keeping, Goebel, & Byrne, 2007a; Ma & Yamaji, 2006; Ranganathan et al., 2006). The requirement for, and subsequent uptake of, Si within cropping plants varies among plant species. High and intermediate Si-accumulating crops have a greater requirement for Si compared to low Si-accumulating crops (Takahashi & Miyake, 1977; Woolley, 1957), which include grapevines. Ma and Yamaji (2008) claim a high accumulation of Si in the plant shoot is required for overcoming biotic and abiotic stresses. Past reviews on the chemical ecology of Si in soil and plants have focused on high and intermediate Si-

accumulator plant species, and there is a gap in our knowledge of the effects of Si fertilisation on low Si-accumulating species. This is surprising considering the potential benefits of Si fertilisation to vineyards, and it highlights the importance of further research in this area.

The Australian wine and grape industry is very important to the Australian economy, with 151,789 hectares of land in Australia under grapevines (Australian Bureau of Statistics, 2010). Domestic sales of Australian wine in 2009/2010 were valued at $2,122 million, and export sales valued at $2,168 million (Australian Bureau of Statistics, 2010). Within vineyards, some pest species are difficult to control using conventional insecticides. For example, leafroller larvae, including larvae of the light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), often feed and pupate within the protection of rolled-up leaves. This makes it difficult for insecticide sprays to reach the pest. As a result, many vineyards are employing integrated pest management techniques, including the mass-release of beneficial insects which can more readily locate and control difficult to reach pests.

Several recent studies have investigated the use of synthetic herbivore-induced plant volatile (HIPV) baits (or lures) in vineyards as a strategy to attract beneficial insects and achieve higher levels of biological pest control (James, 2003b, 2005; James, 2006; James & Price, 2004; Khan, James, Midega, & Pickett, 2008; Simpson, Gurr, Simmons, Wratten, James,

Leeson, & Nicol, 2011; Simpson, Gurr, Simmons, Wratten, James, Leeson, Nicol, et al., 2011). The application of an exogenous substance (HIPV or Si) to act as a precursor for constitutive and induced defences against arthropods pests is a novel biocontrol strategy. Work on these novel pest management strategies are at an early stage generally, and especially in grapevines. However, making this approach viable demands a knowledge of which plant volatile compounds effectively attract which insect species (both pest and beneficial), and further, how Si fertilisation affects the emission of these volatile compounds. A first step in developing this biological knowledge into a cost effective pest management strategy is to determine whether some of the more readily available HIPV compounds are attractive to important predatory and parasitoid arthropods found in Australia. To date there are only two studies which report grapevine leaf Si content in relation to Si concentration in the soil solution (Blaich & Grundhöfer, 1997; Lafos, 1995). Grapevines accumulate low levels of Si in their leaf tissue and it is unknown if this level of Si is sufficient to promote enhanced plant defences in response to arthropod pest attack. There are no reported studies observing the effects of Si fertilisation of grapevines to arthropod pests or their natural enemies.

This chapter provides a comprehensive review on the involvement of Si in constitutive and induced plant defences against arthropod pests. This thesis then goes on to describe four laboratory-based experiments conducted to

identify the effects of Si fertilisation on grapevine constitutive and induced defences (Chapters 2 to 5). The four experiments investigate direct effects of Si fertilisation on a common arthropod pest, and indirect tri-trophic effects of Si fertilisation and grapevine HIPVs on two species of insect natural enemies native to Australia. The concluding chapter of the thesis (Chapter 6) discusses the importance of the findings from this research study, and its potential use within a vineyard context.

1.2 Chemical ecology of silicon–plant interactions

1.2.1 Silicon and soil

Silicon is the second-most abundant element in the earth's crust (Epstein, 1994; Ma & Yamaji, 2006; Raven, 1983) and is mostly inert and only slightly soluble (Savant, Korndörfer, Datnoff, & Snyder, 1999). In comparison to other elements such as nitrogen (N) and carbon (C), Si does not have a true cycle in the environment and behaves in a similar fashion to phosphorus (P); the only biological involvement in Si mobilization–immobilization is the solubilisation of insoluble Si, the release of the element from organic-Si compounds, and the immobilization of Si by bacteria and fungi (Wainwright & Harbi, 2005). A reaction involving soil carbon dioxide (CO_2) dissolves ortho-silicic acid (H_4SiO_4) from the crystalline structure of silicate minerals (Struyf, Smis, Van Damme, Meire, & Conley, 2009).

The soluble, plant-available form of Si is monosilicic acid ($Si(OH)_4$) (Raven, 1983), which is mostly contained in the soil solution as at a pH < 9 (Beckwith & Reeve, 1963, 1964; Jones & Handreck, 1963, 1967; McKeague & Cline, 1963a, 1963b). The concentration of monosilicic acid in the soil solution is dynamic and typically ranges from 0.1-0.6 mM (Richmond & Sussman, 2003). Silicon concentration in the soil solution is largely controlled by an adsorption reaction with soil minerals (especially sesquioxides) which is dependent on the soil pH (Beckwith & Reeve, 1963, 1964; Jones & Handreck, 1967; McKeague & Cline, 1963a, 1963b). In soils with pH >9, monosilicic acid levels in the soil solution increase dramatically as a result of Si monomers transforming to polymers (Neal et al., 2005). The equilibrium of monosilicic acid in the soil solution is also controlled by amorphous Si deposited at the surfaces of soil particles (Brown & Mahler, 1987; Lopes, 1977), and increases with rising soil temperatures (Blaich & Grundhöfer, 1997; Liang, Si, & Römheld, 2005a; Raven, 1983) and water-content of the soil (Blaich & Grundhöfer, 1997; Jones & Handreck, 1967). Monosilicic acid solubility in water is 2 mM at 25^{o}C at pH < 7.5 and polymerisation into silica gel ($SiO_2.H_2O$) occurs when the concentration of monosilicic acid in the soil solution exceeds 2 mM (Jones & Handreck, 1967; Ma & Takahashi, 1991; Raven, 1983). Aluminum (Al) and iron (Fe) oxides (Al_2O_3 and FeO, Fe_2O_3, and Fe_3O_4 respectively) dominate Si adsorption, where a hydrogen atom bonds to an oxygen (O) atom, bridging two Fe (or Al) atoms (Equation 1.1) (Jones & Handreck, 1967).

Equation 1.1

$$2(FeO(OH)) + (OH_3)\text{-}Si\text{-}O\text{-}H \longrightarrow O(Fe_2O_4H_4) + Si + H_2O$$

Therefore, soils with the same pH and clay content, but with varying levels of 'free' sesquioxides, contain varying levels of plant-available monosilicic acid; the soils with the highest levels of 'free' sesquioxides contain the least plant-available monosilicic acid.

1.2.2 Silicon and water

Silicon concentration in rain water is considered insignificant at < 0.2 mg dm^{-3}, whereas monosilicic acid concentration in natural ground water can contain substantial concentrations of Si; some deep ground waters have reportedly contained 23-28 mg dm^{-3} Si (Dapples, 1959). Silicon may occur in ground water in many forms, including ionic and molecular, aggregate (colloid, solid and/or gel), adsorbed onto sesquioxides, organic complexes (humites), metal complexes, and in living organisms, plankton, and detritus (Mitchell, 1975; Tan, 1994). Similar to soils, the overall composition of Si in ground water is influenced by pH, temperature, degree of super-saturation, and the presence of other substances (Savant, et al., 1999).

1.2.3 Silicon and plants

Silicon fertilisation of plants has been shown to mechanically strengthen plant tissue, resulting in improved plant health and fitness in a variety of

ways. These improvements include enhanced erectness of leaves in rice (Mitsui & Takatoh, 1963; Yoshida, Ohnishi, & Kitagishi, 1959) and cucumber (Adatia & Besford, 1986), increased plant mass for shoots and roots in cucumber (Adatia & Besford, 1986), and increased plant height, stem diameter, millable stalks, and yield in sugarcane (Elawad, Street, & Gascho, 1982; Gascho, 1978; Plunknett, 1971). Other benefits of Si fertilisation include reduced lodging in rice (Ma, 2004), increased leaf texture or roughness, enhanced chlorophyll content of leaves, delayed senescence, increased yield in cucumber (Miyake & Takahashi, 1983), higher seed retention rates in grasses, *Phalaris tuberosa* (L.) (McWilliam, 1963) and *Bromus secalinus* (L.) (Gali & Smith, 1992), and the promotion of root nodule formation and function in cowpea, *Vigna unguiculata* (L. Walpers) (Nelwamondo & Dakora, 1999).

Silicon fertilisation can reduce the effects of abiotic and biotic stresses of plants. Examples of abiotic stressors (including chemical and physical stress) reduced by Si application, include enhanced stomatal control resulting in decreased transpiration rate in rice (Ma, 2004; Okuda & Takahashi, 1964) and improved drought tolerance in wheat (Gong, Chen, Chen, Wang, & Zhang, 2003) and corn (or maize), *Zea mays* (L. cv. Nongda 108) (Gao, Zou, Wang, & Zhang, 2004). Silicon fertilisation also reduced the effect of salt stress to grapevines (cv. Cabernet Sauvignon) (Lei, Zhang, Bai, & Han, 2008), improved the tolerance of cucumber (Rogalla &

Römheld, 2002; Q. Shi et al., 2005) and rice (Horiguchi, 1988) to soils containing high levels of manganese (Mn), and alleviated cadmium (Cd) toxicity in rice (X. Shi, Zhang, Wang, & Zhang, 2005) and peanuts, *Arachis hypogaea* (L.) (Shi, Cai, Liu, & Wu, 2010). A range of biotic stresses may also be reduced by Si fertilisation, including plant attack by both arthropod pests and disease, as discussed in detail later.

Silicon is present in green plants at concentrations corresponding in amounts per unit of dry plant matter to the macronutrient elements; around 0.1% Si found in low Si-accumulating plant species corresponds to amounts of P, sulphur (S), calcium (Ca), and magnesium (Mg), and around 10% Si found in high Si-accumulating plant species corresponds to amounts of potassium (K) and N (Epstein, 1994). The content of Si in plant tissue varies depending on the plant species, with dicotyledons generally having lower concentrations ($\leq$1% dry weight) compared to grasses (1-5% dry weight) and wetland grasses (10-15% dry weight) (Epstein, 1994; Jones & Handreck, 1967; Matichenkov & Bocharnikova, 2007). High Si-accumulators are defined as plants which contain 1-10% Si dry weight and show a Si–Ca molecular ratio > 1. Plants which contain 0.5-1% Si (or > 1% Si but Si–Ca molecular ratio < 1) are defined as intermediate Si-accumulators, and plants which contain < 0.5% Si are termed low Si-accumulators (Ma, 2007; Ma & Takahashi, 1991). High Si-accumulators generally contain 8-20 times as much Si in their leaves as low Si-

accumulators (Adatia & Besford, 1986; Takahashi & Miyake, 1977). In higher plants, high Si-accumulators are found in Gramineae and Cyperacea, intermediate Si-accumulators are found in Cucurbitales, Urticales, Commelinaceae and Balsaminaceae, and low Si-accumulators are found in most of the other plant species, including most Dicotyledoneae (Ma & Takahashi, 2002; Mitani & Ma, 2005).

Uncharged monosilicic acid is taken up by plant roots in the transpiration stream, and its concentration in the plant is directly proportional to the concentration of monosilicic acid in the soil solution (Jones & Handreck, 1967; Liang et al., 2005a; Mitani & Ma, 2005). Silicon within the soil solution enters the root at the symplast, at or near the epidermis, and leaves it at the inner side of the endodermis (Jones & Handreck, 1967; Richmond & Sussman, 2003). Silicon contained in the transpiration stream travels via the xylem and is deposited in the intracellular spaces, inside the cells, and in the cell wall of all plant tissue (Kaufman et al., 1981). Polymerised Si is deposited as amorphous phytoliths (or opal) ($SiO_2 \cdot nH_2O$), which is immobile and unable to further translocate within the plant (Epstein, 1994). Phytoliths are Si biominerals, formed by plant proteins, and they increase the mechanical resistance of the plant and enhance plant resistance against biotic and abiotic stresses (da Costa, Guedes, Morris, West, & de Oliveira, 2005). Silicic acid mobility in the phloem is very restricted as it must remain in solution in order to move around the plant (Raven, 1983).

The distribution of Si within the shoots and leaves of the plant is determined by the transpiration rate and the species of plant (Jones & Handreck, 1967). There are three modes of Si uptake by plants; active, passive and rejective. Active Si uptake occurs in high Si-accumulating plants whereby Si is taken up at a faster rate than that of water. Passive uptake occurs in intermediate Si-accumulators and the rate of Si uptake is similar to the uptake rate of water. Rejective uptake occurs in low Si-accumulators and plants exclude Si, resulting in a greater rate of water uptake than Si (Mitani & Ma, 2005). Articles published in Nature state the mechanism by which rice plant roots take up Si is attributed to two Si-transporters, Lsi1 and Lsi2 (Ma et al., 2006; Ma et al., 2007). Lsi1 is an influx transporter of silicic acid, located on the distal side of the root exodermis and endodermis, and Lsi2 is an active efflux transporter of silicic acid, located on the proximal side of the root exodermis and endodermis (Ma, et al., 2006; Ma, et al., 2007). The active uptake of Si from the soil solution by the Si-transporters is driven by a concentration gradient between the endodermis and exodermis, and the cortex and soil solution (Yamaji, Mitani, & Ma, 2008). The transporter, Lsi6, is responsible for the transport of Si out of the xylem and affects the distribution of Si within the shoots of rice (Yamaji, et al., 2008).

Recently, a study by Mitani et al. (2011) identified the molecular mechanism of Si uptake in two cultivars of pumpkin, *Cucurbita moschata* (Duchesne); one intermediate (cv. Shintosa) and one low (cv. Super-unryu)

Si-accumulator. That study found Si accumulation in pumpkin is limited by the capacity of the root influx transporter to take up Si from soil solution. Mitani et al. (2011) found the variation in Si uptake by the two pumpkin cultivars was dependent upon allelic variation in the Si influx transporter, and uptake was diminished by the metabolic inhibitor, mercury(II) chloride ($HgCl_2$). Earlier, a similar study by Liang et al. (2005a) suggested that an energy-dependent active mechanism is involved in Si uptake in cucumber, similar to the Si-transporters in rice. Liang et al. (2005a) reported that although the literature states that cucumber takes up Si passively and it is described as an intermediate Si-accumulator, the cultivar Chinesische Schlange was found to take up Si in quantities significantly higher than that calculated from the transpiration rate. In contrast, the same study reported that a low Si-accumulator, faba beans, *Vicia faba* (L. cv. Divine) predictably measured lower Si uptake than that calculated from the transpiration rate. The influence of a metabolic inhibitor, 2,4-dinitrophenol, and cold temperature on uptake of Si by cucumber and faba beans was measured by Liang et al. (2005a). They found that Si uptake by cucumber was strongly inhibited by both 2,4-dinitrophenol and cold temperatures, however, Si uptake by faba beans was not, suggesting there was no mechanism for active Si-uptake present in faba beans (Liang et al., 2005a). It is likely that in low Si-accumulators Si uptake is restricted by the ability of, or lack of, the influx and/or efflux transporters to move Si from the external soil solution through the roots and into plant xylem. Jones and Handreck (1967) found crimson

clover, *Trifolium incarnatum* (L.) growing in soils containing 7-67 mg/kg silicic acid in solution, contained only 5-10% of the Si concentration found in oats, *Avena sativa* (L.) growing in soils containing the same Si levels. That study found the concentration of total Si in the roots of clover plants was about eight times that in the corresponding foliage. This indicates Si content in the leaves of clover was restricted by an efflux transporter, preventing the movement of silicic acid from within the roots to the xylem.

Takahashi and Miyake (1977) determined the uptake of Si in 175 plant species, comparing uptake between high Si-accumulators and low Si-accumulators. That study found that low Si-accumulators (specifically tomato) also had a requirement for Si, displaying symptoms of Si-deficiency after the first bud of flowering stage, including reduced growth, deformed and brittle leaves, chlorosis in upper leaves, necrotic spots, failure to pollinate, malformed fruits or no fruits, degeneration of stamens, abnormal shape of pollen grains, and depressed root growth. However, overall, the Si-requirement in low Si-accumulating

plants appears to be a lot lower than that for high Si-accumulators. Takahashi and Miyake (1977) reported the application of 20 ppm Si sufficiently prevented Si-deficiency in tomato, and Woolley (1957) reported that 'control' tomato plants inadvertently receiving 0.5 ppm Si had the same yields as Si treated plants. In comparison, the high Si-accumulating crop,

sugarcane requires around 2.1% Si (dry leaf matter) for optimal cane yield (Anderson, 1991).

1.2.4 Silicon uptake by grapevines

Grapevines appear typical of other dicotyledons and low Si-accumulators, with the uptake of Si a rejective process. Lafos (1995) found lower concentrations of Si in grapevine leaf tissue than the soil solution. That author also observed that the concentration of silica in the roots was correlated with, but considerably higher than, Si content in the soil solution. This indicated that grapevines restrict silicic acid leaving the roots, resulting in concentrated Si in the root-symplasts around the xylem from where it diffuses into the transpiration stream according to the speed of the water flow (Lafos, 1995).

In a field trial, Blaich & Grundhöfer (1997) applied potassium silicate (K_2SiO_3) to the soil surrounding grapevines at 10 and 112 mg/kg SiO_2. In addition, supplementary experiments were conducted using 200 and 400 mg/kg SiO_2, forming an oversaturated solution. The results of that study indicated that 1) silicon solubility is dependent on soil temperature – solubility at 5^{o}C was 50% less than solubility at 20^{o}C; 2) silicon solubility in soil is reflected in Si content in grapevine leaves; 3) silicon content in different grapevine cultivars did not vary significantly; 4) uptake of Si by grapevine depends on transpiration rate of plant and decreased in dry

conditions; 5) older leaves of plants grown on 112 ppm SiO_2 contained higher Si content (2% SiO_2) than younger leaves (0.5% SiO_2) and greater Si content in leaf periphery; 6) silicon content in shoots and petioles was less than 10% Si content in leaves; 7) soluble Si in grapevine leaves grown on 112 ppm SiO_2 made up 30% total Si content in young leaves, 15% in medium age leaves, and 7% in older leaves; and 8) silicon content in plants could be further enhanced when applied to the soil at 200 and 400 ppm SiO_2.

1.3 Role of silicon in plant defences

Plants are sessile organisms, unable to move away from attackers, and have therefore evolved to use other means to protect themselves, both physically and chemically, from attack (Dicke, 2009). There is considerable evidence that Si plays an important role in enhancing plant resistance against a range of arthropod herbivores and pathogens. Plant defences are either constitutive, meaning they are always expressed, or induced, meaning they are expressed following biotic or abiotic stress (Arimura, Kost, & Boland, 2005). Fauteux et al. (2005) and Fawe et al. (2001) have comprehensively reviewed the role of Si in plant disease resistance, outlining its effects on both physical and chemical defences. The present literature review will be primarily focussing on the effects of Si fertilisation on plant resistance to arthropod pests.

1.3.1 Role of silicon in constitutive plant defences

Silicon provides a mechanical barrier to plants, directly protecting them against arthropod and fungal attack. Silicon increases the compression-resistance and strength of the plant in the cell wall by replacing water in the space between the microfibrils and carbohydrate components in the cell wall (Raven, 1983). Silicon fertilisation of plants results in higher content of silica deposits in stem and leaf tissue, increasing leaf abrasiveness and enhancing plant constitutive defences against both small and large herbivores (Hummel et al., 2011; Keeping, Kvedaras, & Bruton, 2009; Keeping & Meyer, 2003; Kvedaras, Keeping, Goebel, & Byrne, 2007b; Massey, Ennos, & Hartley, 2006, 2007) and pathogens (Adatia & Besford, 1986; Bowen, Menzies, & Ehret, 1992b; Chérif, Asselin, & Bélanger, 1994; Chérif, Menzies, Benhamou, & Bélanger, 1992; Dann & Muir, 2002; Fauteux, et al., 2005; Liang, Sun, Si, & Römheld, 2005b; Menzies et al., 1991; Reynolds, Veto, Sholberg, Wardle, & Haag, 1996). Silicon fertilisation significantly reduces arthropod pest longevity, population growth rate, reproductive success and pest numbers (Costa & Moraes, 2006; Hou & Han, 2010). The increased physical strength of plant tissue indirectly enhances plant defences as arthropods pests take longer to penetrate the plant and to chew and digest food, resulting in the arthropods being exposed on the plant for longer periods, increasing their susceptibility to weather conditions, predators, and chemical sprays (Correa, Moraes, Auad, & Carvalho, 2005; Kvedaras & Keeping, 2007). For example,

Korndörfer et al. (2011) found Si-treated sugarcane increased nymphal mortality, increased duration of nymphal stage, and decreased longevity of male and female spittlebug, *Mahanarva fimbiolata* (Stål) (Hemiptera: Cercopidae).

1.3.2 Role of silicon in induced plant defences

Plant induced defence mechanisms are generated upon attack by herbivore feeding, arthropod oviposition or disease. Silicon directly enhances plant resistance to such stressors by acting as a signal for induced chemical defences in plants (Gomes, de Moraes, dos Santos, & Goussain, 2005; Kvedaras & Keeping, 2007; Kvedaras, Keeping, & Meyer, 2009; Ma, 2004; Samuels, Glass, Ehret, & Menzies, 1991b). Induced defences can improve the physical strengthening of plant tissue by increasing silicification of leaf tissue in response to stressors (Massey, et al., 2007; Samuels, et al., 1991b). For example, fungal infection in both high and low Si-accumulators has been found to trigger polymerisation of Si in the walls of infected epidermal cells (Chérif, et al., 1992; Fawe, Menzies, Chérif, & Bélanger, 2001; Samuels, Glass, Ehret, & Menzies, 1991a). Similarly, Kvedaras et al. (2007a) showed that Si fertilised sugar cane plants displayed enhanced resistance against the arthropod pest, *Eldana saccharina* (Walker) (Lepidoptera: Pyralidae) when the plants were water stressed.

Induced plant defences also include defensive enzymes, phenolic acids and flavonols which flock to the site of pathogen infection or arthropod attack, limiting pest damage and killing opportunistic fungi that invade the wound site (Bi & Felton, 1995; Chérif, et al., 1994; Dicke & Vet, 1997; Liang et al., 2005b; Ranger et al., 2009; Walling, 2000). Phenolic compounds contain deterrent, toxic and anti-nutritional properties (Appel, 1993). These compounds are oxidised to secondary metabolites, such as quinones, tannins, gossypol and trypsin protease inhibitors, which possess antibiotic properties and reduce the nutritional quality and protein digestibility of the food source (Barbehenn & Martin, 1994; Broadway & Duffy, 1988; Felton & Duffy, 1990; Felton, Summers, & Mueller, 1994; Jassbi, Gase, Hettenhausen, Schmidt, & Baldwin, 2008). Induced chemical defences also include the emission of volatile compounds that signal to predatory and parasitic arthropods the location of their prey or hosts (Dicke & Vet, 1997).

As early as 1964, Okuda and Takahashi questioned the hypothesis that the role of Si in preventing fungal infection was due to mechanical strengthening of the plant only. A study by Yoshi (1941) found Si-fertilisation reduced blast disease, *Pyricularia oryzae* (Cooke) (synonym: *Magnaporthe grisea* (Herbert)), in rice, however, the mechanism of this enhanced defence could not be correlated to increased leaf toughness. Samuels et al. (1991a) and Menzies et al. (1991) found that Si deposited into the leaves of cucumber previously treated with 100 ppm Si was not

available to enhance fungal disease resistance 24 hours after Si-feeding stopped. This suggested that Si must be in its soluble, mobile form to effectively stimulate host natural defence mechanisms, such as the production of defensive enzymes and phenolic compounds. Similarly, Chérif et al. (1992) found the prophylactic effects of Si on *Pythium*-infected cucumber only continued as long as Si was available to the plant in the soil solution, even though the solid amorphous silica had been irreversibly laid in the epidermal tissue of the plant. As polymerisation of Si results in its inactivation as an inducer of systemic acquired resistance (SAR), a continuous supply of Si is required in the transpiration stream for continued defence responses (Fawe, et al., 2001). Engel (1953) reported that in rye, *Secale cereale* (L.), 60% of the total amount of silica is kept soluble within the plant in the form of a galactose complex, although it remains unclear how plant cells manage to do this (Blaich & Wind, 1989).

The effect of Si fertilisation on plant induced defences is apparent when the plant is affected by abiotic or biotic stress. Upon both pathogen or arthropod attack, the damaged tissue will synthesise chemical defence compounds together with systemic stress signals such as salicylic acid (SA), jasmonic acid (JA) and ethylene (Arimura et al., 2008; Fauteux, et al., 2005). Silicic acid is the primary elicitor for generating both local and systemic signals leading to the synthesis of defence compounds, heightening plant response to attack (Fauteux, et al., 2005; Fawe, et al., 2001; Hammerschmidt, 2005;

Ma, 2004; Samuels, et al., 1991a). For example, Si-fertilised grapevine and cucumber plants exposed to powdery mildew have been found to exhibit enhanced activity of the defensive enzymes, chitinases, peroxidises and polyphenoloxydases, and increased accumulation of phenolic compounds (Blaich & Wind, 1989; Chérif, et al., 1994; Chérif, et al., 1992) and flavonoid phytoalexins (Fawe, et al., 2001) at the site of infection. Similarly, Liang et al. (2005b) found significant enhanced peroxidase, polyphenoloxidase and chitinase activity in cucumber fertilised with potassium metasilicate (K_2SiO_3) and infected with powdery mildew, *Podosphaera xanthii* (Castagne), resulting in significantly lower incidence of disease in these plants. Peroxidase is related to lignin and suberin synthesis, which are components of the structural strength of the plant tissue, as well as the production of quinones and active oxygen (Bowles, 1990; Goodman, Kiraly, & Wood, 1986; Stout, Workman, & Duffy, 1994). Ranger et al. (2009) used high performance liquid chromatography-mass spectrometry to identify and quantify phenolic acids and flavonols in leaf tissue of *Zinnia elgans* (L.) treated with potassium silicate. That study attributed the slightly reduced total cumulative fecundity and the intrinsic rate of increase of the green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) to the significant elevations of the phenolic acid and flavonol derivatives, 5-caffeoylquinic acid, *p*-coumaroylquinic acid, and rutin found in Si-treated plants.

Although an increasing number of studies have looked at the effect of Si fertilisation on two trophic levels, more recently it has been proposed that Si may also enhance induced chemical defences against arthropod attack on three trophic levels (plant–pest–predator). It is thought that Si may act to qualitatively and quantitatively alter the profile of volatile compounds emitted by an attacked plant (Kvedaras, An, Choi, & Gurr, 2010). These volatile compounds are termed herbivore-induced plant volatiles (HIPVs).

1.4 Herbivore-induced plant volatiles (HIPVs)

1.4.1 Plant volatiles and herbivore-induced plant volatiles

Plant volatiles are generally composed of lipophilic liquids with high vapour pressures, easily able to cross membranes and diffuse into the atmosphere (Pichersky, Noel, & Dudareva, 2006). They comprise green leaf volatiles (GLVs), terpenoids (homo-, mono-, di-, sesquiterpenoids), and phenylpropanoid aromatic compounds, as well as certain alkanes, alkenes, alcohols, esters, aldehydes, and ketones (Arimura, et al., 2005; Arimura, Matsui, & Takabayashi, 2009; Baldwin, Halitschke, Paschold, von Dahl, & Preston, 2006; Holopainen, 2004; Pichersky & Gershenzon, 2002; Wu & Baldwin, 2009). Volatiles are semio-chemicals; they are used to communicate and send messages between and among species of plants and animals (Paré & Tumlinson, 1997).

Plant volatiles differ in quantity and composition when the plant is intact and healthy, compared to when it is attacked by arthropods pests, and the altered emission of volatiles due to arthropod attack is termed herbivore-induced plant volatiles. The change in composition of intact plant volatiles to HIPVs is a result of volatiles being altered either quantitatively (altered ratios of the same volatile components), or qualitatively (the release of different components) (Dicke & van Loon, 2000; Paré & Tumlinson, 1997). HIPVs also differ from volatiles emitted from plants as a result of mechanical or artificial plant-damage; the secretions produced by herbivores during feeding or oviposition, stimulate the production of HIPVs (Dicke & van Loon, 2000; Howe & Jander, 2008; Schmelz, Alborn, Engelberth, & Tumlinson, 2003; Thaler, Fidantsef, & Bostock, 2002; Tumlinson & Lait, 2005). Compounds found in herbivore secretions, such as β-glucosidase, *N*-(17-hydroxylinolenoyl)-L-glutamine (volicitin), and N-linolenoyl-Glu have been identified as elicitors of HIPVs (Alborn et al., 1997; Halitschke, Schittko, Pohnert, Boland, & Baldwin, 2001; Mattiacci, Dicke, & Posthumus, 1995). HIPVs are emitted by the plant at the site of infestation, as well as systemically from undamaged leaves (Turlings & Tumlinson, 1991).

It was discovered that plants emit volatiles in response to stress over 35 years ago (Green & Ryan, 1972). Since that time, considerable research on the identification of HIPVs and understanding how these volatiles are

produced, transported and perceived has gained momentum. One of the first research studies investigating how predatory mites respond to these volatiles was conducted by Sabelis and Van de Baan (1983). Since that time, studies have focused on HIPV production in response to attack by a range of arthropod pests, including folivores (Bi & Felton, 1995; Drukker, Scutareanu, & Sabelis, 1995; Du, Poppy, Powell, & Wadhams, 1997; Horiuchi et al., 2003; Pareja, Moraes, Clark, Birkett, & Powell, 2007; Puente, Magori, Kennedy, & Gould, 2008; Turlings, Tumlinson, & Lewis, 1990; Van Loon, De Vos, & Dicke, 2000), stem borers (Hou & Han, 2010; Kvedaras & Keeping, 2007; Kvedaras, et al., 2007b; Potting, Vet, & Dicke, 1995), phloem feeders (Gatarayiha, Laing, & Miller, 2010; Goussain, Prado, & Moraes, 2005; Walling, 2000), seed feeders (Steidle, Fischer, & Gantert, 2005), and root feeders (Rasmann et al., 2005), and also as a result of oviposition by herbivorous insects (Hilker & Meiners, 2006).

HIPVs are used as a common language, and the volatiles emitted from plants are used to communicate to both herbivorous (pests) and carnivorous (‘beneficial’ predatory and parasitoid natural enemies of the pests) arthropods, as well as entomophagous nematodes (Rasmann, et al., 2005) and possibly insectivorous birds (Mäntylä et al., 2008). Beneficial arthropods have been shown to respond to the volatile cues relating to their specific host pest, on a variety of plant species (Du, et al., 1997; W. J. Lewis & Tumlinson, 1988; Takabayashi, Sabelis, Janssen, Shiojiri, & van Wijk,

2006). HIPVs indicate to herbivorous arthropods that the plant is being attacked, that there may be strong competition from other herbivores for food, and that it will be a predator–parasitoid dense area (Dicke & van Loon, 2000). Plant species, herbivore species and density of herbivores appear to be the variables influencing whether HIPVs attract or repel other herbivores to the plant (Dicke & van Loon, 2000).

There are thousands of identified plant volatile compounds, and the complex blends (composed of 20 to over 200 compounds) of HIPVs emitted are dependent on the interaction between plant and herbivore species (Dicke, 2009; Dicke & van Loon, 2000; Paré & Tumlinson, 1997; Takabayashi, et al., 2006; Turlings, McCall, Alborn, & Tumlinson, 1993). A study by De Moraes et al. (1998) revealed that the parasitic wasp, *Cardiochiles nigriceps* (Viereck) (Hymenoptera: Braconidae), could distinguish plants infested with its host, *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae), from plants infested with a closely-related caterpillar, but non-host, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae).

Herbivore-induced plant volatiles also signal to surrounding plants that pests are near, allowing them to prepare ("prime") and make ready for possible herbivory (Howe & Jander, 2008). Priming is a mechanism whereby a treatment (or stimulant) prepares a plant to respond more quickly or aggressively to biotic and abiotic stress (Beckers & Conrath, 2007; Frost, Mescher, Carlson, & De Moraes, 2008). While the mechanisms responsible

for priming are not known (Dudareva, Negre, Nagegowda, & Orlova, 2006), it is believed they are due to the up-regulation of defence-related genes or other metabolites which initiate a biochemical signal transduction leading to a primed state (Frost, Mescher, Carlson, et al., 2008). Studies by Engelberth et al. (2004) found corn seedlings previously exposed to volatiles from neighbouring plants displayed enhanced production of HIPV (including JA) when exposed to mechanical damage and caterpillar regurgitant. The plant expends considerable energy in producing volatile blends, and the metabolic cost is thought to compromise other plant processes which require metabolic energy, such as growth, flowering and yield. Primed plants expend less energy than those producing the full induced defence response (Frost, Mescher, Carlson, et al., 2008), and it is ecologically sound and in a plant's best interest to emit volatiles only as needed and for short periods only (Dicke, 2009).

Research suggests that volatile chemicals are synthesised on site as needed, which is demonstrated by a consistent several-hour delay between arthropods feeding and emission of volatile chemicals by the plant (Paré & Tumlinson, 1999). It has been shown that the volatiles emitted from a wide range of plant species are uniform in their chemical structure, suggesting a common set of biosynthetic signalling pathways (Paré & Tumlinson, 1999).

1.5 Metabolic pathways that drive induced plant defences

Many plant volatile compounds, particularly monoterpenes and sesquiterpenes, are synthesised and stored in resin ducts or glandular trichomes on the leaf surface, ready to be released in large quantities upon arthropod herbivory or tissue damage (Duke et al., 2000). However, *de novo* biosynthesis of volatile compounds is a consequence of major metabolic pathways (Figure 1.1) (Browse & Howe, 2008; Howe & Jander, 2008; Maffei, 2010; Paré & Tumlinson, 1999; Pichersky, et al., 2006; Reymond & Farmer, 1998; Rostás & Turlings, 2008). The three major groups of volatile compounds, and their associated pathways, are as follows:

1.5.1 Isoprenoids (terpenoids)

Isoprenoids are produced from the precursors dimethylallyl diphosphate (DMAPP) and its isomer, isopentenyl diphosphate (IPP) (Maffei, 2010). Their biosynthesis occurs along two alternate pathways: (i) sesquiterpene biosynthesis occurs in the cytoplasm/endoplasmic reticulum via the mevalonic acid (MVA) pathway from acetyl-CoA, and (ii) hemiterpene and

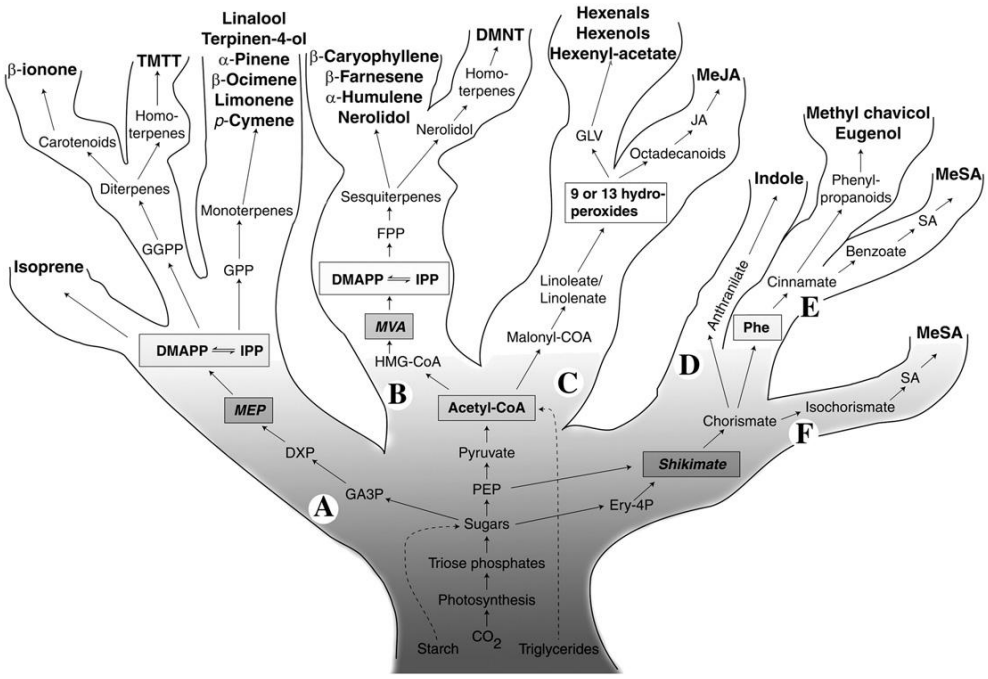

Figure 1.1: The volatilome tree (from Maffei, 2010). Branch (A) volatile organic compounds (VOCs) are produced by different biochemical pathways generated from geranyl diphosphate (GPP); the MEP pathways give rise to the formation of monoterpenes and diterpenes, with the latter being precursors of the homoterpene 4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT) and of the caroteoid-derived β-ionone. Isoprene is generated from DMAPP. Branch (B) sesquiterpenoids are generated by farnesyl diphosphate (FPP) derived from the cytosolic MVA pathway. The homoterpene 4,8-dimethylnona-1,3,7-triene (DMNT) derives from the sesquiterpene nerolidol. Branch (C) oxylipins generate from fatty acids which are cleaved into GLVs and JA derivatives. Branch (D) the volatile indoles generate from anthranilate. Branch (E) aromatic VOCs such as eugenol derive from phelylpropanoids, whereas methyl salicylate (MeSA) derived from SA generated from benzoic acid. Branch (F) MeSA can alternatively be formed by methylation of SA deriving from isochorismate.

monoterpene, and possibly also sesquiterpene, biosynthesis occurs in the chloroplasts via the plastidic 2-*C*-methyl-D-erythritol-4-phosphate (MEP) pathway from glyceraldehyde-3-phosphate (Arimura, et al., 2008; Arimura, et al., 2005; Lichtenthaler, 1999; Maffei, 2010; Sallaud et al., 2009). The

isoprenoid pathway forms pyrophosphate-containing compounds which serve as precursors of many primary metabolites and produces the largest class of plant volatiles, including terpenoid plant volatiles; monoterpenes (ie, linalool and (*E*)-*β*-ocimene), homoterpenes (ie, non-atriene and tridecatetraene), and sesquiterpenes (ie, (*E*,*E*)-α-farnesene, α-humulene and (*E*)-β-farnesene) (Degenhardt, Köllner, & Gershenzon, 2009; Dudareva & Pichersky, 2006; Maffei, 2010; Mumm, Posthumus, & Dicke, 2008; Pichersky & Gershenzon, 2002).

1.5.2 Oxylipins

Oxylipins are produced from α-linolenic acid, present in chloroplast membranes, and include jasmonates (ie, jasmonic acid and methyl jasmonate) and GLVs (ie, *cis*-3-hexenyl acetate and *cis*-hexen-1-ol) (Arimura, et al., 2005; Avanci, Luche, Goldman, & Goldman, 2010; Browse & Howe, 2008; Maffei, 2010). Octadecanoids and jasmonates are produced from 13-allene oxide synthase activity, and aldehydes, fatty acids and alcohols are produced from hydroperoxy lyases. GLVs are synthesised via the lipoxygenase pathway from C_{18} fatty acids (Dudareva, et al., 2006).

1.5.3 Volatile aromatic compounds

Volatile aromatic compounds consist of compounds containing an aromatic ring, and nitrogen or sulphur. They are synthesised by cleavage reactions and decarboxylation of modified amino acids or their precursors (Maffei,

2010), which produces shorter-chain volatiles with aldehyde and ketone moieties, precursors for the biosynthesis of other volatile compounds (Pichersky, et al., 2006). The shikimate pathway produces aromatic metabolites, including indole, eugenol and methyl salicylate.

1.5.4 Interactions between the metabolic pathways

The composition of HIPVs may be affected by biochemical interactions within the plant; plant defences against pathogens and herbivorous arthropods form interacting signal transduction pathways, and both synergistic and antagonistic interactions have been observed between the SA and JA pathways (Beckers & Spoel, 2006). The mode of herbivore damage affects the volatiles produced. Smith and Boyco (2007) and Howe and Jander (2008) state that the extensive tissue damage caused by phytophagous arthropods, including chewing caterpillars and beetles as well as cell-content feeders such as mites and thrips, induce the JA signalling pathway. In contrast, phloem-feeding insects and oviposition of arthropod eggs produce less tissue damage and are perceived by the plant as bacterial, viral or fungal pathogens, activating the SA-dependent and JA/ethylene-dependent signalling pathways.

The synthesis of plant volatiles, including terpenoids, that mediate tri-trophic plant−pest−predator interactions are predominantly regulated by the JA pathway (Erb, Ton, Degenhardt, & Turlings, 2008; Zheng & Dicke,

2008). Terpenoid formation is likely the result of specific defence genes activated by oxylipin compounds (for example, JA) triggered by herbivore-stimulated plant responses (Arimura, et al., 2008; Arimura, Huber, & Bohlmann, 2004).

It has been found that SA-dependent defences may repress JA-dependent defences (Beckers & Spoel, 2006; Thaler, et al., 2002). However, Rostás and Turlings (2008) found that induction of SAR via the SA pathway enhanced the attraction of the parasitoid, *Microplitis rufiventris* (Kokujev) (Hymenoptera: Braconidae) to corn plants infested with *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). The composition of HIPVs may also be affected by abiotic stresses such as light, temperature, availability of water (Gouinguene & Turlings, 2002), nitrogen and phosphorous content (Schmelz, et al., 2003), soil salinity, pH and air humidity (Vallat, Gu, & Dorn, 2005), and heavy metals (Engelberth et al., 2001).

1.6 Use of herbivore-induced plant volatiles as an integrated pest management strategy

The potential to manipulate HIPV production is a new and exciting area of biological pest control research. Recent studies are investigating the use of exogenous applications of synthetic HIPVs on plants to stimulate the plant to produce its own endogenous HIPVs, enhancing plant resistance against

pests and disease (James & Price, 2004; Khan, et al., 2008; Simpson et al, 2011a; Simpson et al., 2011b). Synthetic HIPVs available for exogenous application include methyl salicylate, methyl jasmonate, benzaldehyde, (*Z*)-3-hexenyl acetate, farnesene and octyl aldehyde. Using sticky card traps and canopy shake methods in vineyard blocks baited with methyl salicylate, James and Price (2004) found significantly greater numbers (nearly four times greater) of predatory arthropods, including green lacewings, *Chrysopa nigricornis* (Burmeister) (Neuroptera: Chrysopidae), brown lacewings, *Hemerobius* spp. (Neuroptera: Hemerobiidae), mirids, *Deraeocoris brevis* (Uhler) (Hemiptera: Miridae), lady beetles, *Stethorus punctum picipes* (Casey) (Coleoptera: Coccinellidae), pirate bugs, *Orius tristicolor* (White) (Hemiptera: Anthocoridae), and parasitic wasps (not identified in the study) than in un-baited blocks. That study also found a corresponding reduction in spider mite, *Tetranychus urticae* (Koch) (Acari: Tetranychidae) numbers in the baited blocks (James & Price, 2004). A recent review by Khan et al., (2008) highlights the benefits of exogenously applying synthetic HIPV at low doses to enhance and trigger plants' induced resistance to subsequent pest attack.

Two recent studies have used exogenous applications of both Si and HIPVs and compare their effects on arthropod pests. Correa et al. (2005) evaluated the effect of calcium silicate and a synthetic HIPV, acibenzolar-S-methyl (ASM), on enhanced resistance of cucumber to the pest, whitefly, *Bemisia*

tabaci (Gennadius) (Hemiptera: Aleyrodidae). That study found a significant reduction (more than three times) in ovipositon by *B. tabaci* on cucumber leaves treated with Si, with or without added ASM than on the control leaves. Likewise, Costa and Moraes (2006) found both silicic acid (the authors do not specify what form) and ASM resulted in significant reduction of the number of nymphs, reduced population growth rate, reduced post-reproductive period, and reduced longevity of the aphid, *Schizaphis graminum* (Rondani) (Homoptera: Aphididae) in wheat plants.

1.7 Link between silicon and induced chemical defences

Silicic acid is an elicitor for systemic stress signals such as SA and JA, associated with HIPV production (Fauteux, et al., 2005). Manipulation of HIPVs can be achieved by modifying metabolic pathways, such as by up- or down-regulation of one or more steps in the pathway, or by re-direction of metabolic fluxes by blockages or competing pathways (Maffei, 2010). Silicic acid may also modify HIPV production in a similar way, by the up- or down-regulation of steps in the pathway, resulting in an altered volatile profile.

Silicic acid in the plant's transpiration stream does not trigger induced defences until the plant is stressed. In this regard, Si acts in a similar way to primed plants. Priming initiates a state of readiness that does not confer resistance *per se*, but instead allows for enhanced resistance once the plant

is exposed to biotic or abiotic stress (Frost, Mescher, Carlson, et al., 2008). Figure 1.2 shows the sequence of steps from Si fertilisation to tri-trophic interactions with beneficial arthropods.

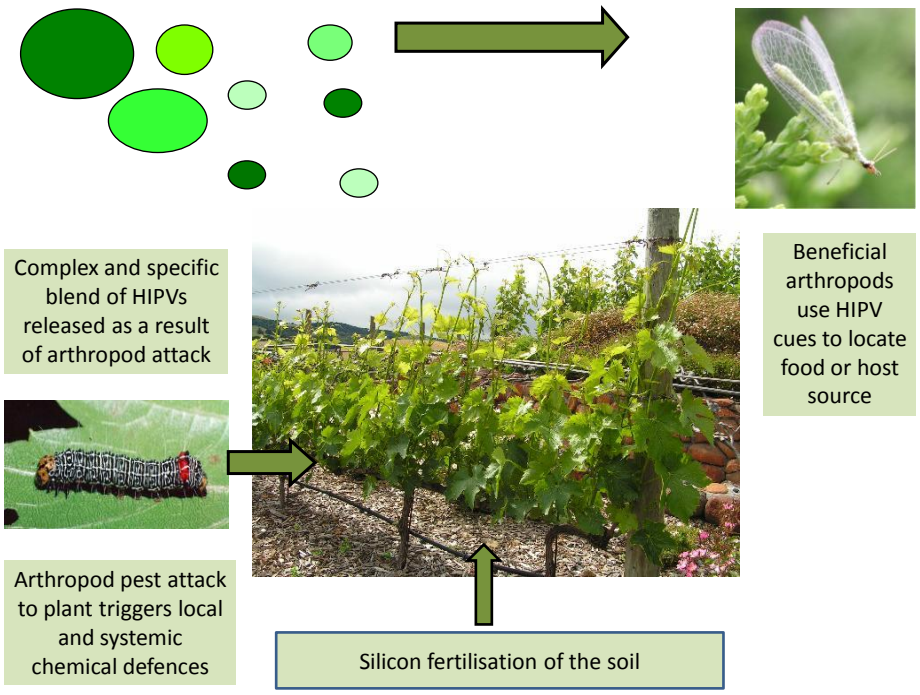

Figure 1.2: Effect of Si fertilisation on chemically-mediated tri-trophic interactions.

For example, Kvedaras et al. (2010) reported no significant difference in attraction of adult predatory red and blue beetle, *Dicranolaius bellulus* (Guérin-Méneville) (Coleoptera: Melyridae) to Si+ or Si- cucumber plants which had no pest infestation. However, once plants were infested with *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) larvae, Si+ plants became significantly more attractive to *D. bellulus* compared to Si-.

1.8 Specific benefits of silicon fertilisation to grapevines

There has been little research on the protective effects of Si fertilisation in non-cucurbit dicots, and fewer studies specifically on grapevines. Those studies on grapevines have focused on the effects of Si treatment on fungal disease and there has been no research undertaken observing the effects of Si treatments on vine-resistance to arthropod pests. Trials on the effects of Si-fertilised grapevines on fungal disease have come to the same conclusions as research on other plant species. That is, Si fertilisation results in the accumulation of Si and phenolic compounds in the epidermal cells of infected plants, enhancing the plant's resistance against the disease (Blaich & Wind, 1989; Bowen, Menzies, Ehret, Samuels, & Glass, 1992a; Reynolds, et al., 1996).

Research has shown that powdery mildew, *Uncinula necator* (Schwein, Burrill), infection stimulated grapevines to deposit phenolic compounds associated with peroxidise activity in the infected and surrounding cell walls (Blaich & Wind, 1989). Both healthy and *U. necator*-infected leaves of grapevines (cv. Riesling) were examined under a scanning electron microscope, revealing local accumulations of Si in epidermal cells of Si+ leaves where mildew papillae or haustoria had entered the cell (with some mildew haustoria completely silicified within the plant cell). In contrast,

uninfected leaves displayed equal distributions of Si. That study also found silica skeletons extended from the upper layer into deeper layers of the leaf. As would be expected, those cultivars of grapevines more resistant to fungal attack displayed greater accumulations of Si within their cell walls (Blaich & Wind, 1989).

Bowen et al. (1992b) applied potassium silicate on both the roots (at the rate 1.7 mM Si) and the foliage (17 mM Si) of grapevines, resulting in significant reduction in the number of mildew colonies on leaves inoculated with *U. necator*, and treated with the foliar application of Si. Grapevines treated with root-fed Si had no reduction in number of *U. necator* colonies compared to control plants, but did have elevated levels of Si surrounding the fungal appressoria (Bowen et al., 1992b). Reynolds et al. (1996) also found a significant reduction in *U. necator* infection on the leaves of vines treated with foliar applications (17 mM Si) of potassium silicate. Both Bowen et al. (1992b) and Reynolds et al. (1996) used scanning electron microscopy to reveal large quantities of Si deposited near infection sites and fungal haustoria on plants treated with both the foliar and root-applied forms of Si.

1.9 Agricultural and commercial uses for silicon

1.9.1 Forms of silicon

There are several forms of Si available for agricultural and commercial use, which are briefly summarised in this review. Potassium silicate can be applied in either a liquid or granular form to either the soil or foliage of plants, and has been shown to significantly reduce the damaging effects of arthropod herbivores (Kvedaras, et al., 2010; Parrella, Costamagna, & Kaspi, 2007; Subbarao & Perraju, 1976), and fungal disease (Chérif, et al., 1994; Chérif, et al., 1992; Ghanmi, McNally, Benhamou, Menzies, & Bélanger, 2004; Guével, Menzies, & Belanger, 2007; Liang et al., 2005b; Menzies, Bowen, & Ehret, 1992; Rémus-Borel, Menzies, & Belanger, 2005; Reynolds, et al., 1996; Schuerger & Hammer, 2003).

Calcium silicate can be incorporated directly into the soil as a solid powder, and has been extensively used in studies investigating the effects on arthropods pests (Goussain, et al., 2005; Keeping, et al., 2009; Keeping & Meyer, 2002; Korndörfer, Cherry, & Nagata, 2004; Kvedaras, et al., 2007b; Redmond & Potter, 2006) with mixed results. Calcium silicate has also been used in a liquid form as a foliar spray (Correa, et al., 2005), resulting in significant reductions in plant damage from arthropods pests. The source of calcium silicate appears to strongly affect plant uptake of Si, and consequently reducing pest and disease attack (Keeping & Meyer, 2006). Calcium silicate was found to perform better than wollastonite (calcium

metasilicate; $CaSiO_3$) which, in turn, performed better than fly ash (inorganic Ca_2SiO_4, which incorporates small quantities of potassium metasilicate, sodium silicate (N_2SiO_3), and magnesium silicate ($H_2Mg_3(SiO_3)_4$) or ($Mg_3Si_4O_{10}(OH)_2$)) (Keeping & Meyer, 2006).

Sodium silicate can be applied in a liquid form to either the soil or foliage, and has been used successfully to manage phloem-feeding arthropods (Moraes et al., 2004; Moraes, Goussain, Carvalho, & Costa, 2005).

1.9.2 Soil versus foliar application of silicon

Studies have shown significant reductions in arthropod pest damage and fungal disease as a result of both foliar and soil applications of Si (Correa, et al., 2005; Costa & Moraes, 2006; Gomes, et al., 2005; Goussain, et al., 2005; Menzies, et al., 1992; Moraes, et al., 2004), however, it has been shown that the uptake of Si is primarily through the root system (Guével, et al., 2007; Liang et al., 2005b). Guével et al. (2007) found that foliar application of Si (1.7 mM Si) reduced fungal infection in wheat, however, it was not as successful as root-applied Si (at the same rate). Similarly, Liang et al. (2005b) found foliar-applied Si had no effects on numbers of colonies of *P. xanthii* or defensive enzyme activity in cucumber, compared to root-applied Si.

In contrast, Bowen et al. (1992b) (as discussed in Section 1.6 above) found that foliar application of potassium silicate at 1000 ppm SiO_2 (17 mM Si) substantially reduced the extent of *U. necator* infection in grapevines, while root-applied Si at 100 ppm SiO_2 (1.7 mM Si) provided little resistance. The results of that study showed significant difference between treatments, with foliar treatment averaging two powdery mildew colonies on grape leaves, compared with root treatment averaging 13.4 colonies. That study suggested the enhanced fungal resistance on plants treated with the high-concentration Si foliar spray could have been due, in part, to a barrier effect on the leaf surface. However, scanning electron micrographs have shown that foliar-applied Si protects leaves not only where thick deposits of Si were laid, but also showed that Si is translocated laterally through the leaf to sites of hyphal penetration (Bowen et al., 1992b; Reynolds, et al., 1996). Correa et al., (2005) found that foliar applications of calcium silicate (200 ml applications at 1% calcium silicate per pot) on cucumber caused a significant reduction (more than three times) in oviposition by *B. tabaci*, compared with soil-applied calcium silicate (at the same rate) and the control.

It appears that the success of the foliar application of Si depends upon the rate it is applied; Bowen et al., (1992b), Reynolds et al., (1996) and Menzies et al., (1992) successfully controlled fungal infection applying Si at 17 mM,

which is ten times that used less successfully by Guével et al., (2007), at 1.7 mM Si.

1.9.3 Adverse effects of silicon fertilisation on beneficial arthropods

There appears to be no reported cases of Si-fertilisation adversely affecting beneficial arthropod populations. Moraes et al. (2004) found Si fertilisation of wheat had no tri-trophic effect on the biological life parameters of two beneficial arthropods; *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) and *Aphidius colemani* (Viereck) (Hymenoptera: Aphididae) were fed a diet of greenbug, *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae), which in turn had been feeding on Si-treated wheat. There has, however, been few studies conducted in this area and the effects of Si fertilisation on the food chain requires further research.

1.10 Grapevine pests of Australia and their natural enemies

The grapevine industry in Australia is spread over every state and, while the vines are grown under wide ranging climatic conditions, the major arthropod pests affecting grapevines (see Table 6.1 in Appendix) are similar across all regions.

1.10.1 Arthropod pests

The major arthropod pests attacking grapevines in Australia include the light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), Australian grapevine moth, *Phalaenoides glycinae* (Lewin) (Noctuidae: Agaristinae), grape phylloxera, *Dactylosphaera vitifoliae* (Fitch) (Hemiptera: Phylloxeroidea), grapevine scale, *Parthenolecanium persicae* (Fabricius) (Hemiptera: Coccidae), mites, *Colomerus vitis* (Pagenstecher) (Acari: Eriophyidae), and mealybugs, *Pseudococcus* spp. (Hemiptera: Pseudococcidae). Only those insect pests used in the present study, their life cycle, geographic location, damage they cause and current control methods will be discussed in this review.

Light brown apple moth (*Epiphyas postvittana*)

Epiphyas postvittana is a leafroller moth native to south-eastern Australia, and has been introduced to Western Australia, South Australia, New Zealand, New Caledonia, the British Isles, Hawaii (Danthanarayana, Gu, & Ashley, 1995) and California (Lewis & Hodges, 2010; Suckling & Brockerhoff, 2010). *Epiphyas postvittana* is more suited to cooler regions, including the Central Tablelands of NSW, with survival limited at temperatures above 30.7^{o}C (Danthanarayana, et al., 1995; Loch, 2007) (Figure 1.3).

A B C D

Figure 1.3: *Epipyhas postvittana* A: adult female, B: adult male, C: pupae, D: larvae (photos courtesy of the University of California, Davis)

Epiphyas postvittana is polyphagous, feeding on a wide range of both Australian native and introduced plant species (Danthanarayana, 1975; Geier & Briese, 1980; Suckling & Brockerhoff, 2010). In vineyards, *E. postvittana* lays its eggs in clusters of 20-50 on the upper surface of leaves or shoots of the plant (Loch, 2007). The larvae roll leaves and shoots together with a silken thread, creating a nest in which they feed on leaves and berries, and sometimes pupate (Loch, 2007; Suckling & Brockerhoff, 2010). One generation cycle can take up to two months to complete, and there can be three or four generations each year, depending on climatic conditions, with a winter generation occurring on broadleaf weeds (Danthanarayana, et al., 1995; Loch, 2007). The damage to grapevines is caused by *E. postvittana* larvae feeding, and the level of damage depends on the timing of the outbreak of larvae; early spring outbreaks affect budburst, while spring/summer outbreaks result in larvae feeding directly on fruit bunches. Larval damage to bunches can increase the incidence of bunch rot infections, including *Botrytis cinerea* (De Bary) and *U. necator*, especially in cool, wet weather (Loch, 2007). A study in South Australia found the presence of *B. cinerea*-contaminated *E. postvittana* larvae at veraison

resulted in up to 59% of bunches infected with *B. cinerea* at harvest, compared with 27% of bunches with uncontaminated larvae, and 18% when no larvae were added (Bailey, Ferguson, McMahon, & Wicks, 1997).

In vineyards, the control of *E. postvittana* larvae using conventional insecticides is difficult due to the broad period of larval emergence making accurate timing of sprays difficult. Larvae are also difficult to control as they are generally contained within leaf rolls and between grape bunches, which protects them from contact with chemicals (Loch, 2007). Control using the biological insecticide, *Bacillus thuringiensis* (Berliner) requires accurate timing because of its specificity for very small larvae and its short field life. Control of *E. postvittana* through the employment of natural enemies has been the focus of recent research. A study by Irvin et al. (2006) found apple, *Malus domestica* (Borkhausen) orchards underplanted with alyssum, *Lobularia maritime* (L.), *Phacelia tanacetifolia* (Bentham), and buckwheat, *Fagopyrum esculentum* (Moench), resulted in significantly greater numbers of the parasitoid, *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae) than control plots. That study found the higher numbers of *D. tasmanica* resulted in increased parasitism of *E. postvittana* larvae and reduced numbers of pupae in the treated plots.

Grapevine moth (*Phalaenoides glycinae*)

Phalaenoides glycinae is also native to Australia, and has been introduced to Canada and South Africa (Figure 1.4). The larvae are not generally a major pest for grapevines, but do feed on leaves and can cause significant damage by feeding on berries if there is a large outbreak and foliage becomes depleted (Loch, 2007).

A B C

Figure 1.4: *Phalaenoides glycinae* A: adult moth, B: larvae, C: pupae (photos courtesy of the Macleay Museum, University of Sydney).

In vineyards, *P. glycinae* pupate in a silken cell in the ground, or in fissures in the vine wood or strainer posts (Loch, 2007). *Phalaenoides glycinae* have two or three generations each year, depending on climatic conditions, preferring hot summers (Loch, 2007). Predators of *P. glycinae* include tachinid flies (Tachinidae), parasitic wasps (Hymenoptera), shield bugs (Pentatomoidae) and birds.

1.10.2 Beneficial arthropods (predators and parasitoids)

Common beneficial arthropods in Australia (see Table 6.1 in Appendix) include the predatory green lacewings, *Chrysopa* spp. (Neuroptera:

Chrysopidae), brown lacewing, *Micromus* spp. (Neuroptera: Hemerobiidae), red and blue beetle, *Dicranolaius bellulus* (Guérin-Méneville) (Coleoptera: Melyridae), shield bugs, *Oechalia schellenbergii* (Guérin-Méneville) (Hemiptera: Pentatomidae), ladybeetles (Coleoptera: Coccinellidae), and mirids, *Deraeocoris brevis* (Uhler) (Hemiptera: Miridae). Beneficial parasitoids include *Trichogramma* spp. (Hymenoptera: Trichogrammatidae), Tasman wasp, *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae), *Brachymeria phya* (Walker) (Hymenoptera: Chalcididae), and *Voriela uniseta* (Malloch) (Diptera: Tachinidae). Only those beneficial insects used in the present study will be discussed further here.

Green lacewing (*Mallada signata*)

Lacewings (Chrysopidae) are found across the world and are important generalist predators in Australian vineyards (Duelli, 2001). The species *Mallada signata* (Schneider) (Figure 1.5) is native to Australia and is

A B C

Figure 1.5: *Mallada signata* A: adult, B: larvae, C: larvae feeding on brown citrus aphid, *Toxoptera citricida* (Kirkaldy) (Hemiptera: Aphididae) (photos courtesy of ipmimages.org)

commercially available, being bred for the use of inundative biological control (Bug Central, 2010; Bugs for Bugs, 2010). However, there have been very few studies conducted on this species of Chrysopidae. *Mallada signata* larvae prey upon a wide variety of grapevine pests, including small lepidopteran larvae and eggs, scale (Coccoidae) and whiteflies (Aleyrodidae) (Bugs for Bugs, 2010). Females lay their tiny, oblong eggs on silken stalks attached to plant tissues, and depending on the species, eggs are laid singly or in clusters, each on an individual stalk (Duelli, 2001). Larvae are pale with dark markings, 3-20 mm in length, possess prominent mandibles with which they attack their prey, and pupate in cocoons attached to plants or under loose bark (Duelli, 2001). Adult Chrysopidae typically feed on nectar and pollen from flowers, and gravid females fly towards and lay eggs on vegetation of preferred plant species (Canard, 2001).

Red and blue beetle (*Dicranolaius bellulus*)

Dicranolaius bellulus are a common Australian native generalist predatory insect (Figure 1.6) which preys on small lepidopteran larvae, insect eggs and occasionally pollen (Hawkeswood, 2011; Horne, Edward, & Kourmouzis, 2000).

Figure 1.6: *Dicranolaius bellulus* adult (photo courtesy of cottoncrc.org.au)

Dicranolaius bellulus are located across Australia, and have been found in grapevines (Simpson et al., 2011a; Simpson et al., 2011b), cotton, *Gossypium* spp. (Perović, Gurr, Raman, & Nicol, 2010), lucerne, *Medicago sativa* (L.) (Hossain, Gurr, & Wratten, 2001; Hossain, Gurr, Wratten, & Raman, 2002), potato, *Solanum tuberosum* (L.) and carrot, *Daucus carota* (L.) crops (Horne, et al., 2000). As their common name suggests, *D. bellulus* have shiny red and blue bands across the body, dark head and legs, and are around 5 mm in length. The eggs, larvae and pupae stages all occur on soil debris and they are therefore very susceptible to farming practices (Hawkeswood, 2011; Horne, et al., 2000; Robertson, Kettle, & Simpson, 1994).

1.11 Aims and scope of this research study

Given there are significant gaps in our knowledge in regard to the effects of Si fertilisation of grapevines, including both direct and indirect effects on grapevine pests and their natural enemies, this thesis aimed to fill some of those voids. Importantly, there has been no research identifying or

qualifying HIPV profiles from Si-treated pest-infested plants. Also, there is no literature reporting the response of *M. signata* to common HIPVs. Therefore, this research study investigated the following hypotheses:

1. Silicon fertilisation would enhance the constitutive and induced defences of grapevines, reducing the level of feeding by *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) larvae on Si-treated (Si+) plants.

2. Silicon fertilisation would enhance or alter the composition of HIPVs emitted by grapevines infested with *E. postvittana* larvae, making the plant more attractive to the generalist predator, *Dicranolaius bellulus* (Guérin-Méneville) (Coleoptera: Melyridae).

3. Silicon fertilisation would enhance or alter the composition of HIPVs emitted by grapevines infested with *Phalaenoides glycinae* (Lewin) (Lepidoptera: Noctuidae) larvae, making the plant more attractive to the generalist predator, *Mallada signata* (Schneider) (Neuroptera: Chrysopidae).

4. As a complement to the work above on plant-mediated effects, another experiment assessed the response of *M. signata* to synthetic volatile compounds, commonly emitted from pest-infested plants, including grapevines.

Chapter 2

Effects of silicon fertilisation of grapevines on feeding damage of the pest, light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae)

2.1 Introduction

Silicon fertilisation protects plants against arthropod attack by two main mechanisms. Firstly, Si may act by improving the plant physical barrier through the deposition of solid amorphous silica, mainly as opaline phytoliths, in the epidermal tissue of the stems and leaves (Blaich & Wind, 1989; Jones & Handreck, 1967). Silicon deposition can make grass leaf tissue more abrasive (Massey, et al., 2006), directly improving resistance to arthropod pests by reducing penetration and feeding by folivores (Goussain, Moraes, Carvalho, Nogueira, & Rossi, 2002; Massey, et al., 2006), borers (Keeping & Kvedaras, 2008; Keeping & Meyer, 2006; Kvedaras, et al., 2007b), phloem (Basagli, Moraes, Carvalho, Ecole, & Gonçalves-Gervásio, 2003; Moraes, et al., 2004; Moraes, et al., 2005), and xylem feeders (Kim & Heinrichs, 1982; Yoshihara, Sogawa, Pathak, Juliano, & Sakamura, 1979) (Table 2.1).

Secondly, Si may enhance the synthesis of defensive chemicals released upon herbivore feeding or oviposition (Correa, et al., 2005; Gomes, et al., 2005). It has been suggested that the role of Si in induced chemical defences may be more important in reducing arthropod attack than the improvements in constitutive defences, particularly for phloem and xylem feeders. Goussain et al. (2005) found the presence of Si in the epidermal tissue of wheat did not impede stylet insertion by the greenbug, *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae). It did, however, result in more frequent

stylet withdrawal, indicating chemical defences had a greater negative effect on *S. graminum* than constitutive defences.

Arthropod pests may take longer to penetrate the plant, and to chew and digest food as a result of Si fertilisation, indirectly affecting reproductive success and lowering population growth rate (Correa, et al., 2005; Costa & Moraes, 2006; Goussain, et al., 2005; Hou & Han, 2010; Massey, et al., 2006) (Table 2.1). Also, arthropod pests may be exposed on the plant for longer periods, increasing their susceptibility to weather conditions, predators, and chemical sprays (Hou & Han, 2010; Keeping & Meyer, 2006; Kvedaras, et al., 2007b; Massey, et al., 2006). There are a wealth of studies demonstrating Si fertilisation reduced feeding efficiency, slightly increased mandible wear, decreased larval survival (Djamin & Pathak, 1967; Goussain, et al., 2002; Hanifa, Subramaniam, & Ponnaiya, 1974; Keeping & Meyer, 2002, 2006; Sasamoto, 1958; Ukwungwu, 1990), reduced percent pupation and adult emergence (Sétamou, Showler, Reagan, Jones, & Bernal, 2005), and reduced feeding preference, adult longevity and fecundity (Basagli, et al., 2003; Correa, et al., 2005; Costa & Moraes, 2006; Gomes, et al., 2005; Goussain, et al., 2005; Moraes, et al., 2004; Moraes, et al., 2005; Salim & Saxena, 1992) (Table 2.1).

Table 2.1: Summary of previous studies observing the effects of Si fertilisation on arthropod feeding, larval growth and life parameters for a variety of feeding guilds and plant species

Pest species	Feeding guild	Plant species	Si content in plant tissue	Feeding damage	Larval growth and life parameters	Reference
Schistocerca gregaria	Folivore	Grass species	High	Increased	Reduced	Massey et al., 2006
Spodoptera exempta	Folivore	Grass species	High	Increased	Reduced	Massey et al., 2006
Spodoptera eridania	Folivore	Artificial diet	High	Increased	Reduced	Peterson et al., 1988
Herpetogramma phaeopteralis	Folivore	Turfgrass species	High	No change	No change	Korndörfer et al., 2004
Agrotis ipsilon	Folivore	Turfgrass species	High	No change	No change	Redmond and Potter, 2006
***Cyclocephala* spp.**	Folivore	Turfgrass species	High	No change	No change	Redmond and Potter, 2006
Eldana saccharina	Borer	Sugarcane	High	Reduced	Reduced	Keeping et al., 2010; Keeping and Meyer, 2006; Kvedaras and Keeping, 2007; Kvedaras et al., 2007b
Chilo suppressalis	Borer	Sugarcane	High	Reduced	Reduced	Hou and Han, 2010
Planococcus citri	Phloem	Fiddleleaf fig	Low	Not tested	No change	Hogendorp et al., 2009

Pest species	Feeding guild	Plant species	Si content in plant tissue	Feeding damage	Larval growth and life parameters	Reference
T. urticae	Phloem	Bean	Low	Reduced	Reduced	Gatarayiha et al., 2010
T. urticae	Phloem	Corn	High	Reduced	Reduced	Gatarayiha et al., 2010
Shizaphis graminum	Phloem	Wheat	High	No change	Reduced	Goussain et al., 2005
S. graminum	Phloem	Wheat	High	Reduced preference	No change	Moraes et al., 2004
Rhopalosiphum maidis	Phloem	Corn	High	Reduced preference	Not tested	Moraes et al., 2005
Myzus persicae	Phloem	*Zinnia elegans*	Intermediate	Not tested	Reduced	Ranger et al., 2009
Sogatella furcifera	Xylem	Rice	High	Reduced	Reduced	Salim and Saxena, 1992
Nilaparvata lugens	Xylem	Rice	High	Reduced	Not tested	Yoshihara et al., 1979

There have, however, been other studies which have shown Si fertilisation to have no effect on larval weight and development (Hogendorp, Cloyd, & Swiader, 2009; Korndörfer, et al., 2004; Redmond & Potter, 2006) (Table 2.1). Silicon fertilisation of various turfgrass species did not affect larval weights of the folivores, tropical sod webworm, *Herpetogramma phaeopteralis* (Guenée) (Lepidoptera: Pyralidae) (Korndörfer, et al., 2004) or black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae), and did not reduce population density or larval weight of natural populations of the masked chafer grub, *Cyclocephala* spp. (Coleoptera: Scarabaeidae) (Redmond & Potter, 2006). Similarly, Si fertilisation of fiddleleaf fig, *Ficus lyrata* (Warburton) had no effect on female size, egg load, and development time of the phloem feeder, citrus mealybug, *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) (Hogendorp, et al., 2009). There have been no studies investigating the effects of Si fertilisation on the important pest, light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae).

The present study assessed whether grapevine, *Vitis vinifera* (L.) gained the same enhanced pest resistance benefits from Si fertilisation found in intermediate or high Si accumulators. Enhanced pest resistance as a result of Si fertilisation has been found in some low Si-accumulators, including eggplant, *Solanum melongena* (L.) and bean, *Phaseolus vulgaris* (L.) (Gatarayiha, et al., 2010). Therefore, this study investigated whether potassium silicate fertilisation of grapevine directly affected the feeding damage and larval weight of *E. postvittana*.

2.2 Materials and methods

2.2.1 Site

The experiment was conducted at the Charles Sturt University Horticulture Centre, situated at the Orange campus in the Central Tablelands of New South Wales, Australia (33^{o}14'36.43''S, 149^{o}06'54.61''E). As potted grapevines spent a period outdoors on a trellis, the mean temperature and rainfall for the experimental site during the years 2009-2010 are given in Table 2.2.

2.2.2 Plant culture

Bare-rooted, one-year old grapevines (cv. Shiraz) grafted to PT23 Polsen rootstock (supplied by Mallee Point Nursery, Yenda, Australia) were potted into 100% palm peat (Magic Soils Pty Ltd, Clayton North, Australia, product of Sri Lanka, a by-product of coconut fibre, pH range 5.4-6.8, lignin content 70%), grown in 30 cm diameter pots (12 L) in a glasshouse from 5 September to 16 November 2009. Potted vines were transferred to an outdoor trellis near the glasshouse from 17 November 2009 to 2 February 2010 and then, due to extreme hot and windy weather conditions experienced on the trellis, were moved back to the glasshouse from 3 February to 1 June 2010. Temperature in the glasshouse was set to 26^{o}C with a photoperiod of 16:8 (light:dark) using 100 Wm^{-2} lighting to extend daylight hours above those experience externally. Vines were fertilised with

Table 2.2: Mean temperature and rainfall data for Orange Agricultural Institute, Orange, NSW

Statistics	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual	Year
Mean maximum temp. (^{o}C)														1976-2009
	28.0	26.6	23.3	17.8	14.4	10.6	9.5	12.8	15.0	17.1	27.2	26.4	19.1	2009
	27.9	24.4	21.9	18.5	14.2	10.4	10.1	9.4	14.0	17.5	19.6	21.6	17.4	2010
Mean minimum temp. (^{o}C)	13.3	13.2	10.6	7.2	4.7	2.6	1.5	2.1	4.2	6.6	9.1	11.3	7.2	1976-2009
	13.6	14.3	10.9	7.6	4.9	3.2	2.3	3.2	5.1	6.2	13.0	12.1	8.0	2009
	15.0	14.2	11.3	8.1	4.5	2.3	2.3	1.5	4.5	7.3	10.0	11.5	7.7	2010
Mean rainfall (mm)	88.3	76.7	58.4	53.2	68.6	70.5	91.5	97.0	79.7	85.4	76.9	78.5	913.5	1976-2009
	62.7	55.7	50.4	62.2	17.6	98.2	73.5	40.9	54.2	54.3	31.2	128.2	729.1	2009
	72.2	151.4	129.3	93.9	77.8	51.6	154.6	214.5	102.6	119.4	124.8	299.9	1592	2010

Source: Australian Bureau of Meteorology, www.bom.gov.au Months potted plants spent outdoors on trellis

a Si-free compound fertiliser (Miracle-Gro® Water Soluble All Purpose Plant Food, Scotts, Baulkham Hills, Australia). The fertiliser was mixed with deionised water (Barnstead B-pure Pressure Cartridge System, Series 583, Barnstead International, Boston, USA, fitted with D0813 and D0809 cartridges) and 1L per pot was applied every 14 days, as per manufacturer recommendations. Deionised water was analysed in May 2009 (in a separate study) by a commercial, National Association of Testing Authorities (NATA) accredited laboratory (SGS Australia, Toowoomba, Australia). The deionised water was tested for plant-available Si using 0.01M calcium chloride ($CaCl_2$) extraction method and inductively coupled plasma-atomic emission spectrometer (ICP-AES) analysis (Haysom & Chapman, 1975). Potted vines were placed on individual plastic trays to catch and allow subsequent uptake of leachate, and were watered using deionised water.

2.2.3 Insect culture

Epiphyas postvittana eggs and pupae were supplied by the South Australian Research and Development Institute (SARDI), Australia. To culture the pest, eggs were placed in plastic containers containing a standard artificial diet (cooked haricot beans, mixed with yeast, agar, hot water, ascorbic acid, paraben, sorbic acid, proprionic acid, orthophosphoric acid, and deionised water) (D'Alberto, 2008) and allowed to develop until pupation. Pupae were then transferred to ridged plastic cups at a ratio of six to ten females to three males per cup. A cotton ball soaked in a honey solution (honey, water, ascorbic acid, sorbic acid, paraben, and ethanol) (D'Alberto, 2008) was placed in each cup as food and was replaced every three days. Emerged

adults remained in the cups and laid eggs in the ridges of the cups. The cups were cut into thin strips containing eggs, which were placed into fresh plastic containers with larval food, thus continuing the life cycle.

2.2.4 Treatments

The potting medium was treated every 14 days from 15 October 2009 to 27 May 2010 (totalling 17 doses) with 1L of either 1) liquid potassium silicate (K_2SiO_3; AGSIL 27, PQ Australia, Dandenong South, Australia) at the rate of 1.7 mM Si (100 ppm Si) mixed with deionised water (Si+), or 2) potassium chloride (KCl; Sigma Aldrich, Castle Hill, Australia) at the rate of 1.1 mM K mixed with deionised water (Si-) to balance potassium fertilisation to plants across the treatments. This resulted in Si+ pots receiving 0.273 ml K_2SiO_3/L of water.

2.2.5 Experimental design

To determine whether Si-fertilised grapevines had any effect on *E. postvittana* feeding, a laboratory trial was conducted on 1 June 2010 under laboratory conditions of 21-24^{o}C room temperature, 55-62% relative humidity (RH). A single leaf, randomly selected individually from 10 Si+ and 10 Si- plants (total of 20 potted plants) was placed inside individual Petri dishes (total 20 Petri dishes). Moist cotton wool was placed around the stem of each leaf. Five, third-fourth instar *E. postvittana* larvae were placed on each excised grapevine leaf and each Petri dish was covered by a lid to

contain larvae and moisture. The combined weight of the five larvae was recorded immediately prior to placement on the leaf. After 24 hours, the five larvae were removed from the leaf and weighed again. The area of leaf consumed by *E. postvittana* larvae during the 24 hour period was measured using Delta-T Scan® (Delta-T Devices Ltd, 128 Low Road, Burwell, Cambridge CB5 OEJ, UK) image analysis software, which accurately gives parameters of the area of objects (ie, grape leaves). The leaves were placed on coloured paper to provide a contrast, and scanned using Delta-T image analysis software to measure the leaf area consumed by the larvae.

Approximate determination of Si content of grapevine leaves used in this study was based on results of Si analysis of separate grapevines, which underwent the same treatment as the plants used in this study (see Chapter 3).

2.2.6 Statistical analysis

Differences between treatments in the area of leaf consumed and larval mass gain (= mass of combined larvae at harvest – mass of same larvae at inoculation) were analysed using a one-way analysis of variance. Simple linear regression analysis with groups was used to correlate area of leaf chewed with larval weight. The least significant difference (LSD) method was used to determine any significant differences between treatments. Statistical analysis of Si content of grapevine leaves was as described in

Chapter 3. All statistical analysis was performed using Genstat 12th Edition for Windows (Payne, Murray, Harding, Baird, & Soutar, 2009).

2.3 Results

2.3.1 Area of leaf consumed

Epiphyas postvittana larvae consumed significantly greater area of Si+ leaves (range = 0.16-22.47%) than Si- leaves (range = 0.47-6.5%) (F = 6.59, $d.f.$ = 1,9, P = 0.03) (Figure 2.1).

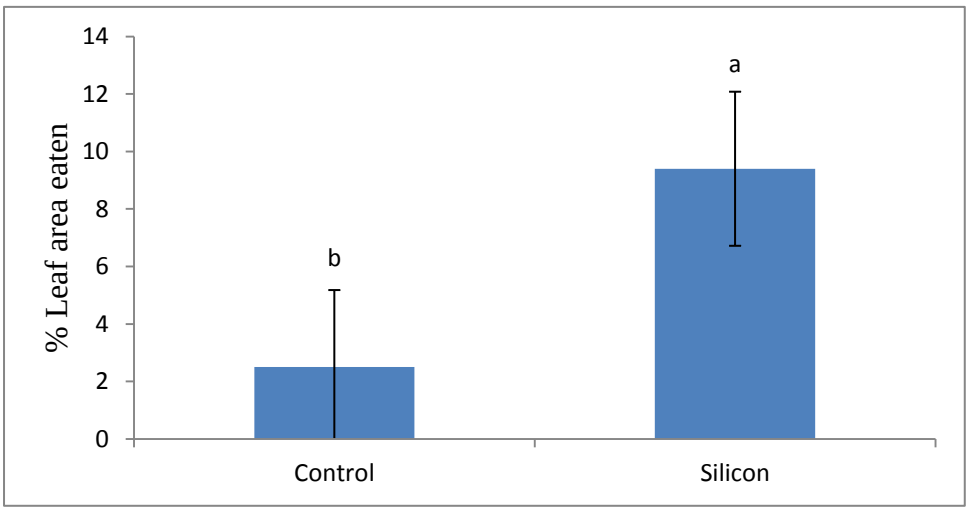

Figure 2.1: Average percent area of silicon-treated and untreated (control) grapevine leaf chewed by *E. postvittana* larvae. Error bars are SE.

2.3.2 Larval weight gain/loss

Average weight loss of *E. postvittana* larvae was less after 24h feeding for larvae feeding on Si+ leaves (range = -16.7 to 15.5 mg) than on Si- leaves (range = -19.9 to -3.4 mg) (F = 8.00, $d.f.$ = 1,9, P = 0.02) (Figure 2.2).

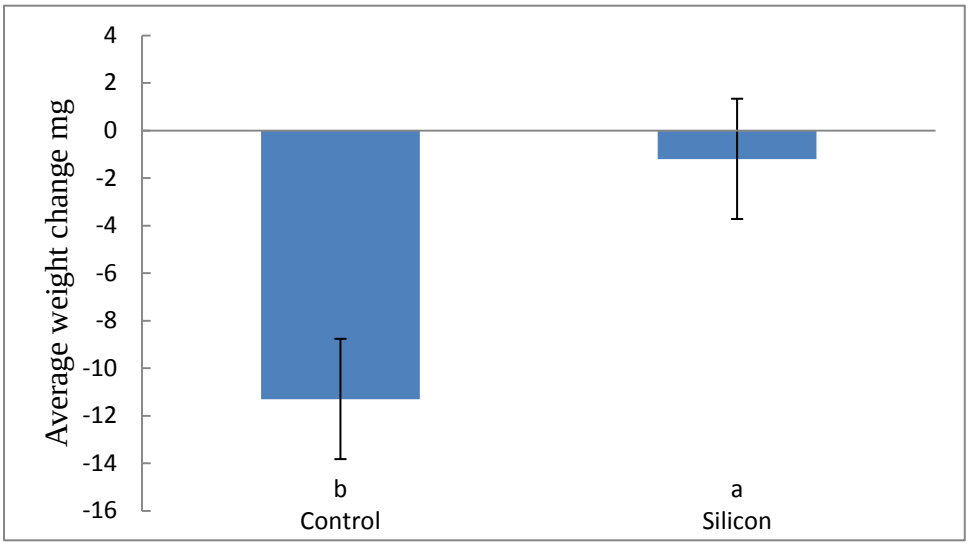

Figure 2.2: Average change in *E. postvittana* larvae weight after 24 hours feeding on silicon-treated and untreated (control) grape leaves. Error bars are SE.

2.3.3 Relationship between leaf area consumed and larval weight

There was a significant positive correlation between larval weight gain/loss and leaf area consumed by *E. postvittana* larvae, which was affected by Si fertilisation (F = 54.01, $d.f.$ = 1,18, $P < 0.001$, adj. R^2 = 73.6) (Figure 2.3).

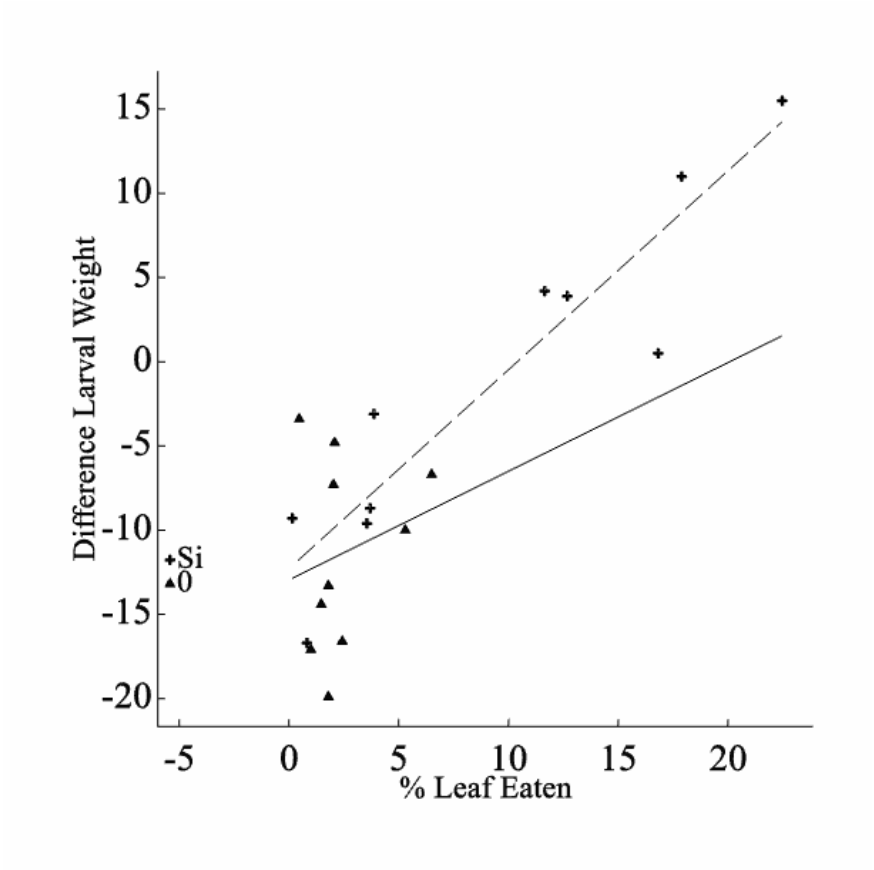

Figure 2.3: Fitted and observed relationship between leaf area eaten and *E. postvittana* larval weight gain or loss. – – – = silicon-treated (Si+), —— = untreated (0).

The mean total Si content of grapevine leaves (see Chapter 3) was higher for Si+ plants (0.315% Si, range = 0.188-0.559% Si) than for Si- plants (0.177% Si, range = 0.065-0.253% Si) (F = 6.51, $d.f.$ = 1,8, P = 0.034). Determination of plant-available Si in the deionised water (separate study) found it contained 2 mg/L Si.

2.4 Discussion

Regression analysis suggests the area of grapevine leaf consumed and *E. postvittana* larval weight were both strongly affected by Si treatment. *Epiphyas postvittana* larvae consumed greater quantities of leaf matter and had a lesser reduction in weight when fed on Si+ grape leaves compared with Si- leaves (Figures 2, 3, and 4). These results contradict our current understanding of the effects of Si fertilisation on feeding damage and larval health. There have been few studies which have reported increased consumption of Si treated plants, with the majority reporting reduced consumption and reduced larval weights as a consequence of Si fertilisation (Table 2.1). For example, Massey et al. (2006) found Si fertilisation resulted in a significant increase in consumption rates by the phloem-feeding grain aphid, *Sitobion avenae* (Fabricius) (Hemiptera: Aphididae), and two generalist insect folivores, the African army worm, *Spodoptera exempta* (Walker) (Lepidoptera: Noctuidae) and the desert locust, *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae) fed on high-Si grass species. Peterson et al. (1988) also found increased consumption rates by *Spodoptera eridania* (Stoll) (Lepidoptera: Noctuidae) fed an artificial diet containing powdered silicic acid at 10-20% dry weight.

In contrast, the majority of reported studies show reduced feeding damage by arthropod pests as a result of Si fertilisation. Keeping et al. (2010), Keeping and Meyer (2006), Kvedaras and Keeping (2007) and Kvedaras et

al. (2007b) found a significant reduction in damage caused by the borer, *Eldana saccharina* (Walker) (Lepidoptera: Pyralidae) in sugarcane (*Saccharum* spp. hybrids) treated with Si compared to untreated cane. Similarly, Hou and Han (2010) found Si fertilisation significantly reduced borer damage by the Asiatic rice borer, *Chilo suppressalis* (Walker) (Lepidoptera: Crambidae) in rice plants, *Oryza sativa* (L.). Silicon fertilisation also significantly reduced leaf damage by the two-spotted spider mite, *Tetranychus urticae* (Koch) (Acari: Tetranychidae) fed on cucumber, eggplant, bean, and corn (Gatarayiha, et al., 2010).

The present study measured larval weight after only a 24 hour period of feeding, and was therefore measuring mass of food in the gut, as an indicator of consumption, rather than actual larval tissue mass. While we did not measure digestion efficiency (plant material converted into larval tissue mass gain) of larvae feeding on Si+ plants, the significant weight loss found in larvae feeding on Si- leaves warrants discussion. The results from the present study suggest that Si fertilisation of grapevines had a greater effect on induced defences compared to constitutive defences. The low Si content in both Si+ and Si- grape leaves, and high consumption of Si+ leaves, indicate that polymerised Si provided little physical resistance to *E. postvittana* feeding. Therefore, it is likely that grapevine induced chemical defences were responsible for the increased consumption of Si+ leaves.

Green and Ryan (1972) first reported that, in response to insect herbivory, potato, *Solanum tuberosum* (L.) and tomato, *Solanum lycopersicum* (L.) plants activate local and systemic expression of proteinase inhibitors that impair digestive proteases in the insect gut. Proteins are very important for lepidopteran larvae nutrition, and tannin (a secondary metabolite enhanced by Si fertilisation) reduces the digestibility of proteins in the larval gut (Barbehenn & Martin, 1994; Broadway & Duffy, 1988; Jassbi, et al., 2008). More recent studies have also suggested that increased consumption of Si+ material is due to reduced digestion efficiency caused by induced plant defensive compounds. For example, Massey et al. (2006) reported significant reductions in the digestion efficiency of both *S. exempta* and *S. gregaria*; even though both species displayed increased consumption rates on some of the grass species tested, *S. exempta* and *S. gregaria* displayed reduced growth rates and lower pupal masses on the high Si diet. Reduced digestion efficiency was also suggested by Goussain et al. (2005) who found *Shizaphis graminum* (Rondani) (Hemiptera: Aphididae) ingested phloem sap for similar periods on Si+ and control wheat plants, however, honeydew excretion was highly reduced in insects feeding on Si+ plants. That study found a clear adverse effect on reproductive period and longevity which Goussain et al. (2005) suggested was due to a lower sap ingestion rate or higher sap retention inside the body.

Review of the literature reveals the increased pest damaged caused by *E. postvittana* to grape leaves in this study was not due to the fact grapevines are low Si-accumulators and, therefore, have lower levels of pest-resistance as a result of Si fertilisation compared to higher Si-accumulating species. Tissue analysis conducted on separate grapevines which underwent the same treatment as grapevines used in this study found total Si content of dry leaf matter averaged 0.315% in Si+ plants and 0.177% in Si- plants. Gatarayiha et al. (2010) reported lower Si content in their tested plant species, eggplant and bean (Si+: 0.057%; Si-: 0.001%, and Si+: 0.024%; Si-: 0.004% respectively). Yet that study reported significantly less leaf damage by *T. urticae* on Si+ compared to Si- plants. Gatarayiha et al. (2010) also reported *T. urticae* feeding on bean had significantly greater mortality in Si+ compared to Si- plants. Notably, it has been shown that some intermediate and high Si-accumulating plant species have received no beneficial effects from Si fertilisation on arthropod pests. Despite moderate Si content of dry leaf matter (Si+: 0.6444%; Si-: 0.4419%), *P. citri* was not adversely affected when fed on fiddleleaf fig leaves (Hogendorp, et al., 2009). Similarly, there was no adverse effect on *H. phaeopteralis* feeding on a variety of turfgrass species (Si+: 1.2% Si+; Si-: 0.6%) (Korndörfer, et al., 2004), or on *A. ipsilon* and *Cycloceohala* spp. also feeding on turfgrass (high Si+: 2.5%; low Si+: 1.2%; Si-: 0.5%) (Redmond & Potter, 2006). These results suggest the effects of Si fertilisation and plant defences vary considerably among different plant−pest combinations.

It is unlikely that the method of using excised grapevine leaves in this study, in place of intact plants, reduced the effect of induced chemical defences on *E. postvittana*. Prior studies which have used both intact and excised leaves have found no difference in results between the methods. For example, Moraes et al. (2005) found that the corn leaf aphid, *Rhopalosiphum maidis* (Fitch) (Hemiptera: Aphididae) had reduced preference in free-choice tests on both intact and detached Si-treated corn leaves compared to the control. Similarly, Korndörfer et al. (2004) found both intact and excised Si-treated turfgrass sheaths had no effect on *H. phaeopteralis* consumption or development compared to the control.

It is difficult to explain why larvae feeding on control leaves consumed significantly less over the 24 hour period and consequently lost a significant amount of weight. A possible explanation could be the water efficiency of Si+ grapevines was enhanced as a result of Si fertilisation (however, water content of plants was not tested in this study). The moisture content of a food source is fundamental to insect larvae growth (Rahmathulla, Tilak, & Rajan, 2006) and has been found to have a direct effect on ingestion, digestion, growth and development of silkworm, *Bombyx mori* (L.) (Lepidoptera: Bombycidae) feeding on mulberry, *Morus* spp. leaves (Paul, Subba Rao, & Deb, 1992; Rahmathulla, et al., 2006). Rahmathulla et al. (2006) found significantly higher larval moisture (79.78%), larval weight (65.65g), and pupal moisture (73.81%) was recorded on larvae feeding on

younger (high moisture) leaves compared to older (lower moisture) mulberry leaves, with a positive correlation between leaf moisture content and larval weight. Silicon fertilisation has been shown to influence stomata movement, improving water use efficiency in corn and wheat through reduction in the transpiration rate via the stomata (Gao, et al., 2004; Gong, et al., 2003). Therefore, in the present study, it is possible that Si+ grapevine leaves had contained higher leaf turgor than Si- leaves, making them more attractive to *E. postvittana* larvae.

The present study demonstrates the complex interactions between plants and arthropods, and the effect of Si on plant defence mechanisms is not yet fully understood. While the majority of research has shown that Si fertilisation reduces arthropod pest damage to plants, the results from the present study show that defence responses by varying plant species cannot be predicted. Identifying the effects of Si fertilisation on grapevine to *E. postvittana* larval growth and development, using plants of known water content, would be a logical continuation of the present study.

Chapter 3

Silicon enhances induced plant defence, attracting the predatory beetle, *Dicranolaius bellulus* (Guérin-Méneville) (Coleoptera: Melyridae) to grapevine plants infested with *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) larvae

3.1 Introduction

The benefits of Si fertilisation to agricultural crops, through enhanced plant defence mechanisms, have been well documented. Recently, work has demonstrated that Si fertilisation may extend induced chemical defences through the likely altered and enhanced production of HIPVs, resulting in increased plant attractiveness to predatory (beneficial) insects (Kvedaras, et al., 2010).

HIPVs are emitted by plants in response to arthropod pest attack. There is a lag time between the onset of herbivore feeding and the emission of HIPVs, with the emission rate increasing to a peak, then gradually dropping and stopping at the cessation of attack (Liu et al., 2006). Loughrin et al. (1997) measured grapevine leaf volatile production over three-hour intervals, during *Popillia japonica* (Newman) (Coleoptera: Scarabaeidae) feeding, with the first measurement taken 20 minutes after *P. japonica* was placed on the leaves. The production of the volatile compounds (*Z*)-3-hexenal, (*Z*)-3-hexenyl acetate, (*E*)-*β*-ocimene, and (*E*)-4,8-dimethyl-1,3,7-nonatriene peaked at 2.3 to 2.8 hours after feeding, which is believed to coincide with the greatest activity of *P. japonica* feeding. In contrast, Van den Boom et al. (2004) placed spider mites, *Tetranychus urticae* (Koch) (Arachnida: Tetranychidae), on grapevine, *Vitis vinifera* (L.) leaves for a period of eight days before testing. Their study did not assess HIPV production over time periods, however, GC-MS analysis revealed grapevines leaves released

several novel compounds after this period, including methyl salicylate (8%), (3*E*)-4,8-dimethyl-1,3,7-nonatriene (37%), *β*-caryophyllene (11%), and α-humulene (12%). The homoterpene, (3*E*)-4,8-dimethyl-1,3,7-nonatriene is believed to contribute to indirect plant defence by attracting natural enemies of pests (Lee et al., 2010). Notably, this compound was produced in concentrations four-times higher in pest-infested leaves compared to non-infested leaves.

The purpose of the current study was to further our understanding of the tri-trophic effects of Si fertilisation on grapevines, an important agricultural crop in Australia. Light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) are difficult to control in vineyards using conventional methods, and there is increased interest in IPM strategies using natural enemy populations to help manage these pests. *Dicranolaius bellulus* (Guérin-Méneville) (Coleoptera: Melyridae) is a generalist predator which feeds on small lepidopteran larvae, as well as insect eggs and pollen (Hawkeswood, 2011; Horne et al., 2000), and have been identified in Australian vineyards (Simpson et al., 2011a; Simpson et al., 2011b). Y-tube olfactometer bioassays were used to determine the effect of Si fertilisation on the attraction of *D. bellulus* to grapevines infested with *E. postvittana* larvae.

3.2 Materials and methods

Site and plant culture are the same as described in Chapter 2.

3.2.1 Insect culture

Epiphyas postvittana culture was the same as described in Chapter 2.

Adult *Dicranolaius bellulus* beetles were collected using a sweep net from lucerne crops near the town of Canowindra, in central-western NSW 24 hours prior to their use. Beetles were kept in mesh cages containing lucerne, *Medicago sativa* (L.), and an abundance of aphids and other arthropod species caught simultaneously in the sweep net. Cotton balls soaked in water provided additional moisture.

3.2.2 Treatment

The potting medium was treated every 14 days from 15 October 2009 to 18 March 2010 (totalling 12 doses) with 1L of either 1) liquid potassium silicate (K_2SiO_3; AGSIL 27, PQ Australia, Dandenong South, Australia) at the rate of 1.7 mM Si (100 ppm Si) mixed with deionised water (Si+), or 2) potassium chloride (KCl; Sigma Aldrich, Castle Hill, Australia) at the rate of 1.1 mM K mixed with deionised water (Si-) to balance potassium fertilisation to plants across the treatments. This resulted in Si+ pots receiving 0.273 ml K_2SiO_3/L of water.

3.2.3 Experimental Design

A laboratory olfactometer bioassay was conducted in April 2010. The experiment observed the tri-trophic effects of Si fertilisation, using a randomised design comparing Si+ and Si- pest-infested grapevines, with nine replicates. The third and fourth youngest true leaves of each plant were inoculated with seven *E. postvittana* larvae (third-fourth instar), and the pest-infested shoots of the plant were then enclosed within a mesh cage

Figure 3.1: Y-tube olfactometer bioassay set-up

(nylon netting, Megaview, Taiwan) to confine larvae to the plant. Pest-infested Si+ and Si- plants were kept in two separate plastic enclosures within a second glasshouse to prevent any possible 'cross-talk' between

plants. Olfactometer bioassays were run between 2.5 and 3.5 hours after pest inoculation.

Y-tube olfactometer (OLFM-YT-2425F, Analytical Research Systems, Gainsville, Florida, USA) bioassays were conducted to provide a two-way comparison between Si+ and Si- pest-infested plants (Figure 3.1). Each end of the Y-tube olfactometer was connected to a glass bell jar (205 mm i.d. x 320 mm height), with each jar containing a Si+ or Si- pest-infested potted vine. To enable comparison, an air stream (set at 200 mL min^{-1}) passed from each odour source through an arm of the Y-tube. Each bell jar stood in a dish of deionised water to ensure it was air-tight at the base. The mesh sleeve was removed from the plant prior to being placed in the bell jar, however, the larvae remained on the plant. To correct for possible directional bias, the allocation of the treatment and control was altered between the left and right arms of the Y-tube olfactometer with each repeat.

Adult male or female *D. bellulus* were randomly selected and introduced singly into the olfactometer port and observed for up to five minutes. The choice by the predator was recorded when it passed a join in the arm of the tube, 4 cm from the olfactometer intersection and did not backtrack for 30 seconds. It was otherwise recorded as a no-choice. Twenty predators were tested for each pair of plants, constituting a single run (replicate), and there were nine replicates overall. Predators were used once, and plants used for one replicate only.

The temperature in the olfactometer room ranged from 18.3^{o}C to 23.8^{o}C and humidity of the air in the olfactometer was stabilised from ambient levels (43% to 67%) by bubbling the airflow through water reservoirs. The room was kept in darkness except for a single fluorescent light box positioned centrally behind the olfactometer. After each replicate, the glass Y-tube and plastic tubing which carried the air to the Y-tube was washed in hot, soapy water, rinsed with hot water, then rinsed with 100% ethanol and oven dried at 60^{o}C.

3.2.4 Plant and potting medium analysis

Upon completion of the experiment, leaves were removed from each tested plant and dried in an oven at 40^{o}C for a period of seven days. Dried plant tissue was sent to a commercial analytical chemistry and testing laboratory (Environmental and Analytical Laboratories, Charles Sturt University, Wagga Wagga, Australia) for determination of total Si content. This was achieved using an acid digestion; 1 g of dried and ground sample was placed into a 50 ml plastic centrifuge tube, and 5 ml concentrated nitric acid was added. The capped tubes were placed into a 60^{o}C oven for 24 hours to digest. To reduce the amount of Si-contamination during the plant digestion process, the digest employed plastic tubing. The sample was diluted to 50 ml using deionised water and Si content determined using an argon-supported inductively coupled plasma-atomic emission spectrometer (ICP-AES; VistaPro, Varian Australia, Melbourne, Australia) (Haysom &

Chapman, 1975). One sample of unamended palm peat potting medium was analysed for plant-available Si content by a commercial, National Association of Testing Authorities (NATA) accredited laboratory (SGS Australia, Toowoomba, Australia) using 0.01M $CaCl_2$ extraction and ICP-AES determination (Haysom & Chapman, 1975).

3.2.5 Statistical analysis

A one-way analysis of variance was used to determine whether there was a difference in the Si content of leaves between the treatments. Insect choice data was analysed using an exact binomial test. Because plant tissue analysis showed high levels of variability of Si content, to the extent that the ranges for each treatment overlapped, insect responses were analysed using regression analysis. This employed a generalised linear model with a binomial distribution and a logit link to determine if there was a relationship between insect choice and leaf-Si content, and whether the relationship differed significantly between Si+ and Si- plants. All analyses were done using Genstat 12th Edition (Payne, et al., 2009).

3.3 Results

A pooled regression analysis of insect responses for all plants in both treatments against Si content in leaf tissue showed a greater attraction of *D. bellulus* to *E. postvittana*-infested plants with the highest Si tissue content (χ^2 = 0.035, *d.f.* = 1,16) (Figure 3.2).

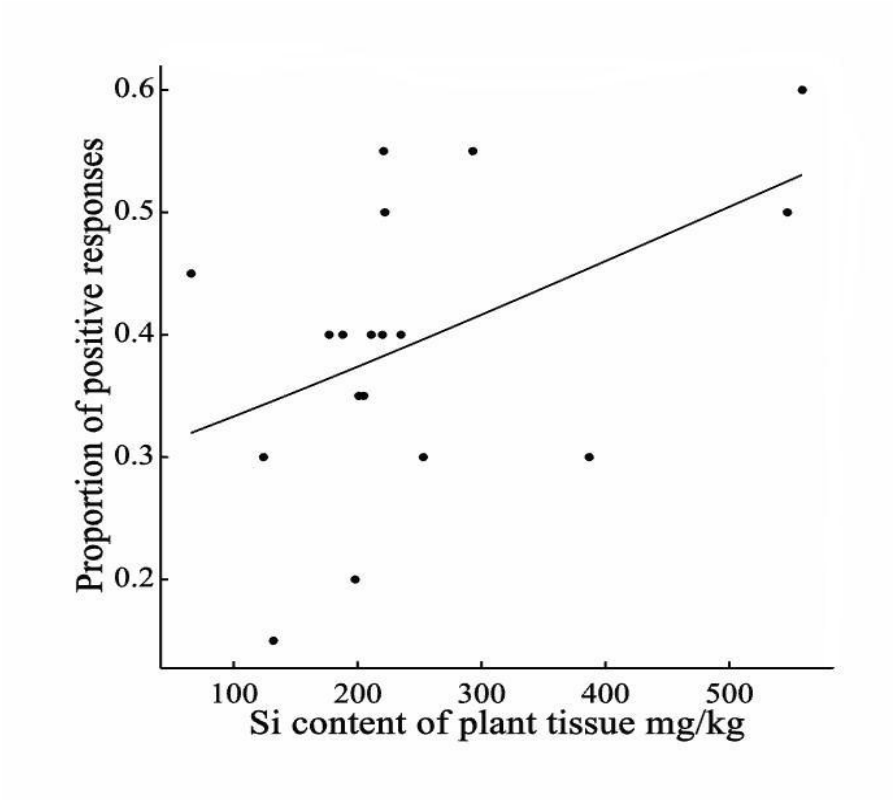

Figure 3.2: The effect of plant tissue silicon level on the attraction of *Dicranolaius bellulus* to *Epiphyas postvittana*-infested grapevines.

However, when comparing olfactometer choice data between Si+ and Si- plants, the exact binomial test showed no significant variation between treatments in individual runs ($P = 0.157$) (Figure 3.3).

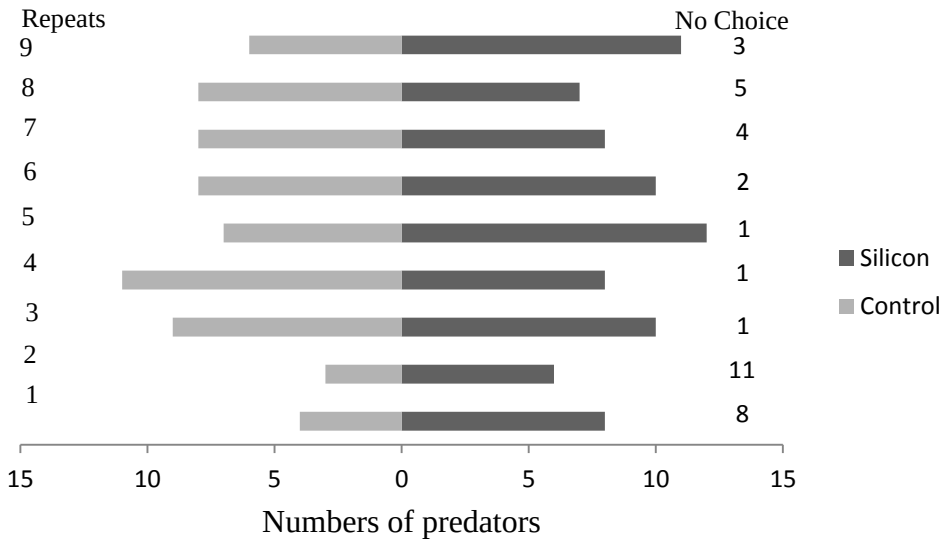

Figure 3.3: results from Y-tube olfactometer bioassays measuring *Dicranolaius bellulus* response to *Epipyhas postvittana*-infested grapevines. No choice are insects which did not choose either silicon-treated (Silicon) or untreated (Control) grapevine.

The mean total Si content of grapevine leaves was higher for Si+ plants (0.315% Si, range = 0.188-0.559% Si) than for Si- plants (0.177% Si, range = 0.065-0.253% Si) (F = 6.510, $d.f.$ = 1,8, P = 0.034). Analysis of plant-available Si in the unamended palm peat found it contained 74 mg/kg Si.

3.4 Discussion

This study found a positive relationship between plant tissue Si concentration and attraction of the predatory *D. bellulus* to *E. postvittana*-infested grapevines. These results are promising and suggest that Si altered the HIPV blend produced by the pest-infested plants. The results from this

study correspond with those from an earlier study by Kvedaras et al. (2010) who observed that Si-treatment significantly enhanced the attraction of *D. bellulus* to *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)-infested cucumber plants. Kvedaras et al. (2010) hypothesised that the increased attraction of *H. armigera* was due to Si altering the HIPV profile in the pest-infested plants. The results from the present study also correspond with the results from the study described in Chapter 2, suggesting Si fertilisation enhances the induced chemical defences in grapevines in response to *E. postvittana* feeding.

Silicon has been shown to enhance defence mechanisms in a range of plant species exposed to pest attack, and the level of plant resistance to pest attack may be correlated with its level of Si content. For example, Gatarayiha et al. (2010) found reduced two spotted spider mite, *Tetranychus urticae* (Koch) (Acari: Tetranychidae) damage in corn, cucumber, eggplant and bean treated with 20, 40, 80, and 160 ppm Si. That study found a significant dose effect of Si in all four plant species tested. Similarly, Keeping and Meyer (2006) investigated the effects of four Si sources, USA-originated calcium silicate, South Africa-originated calcium silicate, Slagment® (at the rates of 5,000 and 10,000 kg/ha), and fly ash (15,000 and 30,000 kg/ha) on boring damage and larval performance of *Eldana saccharina* (Walker) (Lepidoptera: Pyralidae) feeding on four sugarcane cultivars. That study found damage to sugarcane (cv. N30) and larval performance were

significantly reduced at the higher Si treatments compared to the lower treatment and control.

The results from the current study support earlier observations that the benefits of Si fertilisation are only apparent when plants are subjected to stress, including arthropod feeding (Gomes, et al., 2005; Kvedaras, et al., 2010). The present study found there was little *E. postvittana* damage evident on the grape leaves after 2.5-3.5 hours of feeding, although the results of this study show there was sufficient damage to trigger a HIPV response. Liu et al. (2006) stated optimum volatile production from grape leaves after insect attack was between 2.3 and 2.8 hours after feeding. However, Liu et al. (2006) was analysing results of a study by Loughrin et al. (1997) who used *P. japonica* beetles, which are larger and are likely to have caused more feeding-damage than the *E. postvittana* larvae. A possible reason why *E. postvittana* made little chewing damage on the grapevine leaves during this period could be due to the fact that *E. postvittana* larvae were raised on an artificial diet and not exposed to grapevine leaves prior to the experiment. It has been found that the diet of an insect prior to an experiment affects their feeding preference (Ettifouri & Ferran, 1993; Henaut, Alauzet, Ferran, & Williams, 2000).

Importantly, the present experiment has found results consistent with earlier studies in regard to the tri-trophic effects of Si fertilisation. Equally importantly, this study found a similar defence response from a low Si-

accumulator (grapevine) to that of an intermediate Si-accumulator (cucumber) tested in the study by Kvedaras et al. (2010). These findings bring us further in our understanding of an exciting and potentially useful method of arthropod pest control for integrated pest management systems. The potential for using Si fertilisation as an elicitor of induced plant defences, enhancing the attraction of beneficial arthropods into pest-infested crops, whilst simultaneously providing other constitutive benefits to plants, offers a holistic approach to pest management. Further research is warranted to identify and quantify volatiles emitted from pest-infested and intact Si treated grapevines, which will provide further insight into the effects of Si fertilisation on HIPV production of plants.

Chapter 4

The effects of silicon fertilisation on the induced defences of grapevine to grapevine moth, *Phalaenoides glycinae* (Lewin) (Lepidoptera: Noctuidae): HIPV emission and attraction to the predatory green lacewing, *Mallada signata* (Schneider) (Neuroptera: Crysopidae)

4.1 Introduction

It has been well documented that predatory and parasitic arthropods use HIPVs to locate food and/or hosts (Dicke, Gols, Ludeking, & Posthumus, 1999; Kost & Heil, 2005; Moraes et al., 2009; Ozawa et al., 2004). HIPVs include terpenoids (homo-, mono-, di-, sesquiterpenoids), green leaf volatiles (GLVs), and phenylpropanoid aromatic compounds, as well as certain alkanes, alkenes, alcohols, esters, aldehydes, and ketones (Arimura, et al., 2005; Arimura, et al., 2009; Baldwin, et al., 2006; Holopainen, 2004; Pichersky & Gershenzon, 2002; Wu & Baldwin, 2009).

Terpenoids are important volatiles, estimated to constitute more than half the amount of volatile compounds emitted by plants in response to herbivore feeding (Dudareva & Pichersky, 2006; Guenther et al., 1995; Maffei, 2010; Mumm, et al., 2008; Pichersky & Gershenzon, 2002). They are used by beneficial arthropods to locate their prey or host, and they have been found to have direct anti-feedant effects (Jassbi, et al., 2008), as well as detrimental effects on the development of pest insect larvae (Dicke, 1994; Dudareva & Pichersky, 2006; Frost et al., 2008; Hilker & Meiners, 2006; Paré & Tumlinson, 1999; Pichersky & Gershenzon, 2002; Turlings & Tumlinson, 1991). Terpenoids also prime neighbouring plants (Baldwin, et al., 2006; Karban, 2001), inducing them to emit volatiles of their own (Arimura et al., 2000; Engelberth, et al., 2004; Karban & Shiojiri, 2009). Similarly, GLVs directly affect arthropod pests and/or act as signalling

molecules which elicit defence reactions in plants (Arimura, Ozawa, Horiuchi, Nishioka, & Takabayashi, 2001; Bate & Rothstein, 1998; Engelberth, et al., 2004; Farag & Paré, 2002; Hildebrand, Brown, Jackson, & Hamilton-Kemp, 1993). GLVs are released immediately upon wounding of green plant tissue, and consist of saturated and monounsaturated six-carbon aldehydes, formed by the enzymatic degradation of unsaturated fatty acids (Ruther, 2000). Aromatic compounds are very important signalling molecules and are commonly used in cropping environments to trigger plant endogenous HIPVs (James, 2005; James, Castle, Grasswitz, & Reyna, 2005; James & Price, 2004; Simpson et al., 2011a; Simpson et al., 2011b). Similar to terpenoids and GLVs, aromatic compounds have also been found to repel pests (Birkett et al., 2000).

Using gas chromatography-mass spectrometry (GC-MS) analysis, Loughrin et al. (1997) found that grapevines, *Vitis labrúsca* (Linnaeus) infested with Japanese beetle, *Popillia japonica* (Newman) (Coleoptera: Scarabaeidae) released the volatile compounds (*Z*)-3-hexenal, (*Z*)-3-hexenyl acetate, hexanal, (*E*)-2-hexenal, (*Z*)-3-hexenol, hexanol, (*E*)-2-hexenol, hexyl acetate, (*E*)-2-hexenyl acetate, and (*Z*)-3-hexenyl benzoate (GLVs), limonene, (*E*)-*β*-ocimene, linalool, (*E*)-4,8-dimethyl-1,3,7-nonatriene, (*E,E*)-α-farnesene, nerolidol (terpenes), benzyl alcohol (alcohol), methyl salicylate and indole (aromatic compounds). The study found 104 ng of methyl salicylate per plant was released in the first day of feeding damage, compared with 8.5 ng from un-infested vines, and 548 ng of (*Z*)-3-hexenyl

acetate in the first day compared with 17.4 ng. Also using GC-MS analysis, Van den Boom et al. (2004) revealed that grapevines, *Vitis vinifera* (L.) infested with spider mites, *Tetranychus urticae* (Koch) (Arachnida: Tetranychidae) released several compounds, including methyl salicylate, (3*E*)-4,8-dimethyl-1,3,7-nonatriene, *β*-caryophyllene, and *α*-humulene. However, it remains largely unknown whether the attraction of predatory and parasitic arthropods is to a specific compound, or to the ratio of compounds, in the volatile blend.

The Australian grapevine moth, *Phalaenoides glycinae* (Lewin) (Lepidoptera: Noctuidae) is native to Australia and their larvae feed on a variety of native and exotic plant species, including grapevines. The predatory green lacewing, *Mallada signata* (Schneider) (Neuroptera: Crysopidae) are also native to Australia and their larvae feed on many serious grapevine pests, including small lepidopteran larvae and eggs, and scale (Coccoidae). Significantly, *Mallada signata* are mass produced in Australia for inundative biological control in vineyards (and other crops) (Bugs for Bugs, 2010), yet there has been very little research on their response to plant volatiles. Grapevines are an important agricultural crop in Australia and there are great potential benefits of being able to improve the plant's own constitutive and induced defences against arthropod pests through Si fertilisation.

The research study described in Chapter 3 found increasing Si-content in grapevine leaves significantly enhanced the attraction of the predatory red and blue beetle, *Dicranolaius bellulus* (Guérin-Méneville) (Coleoptera: Melyridae) to grapevines infested with light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) larvae. Therefore, the purpose of the present study was to expand on the study described in Chapter 3 and determine 1) whether Si fertilisation affects the attraction of *M. signata* to grapevines, *V. vinifera* cv. Chardonnay, infested with *P. glycinae*, and 2) whether Si fertilisation alters the volatile production from grapevines as a result of *P. glycinae*-infestation.

4.2 Materials and methods

4.2.1 Site

The grapevine cuttings were grown in a heat-bed, located at the Industry and Investment NSW Research Station, Bathurst, in the Central Tablelands of New South Wales, Australia (33^{o} 25'47.49"S, 149^{o}33'23.87"E) from 14 May to 15 July 2010. The potted grapevines were grown in a glasshouse located at the Charles Sturt University Horticulture Centre, Orange campus (as described in Chapter 2) from 15 July 2010 to 10 January 2011.

4.2.2 Plant culture

Hardwood bud cuttings were taken from dormant grapevine plants grown at the Charles Sturt University Orange Vineyard in 13 May 2010. The strike-rate of grapevine cuttings was improved by encouraging the formation of roots while keeping the top of the cutting cool and dormant (Mullins & Rajasekaran, 1981); uniform cuttings of four to six nodes were collected at pruning from dormant grapevine canes, sealed in a plastic bag, and stored at 4^{o}C until required. The cuttings were dipped in a hormone rooting gel (Rootex-G®, SS Laboratories, Bayswater, Victoria, Australia) and placed in a thermostatically-controlled heat-bed, set at 20-25^{o}C. The heat bed was situated in a well-ventilated shed with partially enclosed walls, and exposed to an outside temperature range of 2.9-16.6^{o}C (May 2010), 1.5-12.8^{o}C (June 2010), and 1.0-12.5^{o}C (July 2010) (Australian Bureau of Meteorology, 2010) (Figure 4.1). A 50:50 perlite (coarse grade, Chillacoe Perlite, Mareeba, Queensland, Australia) and sand (superfine washed pure sand, Hothams, Bathurst, New South Wales, Australia) medium was used as the rooting medium (Jeffrey et al., 1997). The rooting-medium was kept moist by regular light watering using deionised water (see Chapter 2). The deionised water was analysed for plant-available Si content in October 2010 by SGS Australia (using methods described in Chapter 2). After eight weeks the dormant cuttings had produced roots, termed ‘pre-rooted’ cuttings (Mullins & Rajasekaran, 1981).

Figure 4.1: cuttings in the heat bed, June 2010

Figure 4.2: Potted grapevines in the glasshouse, November 2010

The pre-rooted cuttings were potted into 12 cm diameter (1L) pots containing medium grade perlite, vermiculite (Exfoliators (Aust.) Pty Ltd, Dandenong, Victoria; perlite contained 50-80% SiO_2, vermiculite 38-46% SiO_2), and palm peat (Magic Soils Pty Ltd, Clayton North, Australia) at the ratio of 6:3:1 respectively. On 15 July 2010 the potted grapevines were transferred to the glasshouse at Orange; temperature set at 26^{o}C, 16:8 light:dark photoperiod (Geny, Broquedis, Martin-Tanguy, & Bouard, 1997), irradiance 100 Wm^{-2} (Figure 4.2). Grapevines were fertilised with a Si-free compound fertiliser (Miracle-Gro® Water Soluble All Purpose Plant Food, Scotts, Baulkham Hills, NSW, Australia) and Magnesium Sulphate – Epsom Salt® (Manutec, Cavan, SA, Australia) mixed with deionised water as per manufacturer recommendations. The fertiliser mix was applied at the rate of

250 ml per pot every seven days. Plants were pruned to a single shoot and were watered, as needed, using deionised water.

4.2.3 Insect cultures

Commercially-available *M. signata* were purchased as eggs and young larvae (Bugs for Bugs, Mundubbera, Queensland). Larvae were reared in mesh cages at 21-24^{o}C, relative humidity 30-62%, and were provided *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) eggs as food and cotton balls soaked in water for additional moisture. Adults were fed a 50:50 mixture of peanut butter and honey smeared onto paper towel and covered with a fine gauze to prevent insects sticking to the food. One week prior to the trial, *P. glycinae* larvae were collected from a private vineyard located near Orange and were kept in mesh cages containing untreated potted grapevines.

4.2.4 Treatment

The potting medium was treated every 14 days from 23 July to 26 November 2010 (totalling 10 doses) with 250 ml of one of two treatments; 1) liquid potassium silicate (K_2SiO_3; AGSIL 27, PQ Australia, Dandenong South, Australia) at the rate of 1.7 mM Si (100 ppm Si) mixed with deionised water (Si+), or 2) potassium chloride (KCl; Sigma Aldrich, Castle Hill, Australia) at the rate of 1.1 mM K mixed with deionised water (Si-) to

balance potassium fertilisation to plants across the treatments. This resulted in Si+ pots receiving 0.273 ml K_2SiO_3/L of water.

4.2.5 Experimental design

In October 2010 samples were taken from unused potting medium and were analysed for plant-available Si using 0.01M $CaCl_2$ extraction method and ICP-AES analysis (see Chapter 3) (Haysom & Chapman, 1975). In December 2010 grapevine leaf samples (using every second leaf starting with the oldest leaf) were taken across both treatments (four x Si+, four x Si-) to determine total Si content. Leaves were dried in an oven at 40^{o}C for 4 days, then analysed for total Si using rapid, wet oxidation procedure (Haysom & Ostatek-Boczynski, 2006) and ICP-AES analysis. All plant and soil analysis was conducted by SGS Australia (see Chapter 3). In December 2010 the pH of ten Si+ and ten Si- potting mediums was taken using a pH meter (Inoculo Laboratories, Box Hill North, Australia).

The experiment observed the tri-trophic effects of Si fertilisation, and compared randomly selected Si+ and Si- grapevines, using four temporal replicates. All plants were inoculated with five *P. glycinae* (third to fifth instar) larvae, with the first larvae placed on the third youngest leaf, and a single larvae per leaf placed on each consecutively older leaf. The pots were individually placed in a glass bell jar (205 mm i.d. x 320 mm h.) to confine the larvae to the plant and to trap any volatiles emitted. Two bell

jars containing randomly selected Si+ and Si- grapevines were placed in a research laboratory which had temperature and lighting set to match the glasshouse. Twenty-four hours after inoculation, the volatiles emitted from *P. glycinae*-infested grapevines were adsorbed onto solid phase micro-extraction (SPME) fibres.

SPME/GC-MS analysis

Single SPME fibres (Supelco fibre PDMS red; Bellefonte, Pennsylvania, USA) were inserted into each glass bell jar (containing either Si+ or Si- plant) for one hour to adbsorb the grapevine volatiles. The fibres were then analysed using GC-MS (GC-17A Gas Chromatograph and QP5050A Gas Chromatograph Mass Spectrometer, Shimadzu, Rydalmere, NSW, Australia) using helium (1 ml/min) as the carrier gas. The GC was equipped with a DB-5 ms column (30 m x 0.25 μm i.d., 0.25 μm film thickness). Volatiles were directly desorbed from fibres in the GC injector at 250^{o}C for 3 min, in splitless mode. Following injection, the column was programmed from 40^{o}C held for 2 min, 6^{o}C/min to 250^{o}C and hold for 5 min, then 40^{o}C/min to 280^{o}C and hold for 5 min. All compounds were analysed by the MS set in the scan mode, interface temp 260^{o}C, 0.2 sec interval, start time 3 min, end time 47 min, start m/z 41, end m/z 450, and scan speed 2000. The SPME fibres were desorbed (cleaned) immediately prior to each volatile collection by completing the same 47 min program as used for volatile analysis.

To identify the volatile compounds emitted by *P. glycinae*-infested Si+ and Si- grapevines, retention indices (Kovats Index; KI) were calculated with the use of a homologous series of *n*-alkanes with a chain length from C8 to C20 (Table 4.1).

Table 4.1: List of *n*-alkane standards used for GC-MS identification of volatile compounds.

Carbon Number	Compound
C8	*n*-octane
C9	*n*-nonane
C10	*n*-decane
C11	*n*-undecane
C12	*n*-dodecane
C13	*n*-tridecane
C14	*n*-tetradecane
C15	*n*-pentadecane
C16	*n*-hexadecane
C17	*n*-heptadecane
C18	*n*-octadecane
C19	*n*-nonadecane
C20	*n*-eicosane

The range of *n*-alkanes used in this study is suitable for identifying plant HIPVs (An, 2010). The identification of the compounds was confirmed on the basis of the uniformity of their retention times and mass spectra with standard substances (Adams, 2007). In addition, identification was confirmed with the retention indices of identified compounds reported in the literature.

Quantification of volatiles produced from grapevines was determined by measuring the area of the highest 20 peaks. This was achieved by integrating peaks which fell between 5 and 30 minutes retention time, and disregarding the highest peak which represented volatiles from the rubber plug used to prevent air escaping the glass bell jar. The integration peak width was set at 2 seconds, minimum area height at 0, using a standard smoothing method. A raw spectrum mode was set at 'peak top spectrum' (number of average points = 3), and background spectrum mode set at 'calculated from peak' (number of average points = 1).

Y-tube olfactometer bioassays

Immediately after removing the SPME fibre from the bell jar, the attraction of *P. glycinae*-infested grapevines to naïve (ie, the herbivores had no experience with the prey) adult *M. signata* was detected using a Y-tube olfactometer (as described in Chapter 3). The Y-tube olfactometer bioassays provided a two-way comparison between a Si+ *P. glycinae*-infested grapevine and a Si- *P. glycinae*-infested grapevine. A randomly selected male or female adult *M. signata* was introduced individually into the Y-tube olfactometer port and observed for up to five minutes, using the same recording criteria employed in Chapter 3. Twenty predators were tested for each pair of plants, constituting a single run. Predators were used once, and plants used for one run only. To correct for possible directional

bias, the allocation of the treatment and control was alternated between the left and right sides of the olfactometer with each repeat.

The temperature in the olfactometer room ranged from 21.4^{o}C to 24.3^{o}C and humidity of the air in the olfactometer was stabilised from ambient levels (60% to 71%) by bubbling the airflow through water reservoirs. The room was kept in darkness except for a single fluorescent light box positioned centrally behind the olfactometer. After each replicate, the glass Y-tube and plastic tubing which carried the air to the Y-tube was washed in hot, soapy water, rinsed with hot water, then rinsed with 100% ethanol and oven dried at 60^{o}C. All bioassays were run between 9.00 am and 5.00 pm to minimise any variation in diurnal and nocturnal behaviour by the insects.

4.2.6 Statistical analysis

A one-way analysis of variance (ANOVA) was used to determine whether there was a difference in the Si content of leaves between Si+ and Si- grapevines. Unbalanced two-way ANOVA provided predicted means of the quantity of volatile produced (% area) for the treatments. Least significant difference (LSD) tests compared the average Si+ and Si- area within each individual compound. Olfactometer data was analysed using generalised linear model (GLM) regression reporting the cumulative number of predators which was fitted to the choice the predator made. All analysis was done using Genstat 12th Edition (Payne, et al., 2009).

4.3 Results

4.3.1 Climate conditions experienced in glasshouse

Potted grapevines within the glasshouse experienced a temperature range of 10-33^{o}C, and relative humidity of 22-100%.

4.3.2 Si content in deionised water, potting medium and plant tissue

The deionised water contained 5 mg/L plant-available Si. The unamended potting medium measured an average of 110.5 mg/kg plant-available Si, pH 6.2. Analysis of grapevine leaf tissue samples found the mean total Si content of Si+ leaves (0.217% Si, range = 0.20-0.23% Si) was greater than for Si- leaves (0.132% Si, range = 0.13-0.14% Si) (F = 157.64, $d.f.$ = 1,6, P < 0.001). The pH of Si+ potting mediums averaged 7.0 (range = 6.8-7.2) and Si- potting mediums averaged 6.9 (range = 6.7-7.1).

4.3.3 SPME/GC-MS analysis of volatiles

Twenty major compounds were collected from the headspace of Si+ and Si- grapevines by SPME, among which seven compounds were consistently identified in all repeats (Figure 4.3). These compounds were the monoterpenes, *cis*-thio rose oxide and *cis*-2,3-pinanediol, the sesquiterpenes α-humulene and 9-*epi*-(*E*)-caryophyllene, and the alkanes *n*-pentadecane, *n*-hexadecane, and *n*-heptadecane. *N*-heptadecane was produced in significant

amounts only by Si+ plants. All other compounds were produce from both Si+ and Si- grapevines.

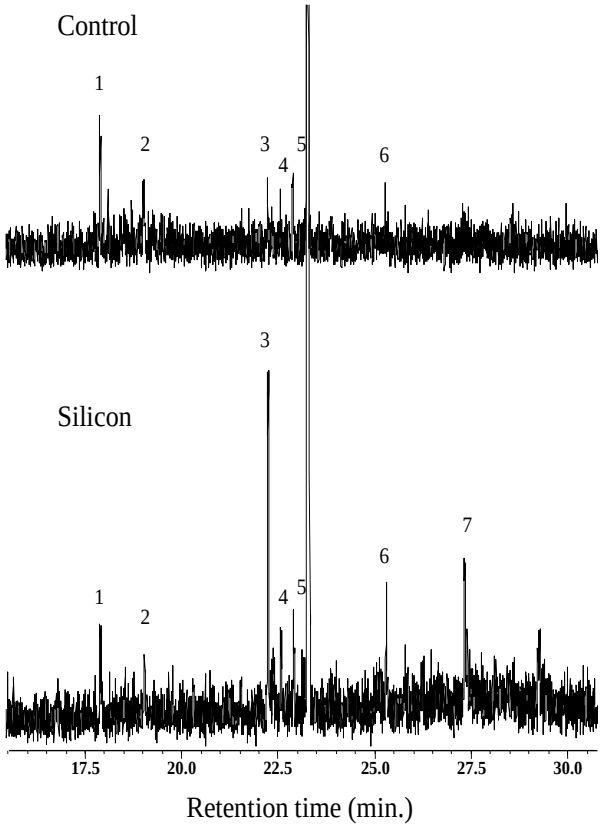

Figure 4.3: Comparison of chromographic profiles of volatiles released by silicon-treated and untreated (control) grapevines infested with *Phalaenoides glycinae* larvae for 24 hours. The identities of the various compounds are, 1: *cis*-thio rose oxide; 2: *cis*-2,3-pinanediol; 3: *α*-humulene; 4: 9-*epi*-(*E*)-caryophyllene; 5: *n*-pentadecane; 6: *n*-hexadecane; 7: *n*-heptadecane.

Cis-thio rose oxide was produced in significantly greater amounts in Si- grapevines compared to Si+ (F = 13.07, $d.f.$ = 1,5, P = 0.015). Within treatments, the quantities of volatile emission varied among the identified compounds (F = 4.28, $d.f.$ = 1,6, P = 0.004), however, there was no overall

signifcant difference between the treatments (F = 0.25, $d.f.$ = 1,6, P = 0.623) (Table 4.2).

Table 4.2: Prediction of quantification values (% Area) of compounds emitted from silicon-treated (Si+) and untreated (Si-) grapevines infested with *Phalaenoides glycinae* larvae for 24 hours. Numbers within the same row followed by the same letter do not differ significantly ($P < 0.05$).

Identified Compound	Compound Class	Kovats Index	Prediction % Area	
			Si+	Si-
cis-thio rose oxide	Monoterpene	1276	7.12[a]	14.49[b]
cis-2,3-pinanediol	Monoterpene	1320	6.40[a]	7.83[a]
α-humulene	Sesquiterpene	1454	10.37[a]	8.21[a]
9-*epi*-(*E*)-caryophyllene	Sesquiterpene	1466	3.32[a]	4.56[a]
n-pentadecane	Alkane	1500	9.02[a]	9.14[a]
n-hexadecane	Alkane	1600	5.83[a]	5.29[a]
n-heptadecane	Alkane	1700	6.07[b]	0.00[a]

4.3.4 Olfactometer bioassays

GLM analysis found no difference in the choice of *M. signata* between treatments (P = 0.227), with a total of 34 out of 80 insects choosing Si+ plants and 35 out of 80 insects choosing Si- plants (Figure 4.4).

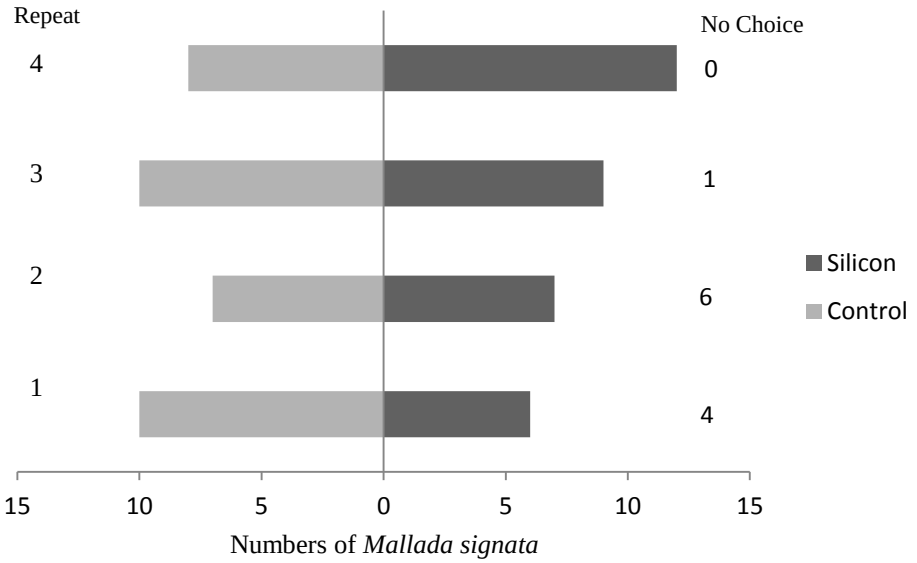

Figure 4.4: Behavioural responses of *Mallada signata* to volatiles produced from silicon-treated versus untreated (control) grapevines infested with *Phalaenoides glycinae* for 24 hours.

4.4 Discussion

Research into the tri-trophic effects of Si fertilisation is very new, and the present study is the first to examine the effects of Si on volatile emissions. This study identified seven volatile compounds emitted from Si+ *P. glycinae*-infested grapevines and six compounds from Si- plants. Of the seven identified compounds, six were novel compounds, not previously identified in the literature as emitted from pest-infested grapevines, while *α*-humulene was identified in Van den Boom et al. (2004). *N*-heptadecane was produced only by Si+ plants, and *cis*-thio rose oxide was produced in greater quantities by Si- plants compared to Si+ plants (Table 2). Despite the effect

of Si on grapevine volatiles, with the production of one compound apparently suppressed and that of another triggered, *M. signata* was equally attracted to infested grapevines from both treatments. The study described in Chapter 3 found increasing Si content in grapevine leaf tissue enhanced *D. bellulus* attraction to *E. postvittana*-infested grapevines, which was attributed to Si fertilisation altering the HIPV profile of pest-infested plants. The results from the present study are, therefore, a significant step in substantiating that hypothesis.

The present study identified terpenoids and alkanes emitted from *P. glycinae*-infested grapevines. Silicic acid is reported as being an elicitor for systemic stress signals such as SA and JA, associated with HIPV production (Fauteux, et al., 2005). The mode of tissue damage affects the volatiles produced by pest-infested plants, and the extensive tissue damage caused by lepidopteran larvae induces volatiles produced using the JA signalling pathway (Howe & Jander, 2008; Smith & Boyco, 2007). HIPVs typically induced by herbivory include GLVs, methyl salicylate, phenylpropanoids and terpenoids (Arimura, et al., 2008; Mumm, et al., 2008). Terpenoid formation is the result of specific defence genes activated by oxylipin compounds (for example, JA) triggered by herbivore-stimulated plant responses (Arimura, et al., 2008; Arimura, et al., 2004). Terpenoid volatiles are produced from the MEP and MVA pathways, in response to phytophagous feeding (Arimura, et al., 2000; Bolter, Dicke, van Loon,

Visser, & Posthumus, 1997; De Moraes, Mescher, & Tumlinson, 2001; Lou, Ma, & Cheng, 2005; Loughrin, et al., 1997; Mumm, et al., 2008; Van Den Boom, et al., 2004). For example, Loughrin et al. (1997) reported several terpenoid volatiles emitted from grapevines, *Vitis labrúsca* (L.) infested with Japanese beetles, *Popillia japonica* (Newman) (Coleoptera: Scarabaeidae). Similarly, Mumm et al. (2008) identified terpenoids emitted from brussel sprout, *Brassica oleracea* (L.) plants infested with *Pieris brassicae* (L.) (Lepidoptera: Pieridae) or *Pieris rapae* (L.) (Lepidoptera: Pieridae) larvae, and lima bean, *Phaseolus lunatus* (Fabaceae) infested with *Tetranyichus urticae* (Koch) (Acari: Tetranychidae). The study by Mumm et al. (2008) found the predatory mite, *Phytoseiulus persimilis* (Athias-Henriot) (Acari: Phytoseiidae) was more attracted to pest-infested lima bean volatile emissions containing terpenoids compared to the volatile emissions which had terpenoid production suppressed. In contrast, the same study found no such preference for terpenoids by the larval parasitoid, *Cotesia glomerata* (L.) (Hymenoptera: Braconidae) to the pest-infested brussel sprout plants.

There has been limited work identifying volatiles emitted from pest-infested grapevines. Van den Boom (2004) identified terpenoids and aromatic compounds emitted from pest-infested grapevines, while Loughrin et al. (1997) identified aliphatic aldehydes, alcohols, esters, terpenoids and aromatic compounds. The sesquiterpene, α-humulene (identified in the

present study) is commonly emitted from a range of plant species attacked by insects of varying feeding guilds (Bolter, et al., 1997; De Moraes, et al., 2001; Van Den Boom, et al., 2004). De Moraes et al. (2001) found *α*-humulene released from tobacco, *Nicotiana tabacum*, plants infested with larvae of the lepidopteran species, *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae) and *Manduca sexta* (L.) (Lepidoptera: Sphingidae). The monoterpene, *cis*-thio rose oxide, that was apparently suppressed in Si+ plants, has been identified in emissions from the berries of *V. vinifera* cv. Muscat (Schwab, Davidovich-Rikanati, & Lewinsohn, 2008), and *cis*-2,3-pinanediol has been identified in emissions from the flowers, *Achillea monocephala* (Gogus, Ozel, & Lewis, 2006) and *Opuntia ficus-indica* (De Leo, Bruzual De Abreu, Pawlowska, Cioni, & Braca, 2010). However, neither *cis*-thio rose oxide nor *cis*-2,3-pinanediol have been reported as emitted as a result of pest infestation, and therefore may not be HIPVs.

The sesquiterpene, 9-*epi*-(*E*)-caryophyllene has been identified as an essential leaf oil of *Protium heptaphyllum* (Pontes et al., 2007). Pontes et al. (2007) found that *T. urticae* exposed to the leaf oils of *P. heptaphyllum* (consisting of 21.4% 9-*epi*-(*E*)-caryophyllene) resulted in significant mortality and reduced fecundity. The present study also identified the alkanes, *n*-pentadecane, *n*-hexadecane, and *n*-heptadecane in the grapevine volatile blends. Alkanes are commonly found in the volatile emissions of a

range of plant species as a result of herbivore feeding (De Leo, et al., 2010; Lou, et al., 2005; Lu, Wang, Lou, & Cheng, 2006; Moraes, et al., 2009; Pareja, et al., 2007). Lu et al. (2006) identified enhanced levels of *n*-pentadecane, *n*-hexadecane, and *n*-heptadecane in the volatile blend emitted from rice plants infested with the rice brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae).

The absence of GLVs from the volatile blend in the present study may be due to the fact that GLVs are released rapidly at the beginning of herbivore damage (D'Auria, Chen, & Pichersky, 2002; Ruther, 2000) and the present study measured volatiles emitted after a 24 hour period of larval feeding. This time-period was chosen because an earlier study (described in Chapter 3) found three hours of infestation of *E. postvittana* larvae on grapevines resulted in little feeding damage. Therefore, in the current study, the aim was to maximise feeding damage. However, Mumm et al. (2008) identified GLVs, as well as terpenoids and methyl salicylate, emitted from brussel sprouts after 48 hours infestation with *Pieris brassicae* (L.) (Lepidoptera: Pieridae) larvae. There have also been several studies which have identified enhanced volatile production after period greater than 24 hours of herbivore feeding (Arimura, et al., 2004; Brilli et al., 2009; Van Den Boom, et al., 2004).

The results from olfactometer bioassays conducted in the present study, however, do not correspond with the earlier study described in Chapter 3 or

the study by Kvedaras et al. (2010). There have been many studies observing the response of generalist predatory lacewing species to a variety of volatile cues (Han & Chen, 2002; James, et al., 2005; Lee, 2010; Yu, Zhang, Wu, Gao, & Gou, 2008; Zhu et al., 2005). However, the study described in Chapter 5 is the first to observe the response of the Australian native *M. signata* to synthetic HIPVs. The lack of significant response by *M. signata* to volatile cues in the present study could have been due to the fact that naïve adults were used. It has been found that the response of generalist carnivorous arthropods to volatiles is a learned process, particularly for those species whose feeding substrate is subject to temporal variability (Smid, 2006; Steidle & van Loon, 2003; Takabayashi, et al., 2006).

The present study has identified volatile compounds emitted from grapevines as a result of *P. glycinae*-infestation and, excitingly, has found a significant variation between the emissions from Si+ and Si- plants. However, the tri-trophic effects of Si fertilisation have only just begun to be investigated and further research into this potentially important method of pest management is warranted.

Chapter 5

Response of the green lacewing, *Mallada signata* (Schneider) (Neuroptera: Chrysopidae) to synthetic herbivore-induced plant volatiles

5.1 Introduction

Plants emit HIPVs as an induced, systemic defence when responding to arthropod attack (Arimura, et al., 2004; De Moraes, et al., 2001). The HIPVs attract natural enemies and may also induce neighbouring plants to emit volatiles and so constitute a form of communication between and amongst surrounding plants and arthropods (Engelberth, et al., 2004; Karban, 2001).

The sesquiterpene *β*-caryophyllene, the green leaf volatile (*Z*)-3-hexenyl acetate, and the phenolic compound methyl salicylate are common volatiles identified in HIPV blends emitted by many plant species as a result of herbivore attack. *β*-caryophyllene has been identified in the HIPV blend of grapevine, *Vitis vinifera* (L.) (Van Den Boom, et al., 2004), corn, *Zea mays* (L. cv. Delprim) (Gouinguene & Turlings, 2002), pear trees, *Pyrus communis* (L. cv. Conference) (Scutareanu, Drukker, Bruin, Posthumus, & Sabelis, 1997), potato, *Solanum tuberosum* (L. cv. Surprise) (Bolter, et al., 1997), lima beans, *Phaseolus lunatus* (L.) (Arimura, et al., 2000), and alfalfa (or lucerne), *Medicago sativa* (L.) (Zhu, et al., 2005). (*Z*)-3-hexenyl acetate has been identified in grapevine, *Vitis labrúsca* (L.) (Loughrin, et al., 1997), corn *(Gouinguene & Turlings, 2002)*, lima beans (Arimura, et al., 2000; Shimoda, 2010), potato (Bolter, et al., 1997), tea, *Camellia sinensis* (L.) (Han & Chen, 2002), and alfalfa (Zhu, et al., 2005). Methyl salicylate has been identified in the HIPV blends emitted by grapevines (Loughrin, et al., 1997; Van Den Boom, et al., 2004), pear (Scutareanu, et al., 1997), lima

beans (Arimura, et al., 2000; Shimoda, 2010), potato (Bolter, et al., 1997), soybeans, *Glycine max* (L. Merr) and alfalfa (Zhu & Park, 2005).

Using GC-MS analysis, Loughrin et al. (1997) found that *V. labrúsca* infested with Japanese beetle, *Popillia japonica* (Newman) (Coleoptera: Scarabaeidae) released a range of novel volatile compounds, including (*Z*)-3-hexenyl acetate and methyl salicylate. The study found 548 ng (*Z*)-3-hexenyl acetate in the first day of feeding damage compared with 17.4 ng from un-infested vines, and 104 ng methyl salicylate compared with 8.5 ng. Also using GC-MS analysis, Van den Boom et al. (2004) revealed that *V. vinifera* L. infested with spider mites, *Tetranychus urticae* (Koch) (Arachnida: Tetranychidae) released several novel compounds, including *β*-caryophyllene and methyl salicylate.

In Australia, the native predatory green lacewing, *Mallada signata* (Schneider) (Neuroptera: Chrysopidae) is an important generalist predator (Canard, 2001). There have been many studies reporting the response of various Chrysopidae species to HIPVs, however, no information is available for *M. signata*. Studies observing Chrysopidae response to various HIPVs vary with lacewing species (Table 5.1), so cannot be relied upon to predict the response of *M. signata* to HIPVs.

Table 5.1: Summary of previous studies observing Chrysopidae response to HIPVs.

Predator	Methyl salicylate	(*Z*)-3-hexenyl acetate	*β*-caryophyllene	Reference
Chrysopidae	Attracted $^{\Delta}$			Lee, 2010
Chrysopa nigicornis	Attracted $^{\Delta}$	Not attracted $^{\Delta}$		James, 2003
	Attracted $^{\Delta}$			James et al., 2005
	Attracted $^{\Delta}$			James and Price, 2004
	Attracted $^{\Delta\Delta}$	Not attracted $^{\Delta\Delta}$		Jones et al., 2011
Chrysoperla carnea			Attracted	Flint et al., 1979
			Low response* Low attraction$^{\Delta}$	Zhu et al., 1999
	Not responsive*	Not responsive*	Not responsive* Low attraction $^{\Delta}$	Zhu et al., 2005
	Not attracted $^{\Delta}$			Zhu and Park, 2005
Chrysopa sinica		Responsive *		Han and Chen, 2002
	Not attracted $^{\Delta}$	Not attracted $^{\Delta}$		Yu et al., 2008
Chrysoperla plorubunda	Not attracted $^{\Delta}$			Lee, 2010
	Attracted $^{\Delta}$			Jones et al., 2011
Chrysopa oculata	Not attracted $^{\Delta}$			Jones et al., 2011
	Attracted $^{\Delta}$			James, 2006

* electroantennogram response
$^{\Delta}$ Attraction in field trials
$^{\Delta\Delta}$Numbers of *C. nigricornis* caught in traps varied within sites

Several recent studies have investigated the use of synthetic HIPV application to grapevines as a strategy to attract natural enemies and achieve higher levels of biological pest control (James, 2003a; James, 2006; James, et al., 2005; James & Price, 2004; Simpson et al., 2011a; Simpson et al., 2011b). Work on this novel pest management strategy is at an early stage

generally, and especially in grapevines. Though there is some information on the HIPVs emitted by grapevines when fed upon by insects (Loughrin, et al., 1997; Van Den Boom, et al., 2004), plus the study described in Chapter 4, there is a dearth of knowledge on the response of natural enemy attraction to these compounds. A first step in developing this biological knowledge into a cost-effective pest management strategy is to determine whether some of the more readily-available HIPV compounds known to be produced by arthropod-attacked grapevines are attractive to important predatory and parasitoid arthropods.

The purpose of the present study was to investigate the response of *M. signata* to synthetic *β*-caryophyllene, (*Z*)-3-hexenyl acetate, and methyl salicylate, previously identified as emitted from grapevines as a result of arthropod herbivory, and which have been found to be attractive to a range of predatory and parasitic arthropods, including green lacewings (Chrysopidae) (Flint, Salter, & Walters, 1979; Han & Chen, 2002; James, 2003a; James, 2006; James, et al., 2005; James & Price, 2004; Lee, 2010) and brown lacewings (Hemerobiidae) (James & Price, 2004). The three chosen compounds are commercially available to use in crops for the attraction of natural enemies, however, the response of *M. signata* to these volatiles is unknown.

5.2 Materials and methods

Y-tube olfactometer bioassays were conducted from July to October 2010 at Charles Sturt University (site as described in Chapter 2). *Mallada signata* culture was as described in Chapter 4.

β-caryophyllene (98.5% GC), (*Z*)-3-hexenyl acetate (98% GC), methyl salicylate (99% GC), and *n*-hexane (98% GC) (Sigma Aldrich, Castle Hill, NSW, Australia) were used to prepare 0.1 ml aliquots containing each HIPV at 200, 20, 2, and 0.2 μg dissolved in 0.1 ml hexane. The control was *n*-hexane. The behavioral response of *M. signata* to the following pairs of odours was tested in a Y-tube olfactometer (see below): control (*n*-hexane) vs. methyl salicylate, (*Z*)-3-hexenyl acetate, and *β*-caryophyllene, respectively for each of the four HIPV concentrations, giving a total of 12 treatment combinations. Three replicates were completed for each odour source pair, with 20 insects tested per replicate. The treatment combinations were tested in a completely randomized fashion throughout the testing period.

5.2.1 Experimental design

A choice experiment using a Y-tube olfactometer (as described in Chapter 3) measured the response of naive *M. signata* adults to a two-way comparison between a HIPV volatile and the control. HIPV and *n*-hexane aliquots were applied to cotton rolls (Roeko Luna non-chlorine bleached;

Henry Schein Halas, Sydney, Australia) and left for three minutes to allow *n*-hexane to evaporate. Treated rolls were placed in the arms of the Y-tube olfactometer so that the air flow (200 mL min^{-1}) carried volatiles from a given treatment into the last section of one arm of the apparatus (De Boer & Dicke, 2004). Cotton rolls were replaced hourly (Scutareanu, et al., 1997).

A randomly selected male or female adult *M. signata* was introduced individually into the Y-tube olfactometer port and observed for five minutes, using the same recording criteria employed in Chapter 3. Each insect was used once only. To control for potential directional bias, the HIPV and hexane control treatments were randomly allocated to the arms of the olfactometer for each replicate. The temperature in the olfactometer room ranged from 16.6^{o}C to 23.3^{o}C and humidity of the air in the olfactometer was stabilised from ambient levels (28% to 60%) by bubbling the airflow through water reservoirs. The room was kept in darkness except for a single fluorescent light box positioned centrally behind the olfactometer. After each replicate, the glass Y-tube and plastic tubing which carried the air to the Y-tube was washed in hot, soapy water, rinsed with hot water, then rinsed with 100% ethanol and oven dried at 60^{o}C. All bioassays were run between 9.00 am and 5.00 pm to minimise any variation in diurnal and nocturnal behaviour by the insects.

5.2.2 Statistical analysis

Numbers of *M. signata* positive (HIPV) and negative (control) choices were analysed individually for each HIPV/rate using chi square goodness of fit, with a two-way comparison. Analysis used Genstat 12th Edition (Payne, et al., 2009).

5.3 Results

Chi square goodness of fit analysis found that significantly greater numbers of *M. signata* chose the HIPV over the control for *β*-caryophyllene at the rates of 20 μg ($\chi^2 = 6.29$, *d.f.* = 1, $P < 0.05$) and 0.2 μg ($\chi^2 = 5.01$, *d.f.* = 1, $P < 0.05$), and for methyl salicylate at the rates of 200 μg ($\chi^2 = 6.58$, *d.f.* = 1, $P < 0.05$) and 0.2 μg ($\chi^2 = 5.62$, *d.f.* = 1, $P < 0.05$) (Figure 5.1).

There was no difference found between positive and negative choices for (*Z*)-3-hexenyl acetate at any of the rates tested. The number of *M. signata* that did not make a choice in the olfactometer bioassays are also shown in Figure 5.1.

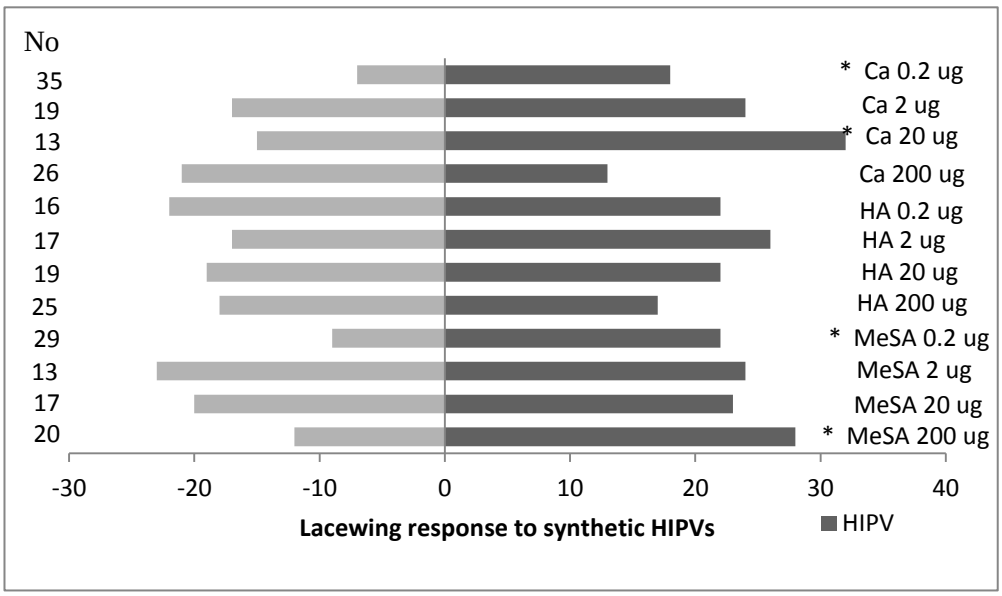

Figure 5.1: Y-tube olfactometer response of *M. signata* to *β*-caryophyllene (Ca), (*Z*)-3-hexenyl acetate (HA), and methyl salicylate (MeSA) at 200, 20, 2 and 0.2 µg, or *n*-hexane (Control). * indicates significant difference (*P* < 0.05)

5.4 Discussion

This study found *M. signata* was more attracted to *β*-caryophyllene at 20 and 0.2 µg, and to methyl salicylate at 200 and 0.2 µg compared with the control. There was, however, no difference in *M. signata* choice between (*Z*)-3-hexenyl acetate and the hexane control at any of the rates tested. Knowledge of Chrysopidae response to the HIPVs used in the present study under laboratory conditions is limited as many of the prior studies have been field trials, with only three studies conducted in a laboratory (Han & Chen, 2002; Zhu, Cosse, Obrycki, Boo, & Baker, 1999; Zhu, et al., 2005). Han and Chen (2002) found a strong response by *Chrysopa sinica* (Tjeder)

(Neuroptera: Chrysopidae) to (*Z*)-3-hexenyl acetate (at doses ranging from 10^{-6} and 10^{-4} g/ml hexane) in electroantennogram bioassays, and that increasing the doses from 10^{-6} to 10^{-2} g/ml hexane resulted in decreased electro-antennogram response. However, olfactometer bioassays (10^{-4} g/ml (*Z*)-3-hexenyl acetate versus a hexane control) conducted in the same trial showed no significant response by *C. sinica* (Han & Chen, 2002). Zhu et al. (1999) found a relatively low electroantennogram response by both adult male and female *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) to *β*-caryophyllene at doses ranging from 0.1 to 1,000 μg/10 μl hexane, and Zhu et al. (2005) found no significant electroantennogram response by *C. carnea* to *β*-caryophyllene, (*Z*)-3-hexenyl acetate, and methyl salicylate (at the rate of 100 μg/10 μl methylene chloride).

Prior studies have found Chrysopidae to be attracted to methyl salicylate and *β*-caryophyllene in field trials. Flint (1979) found *C. carnea* attracted to *β*-caryophyllene (0.2-8 g/load) in Delta traps in cotton, *Gossypium* spp. fields, and both James (2003) and Jones (2011) found *Chrysopa nigricornis* (Burmeister) (Neuroptera: Chrysopidae) significantly attracted to methyl salicylate-baited (2 mg/load) sticky traps in hop, *Humulus* spp. fields and apple, *Malus domestica* (L.) orchids respectively. Other work on *C. nigricornis* found them attracted to sticky traps in blocks baited with methyl salicylate (5 mg/load) in both hop fields and vineyards (James, et al., 2005; James & Price, 2004). Lee (2010) found Chrysopidae abundance greater

near methyl salicylate-baited (2g/load) lures positioned in the centre of experimental plots located in a strawberry, *Fragaria vesca* (L.) field than in the immediate vicinity of the lures, indicating that they were attracted to the point source of methyl salicylate directly.

The results from the present study found a very low positive response of *M. signata* to *β*-caryophyllene at the highest rate of 200 µg, with less than 22% attraction occurring. Flint et al. (1979) carried out dose-related tests in a field situation using *β*-caryophyllene at various rates from 0.002 to 8 g/2 ml acetone on *C. carnea*, and found that attraction did not occur at rates of 0.002 and 0.02 g *β*-caryophyllene, but that rates of 0.2 g to 8 g *β*-caryophyllene were equally attractive. The present study found a large proportion of no choices made by *M. signata* to the HIPVs tested, and there are several other studies which have also found no response by Chrysopidae to these HIPVs (Table 1). James (2003) found *C. nigricornis* were not attracted to (*Z*)-3-hexenyl acetate-baited (2 ml/load) sticky traps placed in hop fields. Zhu et al. (2005) found low catches of *C. carnea* in Rebell Traps baited with *β*-caryophyllene (50 mg/load) located in alfalfa fields, and Zhu and Park (2005) found that *C. carnea* were not attracted to methyl salicylate-baited (100 mg/load) sticky traps located in a soybean field. Lee (2010) found *Chrysoperla plorabunda* (Fitch) (Neuroptera: Chrysopidae) not attracted to methyl salicylate lures (2 g/load) placed in strawberry fields, and a lack of response by *C. sinica* to methyl salicylate and (*Z*)-3-hexenyl

acetate was also found by Yu et al. (2008) who used baited sticky traps (10 mg HIPV/ml diluted in lanolin) located in an open field. Jones et al. (2011) found contrasting results depending on the location of the field trial, with some sites reporting Chrysopidae attraction to methyl salicylate and (*Z*)-3-hexenyl acetate, while other sites found no difference between the HIPV and control (Table 1). That study also found significantly greater numbers of *C. nigricornis* caught in traps compared to *Chrysopa oculata* (Say) (Neuroptera: Chrysopidae) and *C. plorabunda*, reportedly due to a number of possible factors, including seasonal phenology of the species or that it may be less abundant in that area (Jones, et al., 2011).

The present study found that under laboratory conditions, *M. signata* response to *β*-caryophyllene and methyl salicylate differs from the response found in other studies of Chrysopidae species to these HIPVs tested under field conditions. This suggests that *β*-caryophyllene and methyl salicylate as single compounds become more attractive to Chrysopidae in field conditions, possibly due to their interaction with neighbouring plants resulting in a range of endogenous HIPVs being produced. A number of studies have revealed that exposure to HIPVs prime plants for augmented defence expression (Arimura, et al., 2001; Arimura, et al., 2000; Bi, Zeng, Su, An, & Luo, 2007; Farag & Paré, 2002; Frost, Mescher, Dervinis, et al., 2008; Khan, et al., 2008; Simpson et al., 2011a; Simpson et al., 2011b). It is important to note that priming does not directly trigger endogenous

production of HIPVs, but rather prepares a plant for an enhanced response to any subsequent arthropod attack (Conrath et al., 2006), likely under field conditions. Therefore, HIPVs placed as lures in field experiments may result in enhanced attraction of the HIPV bait due to the endogenous volatiles produced by the nearby plants as a result of the exogenous source of HIPV. Methyl salicylate is an important signalling molecule (Arimura, et al., 2001; Bi, et al., 2007; Engelberth, et al., 2004; Khan, et al., 2008) and it is therefore likely that the attraction of Chrysopidae to methyl salicylate placed as a lure in field trials was boosted by other endogenously produced HIPVs from neighbouring plants in the earlier mentioned studies of James (2003), James (2006), James and Price (2004), James et al. (2005), and Lee (2010).

The discrepancy in the response of Chrysopidae species to *β*-caryophyllene, (*Z*)-3-hexenyl acetate, and methyl salicylate in this and other studies may also be due to the variation of physiological conditions within each study, or to other likely factors, such as the diet of the insect prior to being exposed to the HIPV (Ettifouri & Ferran, 1993; Henaut, et al., 2000). The cotton rolls used to used in the present study may have contributed to the lack of response by *M. signata* as they have a high absorbance capacity but may have allowed low volatile emission; Jones et al. (2011) found a 4 x 4 cm polyester felt wick provided a greater surface area for release of volatiles,

particularly at low volumes, compared to a 3.8 cm dental wicks, similar to what was used in this study.

Within vineyards some pest species are difficult to control using conventional insecticides. For example, leafroller larvae, including larvae of the light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), common in Australia and other parts of the world, often feed and pupate within the protection of rolled-up leaves. As a result, many vineyards are employing integrated pest management techniques, including the mass-release of beneficial insects, to assist in controlling a wide range of pests. Chrysopidae are generalist natural enemies, preying on a broad range of arthropod pests, and thus play an important role in pest suppression (Symondson, Sunderland, & Greenstone, 2002). As HIPVs emitted by pest-infested plants are extremely complex and specific to the species of plant and species of pest, it can be assumed that Chrysopidae are therefore attracted to a broad range of HIPVs. The variation in response by Chrysopidae species to HIPVs in this and prior studies reflects the complexity of the pest–plant–predator tri-trophic interaction. While research is currently attempting to identify specific volatiles that are the most attractive to a range of predatory and parasitic arthropods, it is problematic to attempt to single out a specific volatile from the complex blend of HIPVs as it is likely that this single compound at a specific rate is only telling the beneficial arthropod 'part of the story'. This limitation may

account for the variation in results found in previous studies. Regardless, recent advances in pest management have resulted in commercial products now being available, such as methyl salicylate in the slow-release dispenser Predalure® (AgBio, Westminster, CO) (Lee, 2010). Further research into determining the optimal spacing and dosage of lures, the effects of applied HIPVs on searching behaviour of beneficial arthropods and the potential disruption to their populations is essential to monitor the long-term effects of such commercial products.

The results reported in the present study are a small, but significant, step measuring the response of *M. signata* to single HIPVs at varying rates, and have made a useful contribution to our understanding of the effects of these HIPVs on an important predatory insect in Australia. Results from other studies have shown significant Chrysopidae attraction to these three volatiles under field conditions, suggesting further research is necessary to identify the range of volatiles experienced by Chrysopidae under these field conditions, and individually test each volatile under laboratory conditions to determine which are the most attractive to *M. signata.*

Chapter 6

General Discussion

The chemical ecology of Si-fertilised plants, including the effects on constitutive and induced defences, were reviewed in Chapter 1. This literature review identified significant gaps in our knowledge as to the effects of Si fertilisation on grapevines; there were no studies observing the effects of Si-fertilised grapevines on arthropod pests and their natural enemies. Further, there was an absence of studies identifying the volatile profile of Si-treated, pest-infested plants. The literature review also found significant gaps in our understanding of the response of the Australian native Chrysopidae, *Mallada signata* (Schneider) to commonly emitted HIPVs, whilst the response of other Chrysopidae species is well studied.

Overall, the present study found Si fertilisation to have little effect on constitutive defences in grapevines. However, Si did affect induced chemical defences; Si fertilisation influenced herbivore dynamics by directly affecting herbivore feeding, and by indirectly affecting tri-trophic interactions. These findings indicate Si fertilisation can make a significant contribution to IPM strategies within vineyards; potentially using Si to promote grapevine induced defences, and to recruit beneficial arthropods to assist in biological control of pests. This general discussion highlights the key findings from the research study, and expands on the significance of these findings to the viticulture industry.

6.1 Direct effects of silicon fertilisation of grapevines on the pest, *Epiphyas postvittana*

Chapter 2 describes a study to investigate the direct effects of Si fertilisation on grapevine defences, and addressed the hypothesis that Si-fertilised grapevines would display enhanced constitutive and induced defences, reducing the level of feeding damage by *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) larvae. Delta-T Scan® image analysis software was used to measure the area of grapevine leaf consumed by the larvae, and larvae were weighed immediately prior to and on completion of the 24 hour period of feeding. The results of that study were contradictory to the hypothesis. Silicon fertilisation significantly increased the area of grapevine leaf chewed by *E. postvittana* larvae, suggesting Si fertilisation had little effect on the mechanical resistance or abrasiveness of grapevine leaves. Studies have attributed increased Si content in epidermal tissue and resulting leaf abrasiveness and/or toughness to reduced levels of arthropod feeding and detrimental effects on arthropod life parameters (Hou & Han, 2010; Keeping, et al., 2009; Keeping & Meyer, 2006; Kvedaras & Keeping, 2007; Kvedaras, et al., 2007b; Massey, et al., 2006). Being a low Si-accumulator, grapevines restrict entry of Si from its roots to the xylem, resulting in a low level of polymerised Si in epidermal cells (Lafos, 1995). Therefore, as the study described in Chapter 2 found significant difference in *E. postvittana* larval feeding and weight between Si+ and Si- grapevine leaves, it is a logical assumption that the increased consumption was due to

induced chemical defences. The phenolic compounds and enzymes produced as an induced defence mechanism are believed to be difficult for arthropods to digest and, therefore, it has been suggested that greater quantities of food are eaten to compensate for the reduced digestion efficiency (Goussain, et al., 2005; Massey, et al., 2006).

6.2 Tri-trophic effects of silicon fertilisation of grapevines on the beneficial insects, *Dicranolaius bellulus* and *Mallada signata*

The studies described in Chapters 3 and 4 found Si fertilisation affected the HIPV profile of grapevines when attacked by both *E. postvittana* and *P. glycinae*. The study described in Chapter 3 addressed the hypothesis that Si fertilisation would enhance or alter the composition of HIPVs emitted by *E. postvittana*-infested grapevines, making the plant more attractive to the generalist predator, *Dicranolaius bellulus* (Guérin-Méneville) (Coleoptera: Melyridae). Y-tube olfactometer bioassays were conducted to determine the attraction of *D. bellulus* to Si-treated and untreated *E. postvittana*-infested grapevines. That study found *D. bellulus* attraction to *E. postvittana*-infested grapevines was significantly greater in the plants with the highest Si content. The increased attraction was suggested to be due to Si fertilisation altering the HIPV profile emitted by the pest-infested plant, making the plant more attractive to *D. bellulus.* These results are in accordance with that of an earlier study using the same predator, cucumbers and *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) larvae (Kvedaras, et al., 2010).

Dicranolaius bellulus is a significant predator in Australian crops (Horne, et al., 2000) and, significantly, this study is the second to show enhanced attraction of this predator to Si-treated, pest-infested plants.

The study described in Chapter 4 addressed the hypothesis that Si fertilisation would enhance or alter the composition of HIPVs emitted by grapevines infested with *Phalaenoides glycinae* (Lewin) (Lepidoptera: Noctuidae) larvae, making the plant more attractive to the generalist predator, *Mallada signata* (Schneider) (Neuroptera: Chrysopidae). This study expanded on the study described in Chapter 3 by using SPME fibres to adsorb, and GC-MS to identify and quantify, the volatile emissions from Si+ and Si- *P. glycinae*-infested grapevines. Y-tube olfactometer bioassays were run using the same *P. glycinae*-infested grapevines to determine the tri-trophic effect of the altered volatile profile on *M. signata*. The study found Si fertilisation altered the volatile profile in pest-infested Si+ plants compared to Si- plants. Importantly, that study identified six novel compounds, not previously identified in the literature as being emitted from pest-infested grapevines. The volatile profile contained terpenoids and alkanes and, somewhat surprisingly, did not contain methyl salicylate, an aromatic compound commonly emitted by pest-infested plants and identified from pest-infested grapevines by both Van den Boom (2004) and Loughrin et al. (1997). Within the volatile profile of *P. glycinae*-infested grapevines only α-humulene is commonly listed as a HIPV, and there was

no variation in the levels of this compound between Si+ and Si- plants. This may explain why the Si-altered volatile profile did not affect *M. signata* attraction to the pest-infested plants.

The use of SPME fibres for the collection of volatiles was chosen because of its simplicity in setting up, reliability, selectivity and sensitivity, and reduced analysis time. SPME is one of a range of volatile collection methods available, and comprised 9% of the most commonly used methods from the years 1995-2004 (D'Alessandro & Turlings, 2006; Lou, et al., 2005; Zhu, et al., 2005). The SPME adsorption/desorption technique employs a small fibre coated with an absorbing material which collects volatile compounds in headspace (D'Alessandro & Turlings, 2006). The volatiles adsorbed onto the SPME fibre are then desorbed using gas chromatography-mass spectrometry (GC-MS). GC-MS separates, detects and provides linear results for individual volatile compounds, even when they are present at very low concentrations (Spinhirne, Koziel, & Chirase, 2004).

6.3 Response of *Mallada signata* to HIPVs

The studies described in Chapters 4 and 5 were the first to observe the response of *M. signata* to HIPVs, both synthetic and endogenously produced from pest-infested grapevines. *Mallada signata* larvae are efficient biological control agents with a broad prey range, which includes

lepidopteran eggs and larvae (Canard & Principi, 1984). *Mallada signata* larvae are highly resistant to the effects of pesticides compared to other predatory species and they have a low dispersal ability, making them ideal for use in IPM programs (Nordlund, Cohen, & Smith, 2001). However, before *M. signata* can feasibly be used for inundative biological control, we require a greater understanding of what volatile signals are attractive. In this regard, very little is known.

The study described in Chapter 5 was designed to complement the work described in earlier chapters, and assessed the response of *Mallada signata* to synthetic volatile compounds, commonly emitted from pest-infested plants, including grapevines. That study also used Y-tube olfactometer bioassays to investigate the response of *M. signata* to varying rates of *β*-caryophyllene (terpenoid), (*Z*)-3-hexenyl acetate (GLV) and methyl salicylate (aromatic volatile). This study was conducted prior to the trial described in Chapter 4, and the choice of volatiles used in the study was based on literature identifying HIPVs emitted from grapevines. The bioassays showed *M. signata* was significantly attracted to *β*-caryophyllene at the rates of 0.2 and 20 μg and methyl salicylate at 0.2 and 200 μg, but not to (*Z*)-3-hexenyl acetate at any of the rates tested. The response of *M. signata* was found to vary from that of other Chrysopidae species (Table 5.1), and indicated a greater attraction to terpenoids and aromatic volatiles

than to GLVs. This is an important point for consideration if using synthetic HIPVs and *M. signata* in the field for the bio-control of pests.

However, the study described in Chapter 4 found a lack of response by *M. signata* to an endogenous volatile profile containing predominantly terpenoids. The reasons for the variation in response of *M. signata* to terpenoids are not known. It could simply be that this predator found *β*-caryophyllene (discussed in Chapter 5) more attractive than the other terpenoids reported in Chapter 4, including *cis*-thio rose oxide, *α*-humulene, *cis*-2,3-pinanediol and 9-*epi*-(*E*)-caryophyllene. The response of *Mallada signata* may also have been influenced by the fact that naïve insects were used, that is, they had no prior exposure to plant HIPVs. It has been found that the response of generalist carnivorous arthropods to volatiles is a learned process (Smid, 2006; Steidle & van Loon, 2003; Takabayashi, et al., 2006). Therefore, further research in this area may benefit from exposing *M. signata* to pest-infested grapevines prior to the experiment.

The experiments described in Chapters 4 and 5 originally planned to use *M. signata* larvae in the Y-tube olfactometer bioassays instead of adults. This was because larvae are predatory, while the adults feed on pollen and nectar (Canard, 2001). However, the Y-tube olfactometer bioassays found *M. signata* larvae slow and un-responsive once placed in the glass tube. The use of adult *M. signata* can be justified as gravid females fly towards and lay eggs on vegetation of preferred plant species (Canard, 2001), and numerous

studies have observed a positive response by adult Chrysopidae to HIPVs (Flint, et al., 1979; Han & Chen, 2002; James, 2003a; James, 2006; James, et al., 2005; James & Price, 2004; Jones, et al., 2011; Lee, 2010).

6.4 Silicon content in grapevine leaf tissue

Notably, this research found Si fertilisation can benefit grapevines, a low Si-accumulating species, and contradicts the theory that high accumulation of Si in plant shoots is required to overcome biotic and abiotic stress (Mitani, et al., 2011). There are many studies which have found the benefits of Si fertilisation are positively correlated with increasing Si content in leaf tissue (Gatarayiha, et al., 2010; Korndörfer, et al., 2011; Kvedaras & Keeping, 2007; Ma & Yamaji, 2008). For example, Korndörfer et al. (2011) found Si fertilisation of the sugarcane variety cv. SP79-1011, which represented highest Si content in leaves (2.31% Si), increased nymphal mortality and decreased female longevity of spittlebug, *Mahanarva fimbiolata* (Stål) (Hemiptera: Cercopidae) compared with two lower Si-accumulating sugarcane varieties cv. SP80-1816 and SP81-3250 (1.60% and 1.56% Si respectively). While the current research project is not the first to attribute enhanced defences in a low Si-accumulating plant species, it is the first to show this occurs in grapevines as a result of arthropod pest attack.

Results of grapevine leaf tissue samples described in Chapters 3 and 4 are consistent with literature and confirm that grapevines are low Si-

accumulators, typical of most dicotyledons. Analysis of grapevine leaf tissue described in Chapter 3 found the mean total Si content of Si+ leaves (0.315% Si, range 0.188-0.559% Si) was greater than for Si- leaves (0.177% Si, range = 0.065-0.253% Si), indicating Si uptake by grapevines is increased with increasing availability of Si in the soil medium. Analysis of grapevine leaf tissue samples described in Chapter 4 found the mean total Si content of Si+ leaves (0.217% Si, range = 0.20-0.23% Si) was greater than for Si- leaves (0.132% Si, range = 0.13-0.14% Si). As the Si ferthlisation regime used in all the Trials148was designed to pro6ide a high level of148plant-available Si, grapevine leaf Si content shows Si uptake was restricted by the grapevine roots. Previous studies have also reported restricted Si uptake by grapevine roots (Blaich & Grundhöfer, 1997; Lafos, 1995). It is likely that the low level of Si-accumulation in grapevine leaf tissue is due to the reduced ability of ietabolic influx and efflux transporters located in grapevine roots to transport Si from the external soil solution into the plant xylem (Liang et al., 2005a; Ma, et al., 2006; Ma, et al., 2007; Mitani, et al., 2011).

6.5 Plant-available silicon content in the potting media

Silicon is an ubiquitous contaminant, present as an impurity in macronutrient salts, in distilled and demineralised water, in glass containers, and as dust (Woolley, 1957). It can be very difficult, therefore, to completely exclude Si from plants in experimental trials (Woolley, 1957).

The studies described in Chapters 2, 3 and 4 found significant amounts of Si (palm peat = 74 mg/kg Si, and perlite/vermiculite/palm peat = 110.5 mg/kg Si) in the unamended potting mediums, and a small amount of Si (2-5 mg/kg Si) in the deionised water. Two repeats of soil samples from each unamended potting medium were sent for analysis. Sample analysis of the unamended perlite/vermiculite/palm peat potting medium described in Chapter 4 found plant-available Si was 110.5 mg/kg Si, greater than the Si+ treatment of 100 mg/kg Si. As a result, this experiment effectively had no control, and was instead measuring two levels of Si (high Si and low Si).

Raw sand has been successfully used as a potting medium in other Si-based studies (Keeping et al., 2010; Keeping et al., 2009). However the study described in Chapter 3 used palm peat, the same potting medium described in the Si-based trial conducted by Kvedaras et al. (2010). In the study described in Chapter 4, it was decided to use a perlite/vermiculite/palm peat potting medium, described in Mullins and Rajasekaran (1981), reported to enhance the strike rate and growth of grapevine cuttings. Perlite and vermiculite contain high levels of silicon dioxide, however, this form of Si is reportedly not available for plant uptake and these mediums, as well as palm peat, have been previously used in other Si-based experiments (Dann & Muir, 2002; Jeffrey, et al., 1997; Kamenidou, Cavins, & Marek, 2010; Kvedaras, et al., 2010; Liang et al., 2005b; Q. Shi, et al., 2005; van der Vorm, 1980). For example, Jeffrey et al. (1997) used 50:50 perlite/sand

mixture which was found to contain 1.7 mg/L Si prior to applying treatments. Dann and Muir (2002) also used 50:50 perlite/sand potting medium as their control medium, and this was found to contain 2.7 mg/L Si in one experiment and 4.4 mg/L Si in a separate experiment. Van der Vorm (1980) used 100% perlite, and Liang et al. (2005b) and Q. Shi et al. (2005) both used 100% vermiculite, however, none of these studies tested the potting medium for plant-available Si content. Likewise, Kvedaras et al. (2010) and Kamenidou et al. (2010) did not ascertain plant-available Si levels in their potting mediums, 100% palm peat and 4:1 peat/perlite respectively. Yet, all of the above-mentioned studies reported significantly positive results between Si-treated and untreated plants, indicating there were sufficient difference in Si levels between Si-treated and untreated potting mediums.

However, our results suggest perlite, vermiculite and palm peat contain significant amounts of plant-available Si. The samples of perlite/vermiculite/palm peat potting medium were taken well into our study (described in Chapter 4), in response to receiving analysis results of palm peat used in the previous trial (described in Chapter 3). Therefore, we were forced to accept low and high Si treatments, without a nil-content Si potting medium as a control. Although a number of the studies mentioned above reported high plant-available Si levels in perlite, vermiculite or palm peat, it

seems likely that the Si-availability in these mediums may vary depending on the location of its source.

6.6 Effect of hot, windy weather on potted grapevines

The potted grapevines described in Chapters 2 and 3 were exposed to unusual and extreme hot, windy weather only two days after being moved from the glasshouse and placed on an outside trellis in November 2009 (see Table 2.2). This resulted in the vines losing the majority of their leaves. By the end of January 2010 it became apparent that the vines would not grow new leaves while remaining on the outside trellis, and it was decided to move them back in the glasshouse in February 2010. Once back in the glasshouse, the vines began to grow new leaves. Older leaves contain higher Si content than younger leaves (Blaich & Grundhöfer, 1997), however, due to the irregularity of regrowth amongst the plants, it was decided to use leaves of similar size and appearance for the experiment conducted in Chapter 2, rather than leaves of uniform position on the vine.

6.7 Impact of the present study on the viticulture industry

The possible impact of these results on the viticulture industry is great. The reported benefits of Si fertilisation are widespread; improving the health and strength of the plant, as well as improving its defences against biotic and abiotic stresses. The aim of the current research project was to identify the impact of Si fertilisation on pest and beneficial insect species within a

vineyard environment. The results from this research study have shown that Si fertilisation had limited, if any, effect on the constitutive defences of grapevines, however, Si did affect the induced chemical defences. This research project has shown Si-fertilisation increases the attraction of *D. bellulus* to *E. postvittana*-infested grapevines, as well as altering the volatile profile of *P. glycinae*-infested grapevines. Research described in this thesis has also identified the response of the predatory *M. signata* to common grapevine HIPVs used in IPM systems to attract beneficial arthropods to crops.

It is crucial to have an understanding of the ecology of arthropod populations within an environment in order to effectively promote beneficial species and diminish the impact of pests. One of the current objectives of the Australian wine industry is to reduce the levels of broad spectrum insecticides applied within vineyards, and to enhance and promote the natural populations of beneficial arthropods within vineyards to utilise the contribution they make to sustainable pest control practices (South Australian Wine and Brandy Industry Association Incorporated and Winemakers Federation of Australia, 2002). Studies have demonstrated that the activity of natural enemies in agricultural ecosystems has reduced as a result of declining diversity in these ecosystems (Schmidt, Thies, & Tscharnkte, 2004). Supplementing natural enemy populations to facilitate the implementation of IPM in vineyards is growing in popularity (Bug

Central, 2010; Bugs for Bugs, 2010). However, this approach of pest management demands sound knowledge of the natural enemy to anticipate its response in that environment. The present research study has contributed to this knowledge of important pest and predatory insect species in Australian vineyards, and may ultimately enable enhanced levels of pest control by beneficial arthropods.

6.8 Areas requiring further research

The study described in Chapter 2 found Si fertilisation resulted in increased consumption of grapevine leaves by *E. postvittana* larvae. Increased consumption of Si-treated crop material is not acceptable to farm managers, and further research is vital in this area to determine whether Si fertilisation has similar effects on other grapevine pests. To confirm whether the increased consumption was due to induced chemical defences, further studies should measure chemical defence products, such as enzymes, *β*-glucosidase, chitinase and phenolic compound activity (using similar methods used by Chérif et al., 1994), in response to arthropod feeding. To further expand on the study described in Chapter 2, future work could assess larval growth as opposed to volume (and weight) of plant tissue in larval gut. Therefore future experiments need to run for longer periods, encompassing stages from larval through to adult emergence to gain a more comprehensive understanding of the effects of Si fertilisation.

Research into the effects of Si fertilisation on HIPV production is still in its infancy, however, the promising results identified in this thesis deem this area of research worthy of further study. Additional research should investigate the HIPV profiles of Si-treated and untreated grapevines in response to a variety of common grapevine pests. These studies could be expanded using olfactometer bioassay and field trials to gauge the tri-trophic effect of Si-altered HIPV profiles on natural enemies. A greater understanding of the effects of HIPVs (both exogenous and endogenous) on beneficial arthropods commonly found in vineyards is necessary before this knowledge can be developed into a cost-effective pest management strategy.

The substantial research evaluating the effect of HIPVs on a range of Chrysopidae species has found the response varies between differing species and, therefore, those results cannot be used to predict the response of *M. signata* to HIPVs. Accordingly, further research is required in determining the response of *M. signata* to a range of common HIPVs, using both laboratory and field experiments.

Finally, further research is also required to determine the level of plant-available Si by grapevines needed to provide plant protection against arthropod pests. Further studies expanding on the work of Ma et al. (2006, 2007) on rice by identifying the metabolic processes restricting Si uptake in grapevines, would also be greatly beneficial. As a low Si-accumulator, there may be merit in complementing Si fertilisation of grapevines via the roots

by incorporating a foliar-applied Si program during periods of peak pest and pathogen attack. Bowen et al. (1992b) and Reynolds et al. (1996) found foliar sprays of Si to grapevines enhanced resistance to fungal disease, likely due to Si crystals providing a protective barrier on plant leaves, making hyphal penetration more difficult. Notably, both Bowen et al. (1992b) and Reynolds et al. (1996) found increased deposition of Si in Si+ pathogen-infected leaves, suggesting there was a lateral movement of Si within leaves to the sites of infection. As these two studies were testing grapevine pathogens, further research to identify the effects of foliar-applied Si on grapevine pests would be beneficial.

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Appendix

Book Chapter

Chapter 6

Chemical ecology providing novel strategies against vineyard pests in Australia

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6.1 Introduction

Chemical ecology has been recognized as an important and distinct research area for over three decades and deals with chemical mechanisms which help control intra- and inter-specific interactions amongst forms of life. All organisms use chemical signals to transmit information as a form of communication (Dicke 2009). Research in the field of chemical ecology involves the identification and synthesis of the chemical substances as well as the measurement of the ecological consequences of signal transfer (Dicke and Takken 2006).

Plants have evolved a wide range of constitutive and induced defence mechanisms against herbivore feeding or oviposition, including the production of herbivore-induced plant volatiles (HIPVs) (Karban and Baldwin 1997). Natural enemies use these volatile signals to locate their hosts or prey (Thaler 1999, Bernasconi Ockroy et al. 2001, Dicke 2009, Dicke et al. 1990). HIPV blends differ depending on the attacking herbivore and plant species involved. They can be extremely complex and vary qualitatively and quantitatively, with several compounds released more commonly than others (Dicke et al. 1998, Van Den Boom et al. 2004). There is also evidence of plant-plant and within-plant communication through volatiles (Frost et al. 2008). For instance, specific volatiles such as

methyl salicylate (MeSA), methyl jasmonate (MeJA), ethylene and the green leaf volatiles (GLVs) can activate jasmonic acid-dependent defence reactions in neighbouring plants, or other parts of the same plant, boosting the production of endogenous aromatic and terpenoid volatile compounds that enhance the induced defence response of plants (Yan and Wang 2006, Ton et al. 2007, Tamogami et al. 2008).

This chapter provides a concise review of the ways in which chemical ecology research is generating new avenues for pest management and considers the utility of these novel technologies to major vineyard pest problems. Our emphasis is on vineyard protection in Australia, as wine and table grapes are a substantial and valuable industry, but many of the issues apply generally to other agricultural crops.

6.2 The conservation biological control context

In agriculture, integrated pest management (IPM) is a pest-control strategy which uses a variety of complementary practices. One of these, conservation biological control (CBC), involves cultural practices that preserve and enhance the efficacy of natural enemy populations through the modification of the biotic environment and pesticide usage (Eilenberg et al. 2001, Gurr et al. 2004, Wratten et al., Chapter 7). Interest in biological control as an alternative to pesticides due to environmental and human health concerns, pest resistance and costs has intensified by growers,

researchers and policy makers. Adoption of biological control by growers has been limited by a range of factors, including the cost of mass produced agents (in inundative biological control) and the risks associated with introducing exotic agents (in classical biological control). Both of these issues are avoided through the use of CBC, however this method is a comparatively new approach and therefore is constrained by a relative paucity of information.

Research on CBC in various crop systems is aimed principally at increasing the efficacy and reliability of this pest control method. Many studies have identified that habitat manipulation in the form of floral plant species distributed appropriately within or around the crop leads to increased abundance, residency and diversity of natural enemies, reduced pest numbers and increased parasitism rates (Baggen et al. 1998, Landis et al. 2000, English-Loeb et al. 2003, Bostanian et al. 2004, Lee and Heimpel 2005, Gurr et al. 2005, Berndt et al. 2006). A key challenge is to establish sufficient numbers of natural enemies exactly when and where they are required; an objective similar to biological insecticide use. Natural enemy populations may otherwise be too slow to establish and have limited ability to keep pests below economic thresholds.

6.3 Vineyard pests occurring in Australia and their natural enemies

6.3.1 Important vineyard pest species in Australia

The light brown apple moth (LBAM) *Epiphyas postvittana* Walker (Lepidoptera: Torticidae) is a polyphagous leafroller indigenous to Australia that feeds on native and introduced plant species. It is also widely distributed in New Zealand, Great Britain, and several other countries (Danthanarayana 1975, Buchanan 1977). It is regarded as the most serious pest in Australian vineyards as well as a major pest of other horticultural crops including pome, stone and citrus fruits.

Several common mite species, such as grapeleaf blister mite and grapeleaf bud mite *Eriophyes vitis* Pagenstecher (sin *Colomerus vitis* Pagenstecher) (Acari: Eriophyidae), rust mite *Calepitrimerus vitis* Nalepa (Acari: Eriophyidae), and bunch mite *Brevipalpus* spp. (Acari: Tenuipalpidae) attack grapevines and are considered minor pest species, however under favourable conditions they can cause economic damage (Nicholas et al. 1994, James et al. 1995).

Less frequently occurring pests are the twospotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae), mealybugs *Pseudococcus* spp. (Hemiptera: Pseudococcidae), and grapevine scale *Parthenolecanium persicae* Fabricus (Hemiptera: Coccidae). Regional pests include weevils (Coleoptera: Curculionidae), Rutherglen bug *Nysius vinitor* Bergroth (Hemiptera: Coccidae), fig longicorn *Acalolepta vastator* Newman (Coleoptera: Cerambycidae), larvae of pink cutworm *Agrotis munda* Walker (Lepidoptera: Noctuidae), vine moth *Phalaenoides glycinae* Lewin

(Lepidoptera: Noctuidae), thrips (Thysanoptera), wingless grasshoppers *Phaulacridium vittatum* Sjöstedt (Orthoptera: Acrididae), and katydids *Caedicia* spp. (Orthoptera: Tettigoniidae) (Nicholas et al. 1994, Thomson et al. 2007). Grapevine phylloxera *Daktulosphaira vitifolii* Fitch (Hemiptera: Phylloxeridae) is restricted to a small number of quarantined areas in the states of Victoria and New South Wales, with the majority of the Australian winegrowing regions remaining free of this pest. Phylloxera causes grapevine roots to develop fleshy, yellow galls resulting in weak shoot growth, reduced cropping and premature yellowing of foliage in autumn (Buchanan et al. 1994, Herbert et al. 2008, Forneck and Huber 2009). A summary of the major grapevine pests occurring in Australia is presented in Table 6.1.

Table 6.1 Major grapevine pests, crop damage caused and their natural enemies in Australia

		Major Grapevine Pests		
		Light brown apple moth (*Epiphyas postvittana*) (Lepidoptera: Tortricidae)	Mites (*Brevipalpus* spp., *Colomerus vitis, Calepitrimerus vitis Tetranychus urticae*)	Mealybugs (*Pseudococcus* spp.)
	Crop damage	Larvae eat shoots, flowers and fruit. The wounds to berries make the bunches further susceptible to mould entry by *Botrytis* spp. and *Aspergillus* spp.	Blister mites cause galls on leaves. Bud mites feed on bud scales, leaf, bunch primordia. Rust mites distort leaves, shorten growing roots. Bunch mites and *T. urticae* cause leaf necrosis.	Mealybugs excrete sticky honeydew causing mould covering on grape bunches and leaves.
Natural Enemies				
Guild/Order/Family	**Species**			
Parasitoids				

Hymenoptera				
Braconidae	*Dolichogenidea tasmanica*	Larvae ([1,2,,3,6])		
	Bassus unimaculata	Larvae ([1])		
	Bracon spp.	Larvae ([1])		
Trichogrammatidae	*Trichogramma* spp.	Eggs ([1,2,3])		
Ichneumonidae	*Australoglypta latrobei*	Larvae ([1,2])		
	Exochus spp.	Larvae ([1,2])		
	Eriborus Epiphyas	Larvae ([1])		
	Temelucha minuta	Larvae ([1])		
	Oedemopsis hobartensis	Larvae ([1])		
	Phytodietus celsissimus	Larvae ([1])		
	Glabridorsum stokesii	Pupae ([1])		
	Xanthopimpla rhopaloceros	Pupae ([1,6])		
	Phytodietus spp.	Larvae ([2])		
Bethylidae	*Eupsenella* spp.	Larvae ([1])		
	Goniozus jacintae	Larvae ([1,2,6])		
Chalcididae	*Brachymeria* spp.	Pupae ([1,2,6])		
Encyrtidae	*Anagyrus fusciventris*			([3,5])
	Tetracnemoidea brevicornis			([3,5])
Pteromalidae	*Ophelosia* spp.			([3,5])
Diptera				
Tachinidae	*Voriella uniseta*	Larvae, pupae ([1,2,6])		
	Trigonospila brevifacies	Larvae ([1,2])		
Predators				
Neuoptera				
Hemerobiidae	*Micromus* spp.	Eggs, larvae ([3,6])	([3])	([3,5])
Chysopidae	*Chrysopa* spp.	Eggs, larvae ([1,6])	([3])	([3,5])
	Mallada signata	Eggs, larvae ([4])		
Coleoptera				
Coccinellidae	*Rhizobius ruficollis*			([3,5])
	Cryptolaemus montrouzieri			([3,5])
	Stethorus spp.		([3])	
	Diomus notescens	Larvae [(6)]		
Melyridae	*Dicranolaius bellulus*	Larvae [(6)]		
Hemiptera				
Pentatomidae	*Oechalia schellenbergii*	Larvae ([3])		
Miridae	*Melanotrichus australianus*	([1,6])		
Araneae				
Theridiidae	*Achaearanea veruculata*	Larvae ([1,4,6])		
Thomisidae	*Diaea* spp.	Larvae ([1,4,6])		
Dermaptera				
Forficulidae	*Forficula auricularia*	([1,2,3,6])		
Acari				
Phytoseiidae	*Amblyseius victoriensis*		([3])	
	Typhlodromus doreenae		([3])	
Diptera				
Cecidomyiidae	*Diadiplosis koebelei*			([5])

References: [(1)]Paull and Austin 2006, [(2)]Danthanarayana 1983, [(3)]Geier and Briese 1980, [(4)]Buchanan and Amos 1992, [(5)]Furness 1976, [(6)]MacLellan 1973

6.3.2 Important beneficial parasitoids and predators in Australian vineyards

The pest-predator interactions in grapevines are complex and multidimensional. A summary of the important beneficial parasitoids and predators of major grapevine pests is presented in Table 6.1.

6.4 Plant interactions with the environment and their chemical defence mechanisms

Plants have evolved various direct and indirect responses to arthropod pests. Karban and Baldwin (1997) classified plant defences into constitutive or induced. Constitutive defences exist independently of plant damage and are activated prior to contact with the attacker, whereas induced responses are changes in the plant as a result of damage by an attacker (Levin 1976). A decrease in negative consequences from induced attacks on the plant is termed induced defence (Karban and Baldwin 1997). Plant defence can involve mechanical or chemical mechanisms, however only direct and indirect chemical defence mechanisms will be discussed in this chapter. Chemical volatile compounds can be released from plant leaves, flowers and fruits into the atmosphere, and from roots into the soil. From more than 90 plant families 1,700 volatile compounds have been described (Dudareva et al. 2006). Typically, volatile compounds are lipophilic liquids of sufficiently low molecular weight and high vapour pressure to allow

transport across membranes; thus volatile compounds can be released into the atmosphere or soil (Dudareva et al. 2006). These compounds can be classified into three major groups: terpenoids, phenylpropanoids/ benzenoids and fatty acid derivatives (Dudareva et al. 2006).

The primary function of such volatile compounds is to defend plants against herbivores and pathogens, or to provide a reproductive advantage by attracting pollinators and seed dispersers to the plant. Volatiles emitted by plants in response to herbivore damage are also referred to as HIPVs.

6.5 Induced plant defences

The relationship between HIPV blends and tri-trophic insect- plant interactions has been the subject of research for the past 20 years. The majority of studies have been conducted in laboratory environments. Research has established that two types of volatile blends can be distinguished depending on the type of plant damage; herbivore-induced or mechanical, and that these blends can differ quantitatively and qualitatively with some compounds in common (Whitman and Eller 1990, Van Den Boom et al. 2004, Dudareva et al. 2006). The first type includes the production of novel compounds which are the major components of volatile blends. The second type includes the production of the same components produced in lesser quantities after mechanical damage. Among the induced volatile substances released from plants are green leaf volatiles (GLVs)

which are important signalling cues and are released immediately after the plant tissue has been damaged by either a herbivore or mechanically (Yan and Wang 2006). Green leaf volatiles are six carbon alcohols, aldehydes and derivative esters (Whitman and Eller 1992) and are responsible for the odour of damaged leaves, for example, fresh mowed grass, and therefore are also defined as typical wound signals (Whitman and Eller 1990, Dudareva et al. 2006).

Herbivorous arthropods can be directly affected by emitted HIPVs due to their toxic, repelling and deterring properties which can result in their death or retard development (Dicke 1999). Herbivore-induced plant volatiles can also affect herbivores indirectly by attracting natural enemies of the attacking herbivore, which can protect the plant from further damage (Dicke 1999, Dicke et al. 1999, Turlings and Ton 2006, Dudareva et al. 2006). A schematic representation of an increase in volatile compounds released by plants in response to the application synthetic HIPVs and of silicon or subsequent herbivore feeding is shown in Fig. 6.1.

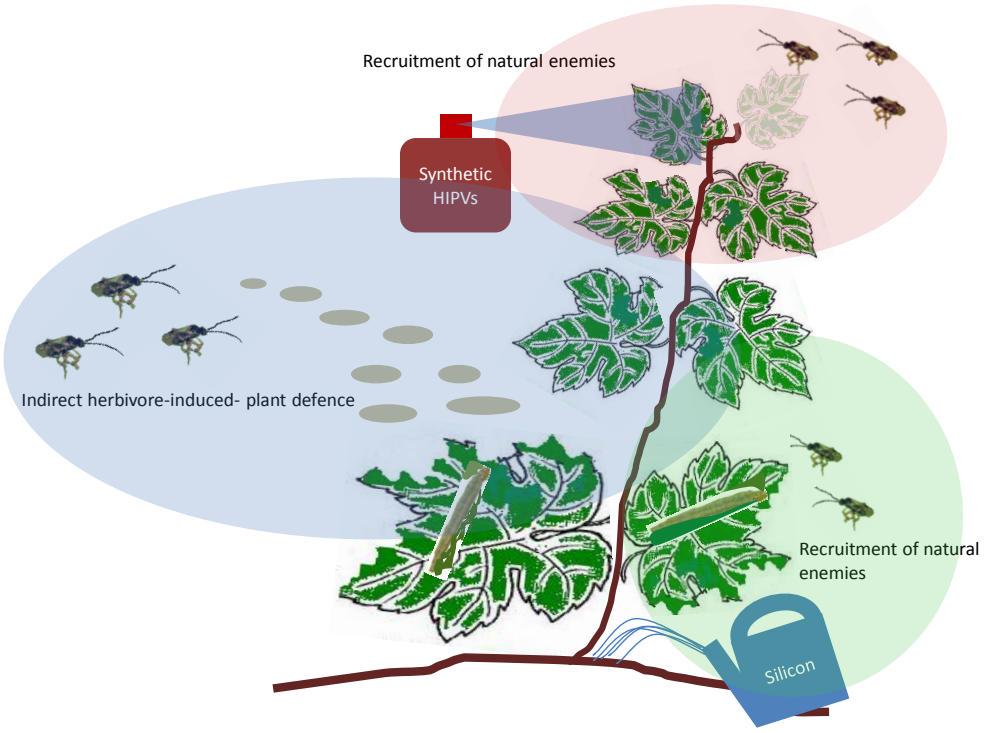

Fig. 6.1 Emission of HIPVs and recruitment of natural enemies in response to herbivore damage, application of synthetic HIPVs and silicon (also see Section 6.8 below)

Emissions of HIPVs occur not only in response to herbivore feeding but also from the deposition of insect eggs on plant parts (oviposition) or from insect feeding on plant roots, thus attracting the natural enemies that use these eggs as hosts or root feeders as prey respectively (Hilker and Meiners 2006, Turlings and Ton 2006). In addition, HIPV compounds also act as plant-to-plant signals (Ruther and Kleier 2005, Dudareva et al. 2006, Yan and Wang 2006), triggering responses in neighbouring undamaged plants, effectively warning of impending attack. Plants may then produce their own direct and indirect defences to respond faster to impending or

future herbivore attack (Engelberth et al. 2004, Turlings and Ton 2006, Baldwin et al. 2006).

However, HIPVs not only attract beneficial insects, but can also attract herbivores that may cause increased damage to the plant (Dudareva et al. 2006). Bolter et al. (1997) demonstrated in a laboratory study the attraction of the herbivorous Colorado potato beetle (CPB) *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) to herbivore-damaged plants during and after feeding; CPB adults were attracted to small potato plants infested with CPB and beet army worm *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) larvae. However, Whitman and Eller (1990) argued that once a plant is under herbivore attack, attracting beneficials may outweigh the disadvantage of attracting additional herbivores.

Variation in composition of HIPV blends occur within an individual plant species as well as between different cultivars of the same species. Takabayashi et al. (1994) demonstrated that predators were preferentially attracted to volatiles of young cucumber leaves infested with *T. urticae* compared to older ones also infested with *T. urticae* as a plant's response of directing predators to its growing parts. The attacking herbivore and abiotic factors such as light intensity, humidity, water stress, availability of nutrients and wind may also have an affect on HIPV composition (Takabayashi et al. 1994, Dudareva et al. 2006, Kessler et al. 2006). Takabayashi et al. (1994) assessed in an olfactometer that uninfested lima

bean leaves placed under high light intensity were more attractive to predatory mites than under lower light intensity. Gouinguene and Turlings (2002) described higher induced volatile emissions by corn plants when the soil was relatively dry, relative humidity was between 45-65% and air temperature was between 22-27 ºC, with high light intensity and continuous fertilisation of the soil.

Little is known about the volatile profile induced by feeding or ovipositing arthropod herbivores on grapevines. Van den Boom et al. (2004) identified the volatile profiles induced by feeding of the spider mite *T. urticae* on several plants species including grapevines. The authors of that study compared the volatile blend induced by spider mite feeding with blends emitted from mechanically damaged and healthy grapevine leaves. Results revealed that spider mite-infested grapevine leaves emitted several dominating novel compounds including MeSA, (3*E*)-4,8-dimethyl-1,3,7-nonatriene, β-caryophyllene and α- humulene. Additionally, the amount of (3*E*)-4,8-dimethyl-1,3,7-nonatriene was four-times higher in spider mite-infested leaves compared with blends released from uninfested leaves. Loughrin et al. (1997) identified 19 compounds emitted from grape vine (*V. labrusca* L.) leaves after damage by Japanese beetle *Popillia japonica* Newman (Coleoptera: Scarabaeidae), the majority comprising aliphatic aldehydes, alcohols, esters, and terpene hydrocarbons, which were produced about 50 times higher in beetle-damaged vines compared to undamaged vines. In recreating Loughrin et al. (1997)'s experiment, Liu et al. (2006)

found peak emission time of volatiles produced from *V. labrusca* damaged by *P. japonica* occurred at 2.3 to 2.8 hours after feeding began.

6.6 Silicon's role in plant defence against pests

The role of silicon (Si) in plant defence has been recognised since the early 1900's, although Si's involvement in induced plant defences against arthropod pests is a relatively new field of study (Reynolds et al. 2009). Silicon is the second-most abundant element in the Earth's crust (Epstein 1994) and is present in plants in amounts equivalent to, and sometimes in excess of, those of Ca, Mg, S, and P (Epstein 1999), although the extent to which it is an essential plant nutirent is not known. However, the importance of Si as an element that is especially beneficial for plants exposed to abiotic (for example, drought, salinity & heavy metal toxicity) and biotic (for example, insect and pathogen) stresses is now beyond doubt. More recently, it has been found that Si also directly enhances induced resistance in plants attacked by pests by acting as a signal in inducing systemic chemical defences in plants (Gomes et al. 2005, Kvedaras and Keeping 2007, Kvedaras et al. 2009).

6.6.1 Silicon uptake by Vitis vinifera

The content of Si in plant tissue varies depending on the plant species, with dicotyledons generally containing lower concentrations ($\leq$1% dry weight) compared to grasses (1-5% dry weight) and wetland grasses (10-15% dry

weight) (Jones and Handreck 1967, Epstein 1994, Mitani and Ma 2005, Matichenkov and Bocharnikova 2007). Silicon-accumulators are defined as plants which contain > 1% Si dry weight and show a Si:Ca molecular ratio > 1; plants which contain 0.5-1% Si (Si:Ca molecular ratio < 1) are defined as intermediate accumulators; and plants which contain < 0.5% Si are termed non-accumulators (Takahashi and Miyake 1977, Ma 2007). Silicon-accumulators generally contain 8-20 times as much Si in their leaves as non-accumulators (Takahashi and Miyake 1977, Adatia and Besford 1986).

There is little published research on the role of Si in *V. vinifera*, a dicotyledon and a Si non-accumulator. However, it is known that Si uptake by *V. vinifera* via the transpiration stream is a passive process; a view reinforced by studies finding constant amounts of soluble SiO_2 in the 'transport regions' of the plant (including stem and petiole), and an accumulation of SiO_2 in older leaves (Blaich and Wind 1989, Blaich and Grundhöfer 1997). *Vitis vinifera* is able to restrict Si levels in the xylem, with Lafos (1995) observing that the concentration of silica in the roots is correlated with, but in considerably higher concentration, than SiO_2 content in the soil solution, indicating that $Si(OH)_4$ might be concentrated in the root-symplasts around the xylem from where it diffuses into the transpiration stream according to the speed of water flow (Blaich and Grundhöfer 1997).

Only one study reports the uptake of varying concentrations of soil-applied Si by *V. vinifera*. This study, by Blaich and Grundhöfer (1997), applied K_2SiO_3 to the soil growing *V. vinifera* at 10 and 112 mg/kg SiO_2, as well as conducting supplementary experiments using 200 and 400 mg/kg SiO_2, forming an oversaturated solution. The results of that study indicated that; 1) Si solubility is dependent on soil temperature – solubility at 5^{o}C was 50% less than solubility at 20^{o}C, 2) Si-solubility in soil is reflected in Si content in *V. vinifera* leaves, 3) Si content in different *V. vinifera* cultivars did not vary significantly, 4) uptake of Si by *V. vinifera* depends on transpiration rate of the plant and decreases in dry conditions, 5) older leaves of plants grown on 112 ppm SiO_2 contained higher Si content (2% SiO_2) than younger leaves (0.5% SiO_2) with greater Si content in the leaf periphery, 6) Si content in shoots and petioles was less than 10% Si content in leaves, 7) soluble silica in *V. vinifera* leaves grown on 112 ppm SiO_2 made up 30% total silica content in young leaves, 15% in medium age leaves, and 7% in older leaves, and 8) Si content in grapevines could be further enhanced when applied to the soil at 200 and 400 ppm SiO_2, although $Si(OH)_4$ in *V. vinifera* tissue saturates at 150 ppm at 20^{o}C. These findings are consistent with what is known about the uptake of Si in plants in general (Jones and Handreck 1967, Takahashi and Miyake 1977). Matichenkov and Bocharnikova (2007) consider soluble-Si in the soil solution at 20-40 ppm to be a ‘low level of deficiency’ and > 40 ppm Si as ‘without deficiency’. However, additional research is required to determine

Si-requirements of grapevines, and the minimum and maximum threshold levels of Si required in the soil solution to provide plant protection against arthropod attack. Also unknown is the time lag between Si application and the resulting effects on enhanced pest-resistance of grapevines.

6.6.2 Role of silicon in induced plant chemical defences

There is recent evidence to suggest that soluble Si is involved in induced chemical defence to insect herbivore attack, through the enhanced production of defensive enzymes (Gomes et al. 2005, Kvedaras and Keeping 2007, Ranger et al. 2009) or possibly the enhanced release of plant volatiles for attraction of biological control agents in pest management (Kvedaras et al. 2009). Silicon either alone or together with *Schizaphis graminum* Rondani (Homiptera: Aphididae) pre-infestation, elicited a significant increase in the defensive enzymes, peroxidase, polyphenoloxidase, and phenylalanine ammonia-lyase, activity in wheat (Gomes et al. 2005). Similarly, Ranger et al. (2009) identified and quantified phenolic acids and flavonols in leaf tissue of *Zinnia elgans* treated with potassium silicate and infested with the green peach aphid *Myzus persicae* Sulzer (Hemiptera: Aphididae). This study found significant elevations in the defensive enzymes 5-caffeoylquinic acid, *p*-coumaroylquinic acid, and rutin, and a slight elevation in guaiacol peroxidase activity. Further, the total cumulative fecundity and the intrinsic

rate of increase of *M. persicae* were reduced on Si-treated plants, which was believed to be caused in part by the defence-related compounds.

More recently, Kvedaras et al. (2009) hypothesised that Si may also enhance induced chemical defences against arthropod attack by altering and enhancing the volatile compounds emitted by an attacked plant. The authors were able to demonstrate that Si-treated plants with a pest-infestation were more attractive to natural enemies than Si-untreated plants with a pest infestation. Further, this effect was reflected in elevated biological control in the field. Additional studies to measure and identify the compounds produced by pest-infested plants, particularly those which may be strongly affected by treatment of the plants with Si are now being pursued. Research aimed at understanding the role of Si in HIPV production is gaining momentum although this area remains novel in horticultural and indeed agricultural research.

6.7 Novel vineyard pest management strategies in Australia

6.7.1 The use of synthetic HIPVs for enhancing recruitment and residency of beneficial arthropods

Laboratory and field trials have assessed the potential and practicality of synthetic HIPVs for application in IPM programs, particularly the increased searching activity of beneficial arthropods and direct recruitment into the crop at certain times of the year (Khan et al. 2008). Several synthetic HIPVs, such as MeSA, MeJA, methyl anthranilate (MA), cis-3-hexen-1-ol (He), cis-3-hexenyl acetate (HA) and benzaldehyde (Be), have been tested for their efficacy to attract beneficial insects in field studies in North American grapevine- and hop-yards. James (2003, 2003a) provided direct field evidence that the abundance of the green lacewing *Chrysopa nigricornis* Burmeister (Neuroptera: Chrysopidae), *Geocoris pallens* Stål (Hemiptera: Geocoridae), hoverflies (Diptera: Syrphidae), and the lady beetle *Stethorus punctum picipes* Casey (Coleoptera: Coccinellidae) were significantly increased on sticky traps baited with controlled released dispensers (CRD) containing MeSA in a hop-yard over several months. The large population of predatory insects in the MeSA-baited hop yard led to a reduction of spider mites below the economic threshold for the rest of the season. Similarly, a subsequent study conducted in vine- and hop-yards by James and Price (2004) demonstrated that brown lacewings *Hemerobius* spp. (Neuroptera: Hemerobiidae), *Orius tristicolor* White (Hemiptera: Anthocoridae), braconid wasps (Hymenoptera) and flies from the families Empididae and Sarcophagidae (Diptera) were attracted to CRD containing MeSA. James (2005) also showed attraction in the field of braconids to MeJA and MeA, the predaceous fruit fly *Thaumatomyia glabra* Meigen

(Diptera: Chloropidae) to MeJA-baited traps and attraction of *O. tristicolor, S. punctum picipes, Anagrus daanei, S triapitsyn* (Hymenoptra: Mymaridae), braconid wasps, and micro Hymenoptera to He. The GLV, HA increased abundance of *O. tristicolor,* the predatory mirid *Deraeocoris brevis* Uhler (Hemiptera: Miridae) and *S. punctum picipes* (James 2003a). Numbers of *Orius tristicolor, S. punctum picipes* and flies from the families Tachinidae and Sarcophagidae (Diptera) were also increased by Be (James 2005).

James and Grasswitz (2005) provided field evidence for attraction of MeSA, MeJA and HA to two parasitic wasp families, *Angarus* spp. (Hymenoptera: Mymaridae) and *Metaphycus* spp. (Hymenoptera: Encyrtidae) in vineyards. However, previous field studies by James (2003a, 2005), which deployed the same HIPVs, did not show any response by these parasitic wasps. The authors suggested the concentrations of HIPVs used could have caused the variance in attraction, with the higher HIPV rates possibly acting as a repellent to the hymenopteran species. Another explanation could have been that the dispersion of HIPVs from an earlier study in the same vineyard (James 2005) led to signalling of the plants to produce their own HIPV blends which then led to attraction of *Angarus* spp. and *Metaphycus* spp. in the latter study (James and Grasswitz 2005). The potential for seasonal differences and concentration effects highlights the need for further research on the role of HIPVs as pest management tools.

James (2011) provided field evidence of plant signalling functions of MeSA and HA in inducing indirect defence responses in hop and grapevine plants when sprayed with botanical oil pesticides containing small amounts of these HIPVs. In that study, the abundance of some carnivorous and parasitic insects was greater near HIPV-treated grape and hop plants. James (2011) explained that the higher numbers of natural enemies was unlikely to have been caused by direct attraction to botanical oils containing HIPVs due to their rapid evaporation after spraying and the small amounts of HIPVs involved. The author hypothesised that the treated plants altered their physiological response by emitting endogenous HIPV blends that lead to the attraction of natural enemies. Similar field studies which use synthetic spray-applied HIPVs are currently being conducted by the authors in Australian vineyards (Fig. 6.2). This work investigates several different synthetic HIPVs, spray-applied to *V. vinifera* at three different release rates, and monitored for their attraction by beneficial arthropods over extended time periods. The work is also testing these spray-applied HIPVs in combination with floral resources to explore whether this combined approach termed 'Attract and Reward' (Kahn et al. 2008) could further boost CBC.

Fig. 6.2(a)

Fig. 6.2(b)

Fig. 6.2(c)

Fig. 6.2 Spray application of synthetic HIPVs (a) and buckwheat integrated (b) in an Australian vineyard to enhance CBC. Olfactometer bioassay with potted grapevines treated with silicon enclosed in air-tight bell-jars to determine their attractiveness to beneficial arthropods (c)

6.7.2 The use of silicon to enhance plant defence mechanisms

Recent studies have started to scrutinise the role that Si plays in enhancing natural enemy attraction to the plant, thought to be through the qualitative and quantitative changes of the volatile profile emitted by plants under attack by arthropod pests (Fig. 6.1, Kvedaras et al. 2009). To date, Kvedaras et al. (2009) is the only published study that demonstrates increased natural enemy attraction to pest-infested plants and subsequent enhancement of field biological control through the plausible role of Si on HIPV production. There are therefore numerous opportunities for research on the role of Si on HIPV production, including in *V. vinifera.*

The authors of this chapter have commenced laboratory and field studies in Australia looking at the function of Si in HIPV production in *V. vinifera*. Grapevines have been treated with varying concentrations of Si, and the volatile compounds emitted as a result of arthropod attack absorbed onto solid-phase-microextraction (SPME) fibres, before being identified and quantified using Gas Chromatography-Mass Spectrometry (GC-MS). Arthropods may respond to HIPVs at low concentrations, below the level of analytical detection (D'Alessandro and Turlings 2005, Gouinguene et al. 2005, Dicke 2009), and therefore, a Y-tube olfactometer was used in conjunction with the SPME/GC-MS analysis to determine the attractiveness of the plant to beneficial arthropods (Fig. 6.2). Field trials conducted in vineyards in the Central West of New South Wales have used sticky traps to

monitor arthropod populations (both pest and beneficial), and egg-bait cards to determine the predation or parasitism levels within the varying Si-treatments. The treated grapes were then harvested and made into wine, which underwent sensory analysis to determine whether the application of Si altered the taste or quality of the wine. This quality assessment on consumer preference to in-field manipulations is an important component of the production cost to sales ratio and a novel approach which only a small number of horticultural enterprises have used.

6.7.3 The role of Functional Structural Plant Models in vineyard chemical ecology research

Simulation models are an important component of agricultural and ecological research, including experimental work using sensitivity analysis. Functional Structural Plant Models (FSPM) are computer-based mechanistic models describing the structure and functions of plants and plant organs, usually using Lindenmayer Systems, a botanical formalism (Prusinkiewicz 2004**)**. Botanical and physiological information drawn from the published literature are used to construct photorealistic three dimensional virtual plants (Prusinkiewicz 2004**)**.

A range of commercially important crops and weeds have already been modelled, including grapevines (*Vitis vinifera*), peach (*Prunus persica*), wheat (*Triticum eastivum*), rice (*Oryza sativa*), corn (*Zea mays*), barley (*Hordeum vulgare*), faba bean (*Vicia faba*), yellow starthistle (*Centaurea solstitialis*), rye grass (*Lolium perenne*), and many others. FSPM are capable of simulating functions such as carbon capture and partitioning, water movements, nitrogen cycling, hormone synthesis as well as acropetal or basipetal flows. In addition, FSPM are able to describe interactions between the plant and their surroundings, such as light interception, air flow movements and exogenous spray depositions (Prusinkiewicz et al. 2007, Dorr et al. 2008). Finally, the behaviour of virtual pests and predators, as well as disease epidemiology can be simulated and new hypothesis tested without the associated cost of complex field experiments (Hanan et al. 2002, Skirvin 2007). FSPM are therefore a multi-variate integration tool rarely available in field studies and positively contribute to pre- experimental studies of complex ecosystems such as vineyards. HIPV research using FSPM has not yet been reported in the literature. This is not due to a computational challenge but to our limited knowledge of the synthesis and emission of these compounds and hence represents an opportunity for investigative ecological research.

6.8 Summary

Plants emit HIPVs as part of their defence mechanisms, which can act as either a direct attractant or repellent to surrounding arthropods, as well as warning nearby plants of impending attack. Novel strategies using chemical ecology include the use of exogenously-applied HIPV compounds or Si, which may trigger the plant to produce its own semiochemicals, or enhance and/or alter the existing volatile profile, thus boosting the plant's own defences against arthropod pests. These inducing agents 'switch on' or 'prime' the plant for imminent pest attack, and are the focus of current research in the field of pest herbivore management in vineyards and provide opportunities to enhance CBC.

In order to apply these novel strategies in vineyards, research needs to identify whether insects from the second and fourth trophic level are attracted or repelled by these semiochemicals which may result in the attraction of organisms other than the target pest species. Research should also focus on the duration of effects of the inducing agents, and blends of HIPVs should be tested in addition to single compounds. Further, studies should address the practicality of field application and whether these strategies are compatible with other grapevine management practices, including the application of fungicide sprays.

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