WESTERN SYDNEY
UNIVERSITY

Hawkesbury Institute
for the Environment

Beyond physical resistance: novel aspects of plant silicon defences against arthropod herbivores

By

Tarikul Islam
BSc (Hons), MS

A thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

Hawkesbury Institute for the Environment

Western Sydney University

February 2022

Declaration of Authenticity

The work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.

Acknowledgements

I would like to express my deepest gratitude and greatest thanks to my primary PhD supervisor, Scott N. Johnson, for his excellent guidance and support throughout my PhD. Without Scott's help and support, this thesis would not have been completed. My secondary supervisor, Ben D. Moore, deserves special thanks for his time, effort, suggestions, and guidance.

My sincere thanks go to my lab mates and friends who helped me a lot throughout my three years at HIE: Fikadu N. Biru, Simranjit Kaur, Rocky Putra, Ximena Cibils-Stewart, Al Emran, Jamie M. Waterman, Dr Rebecca K. Vandegeer, Dr Casey R. Hall, Sidra Anwar, Rhiannon C. Rowe, Mahmud Hossain, Mahfuza Pervin, Mahmuda Sharmin, Golam Kibria, Golam Sarwar, and Kamrul Hassan. I am also grateful to the Bangladeshi community at HIE for their support and love.

I must thank all the students and technical and support staff at HIE. In particular, I would like to acknowledge the help provided by Dr Andrew Gherlenda, Dr Craig Barton, Dr Dominika Wojtalewicz, and Burhan Amiji. I sincerely thank Philip Smith for proofreading this thesis. Special thanks to my examiners, Emeritus Professor Myron Zalucki and Professor Michael J. Stout, for their consideration of this thesis and helpful suggestions for its improvement.

I would like to thank my beautiful wife, Tasnim Mahbub, my sister, Jannatun Nahar, and my parents, Shafi Talukder and Yasmin Ara, for their love, support, and encouragement. I am thankful to Jamil Hossain Biswas for his friendship and visits. To all of you good people who offered help along the way, my sincerest thanks.

Finally, I would like to dedicate this thesis to the memory of my grandfather, Majom Talukder, who always loved me unconditionally.

Table of Contents

List of Tables

List of Figures

Abstract

Silicon (Si) fertilisation has received increasing recognition in recent years for ameliorating biotic stresses in plants, including damage by pest herbivores. Si defences are effective against chewing herbivores as silicification makes plants abrasive and tougher, whereas sap-feeding insects are thought to be less affected. There are still substantial knowledge gaps regarding the effects of Si on direct and indirect (i.e. whereby plants benefit from natural enemies of herbivores) plant defences against herbivorous arthropods. Moreover, it is unknown how anti-predator defences (e.g. morphological, behavioural, and immune defences) of herbivorous insects are affected when feeding on Si-rich diets nor whether Si mediates interactions (e.g. competition, facilitation) between herbivores of different feeding guilds. Using plants with contrasting capacity for Si accumulation (i.e. low, moderate, and high Si-accumulators) and pest herbivores from different feeding guilds (i.e. chewing, sap-feeding, and cell-content feeding), this work explores some novel facets of plant Si defences against herbivorous pests.

Chapter one synthesises general features of, and recent information on, plant Si defences against pest herbivores and highlights the existing knowledge gaps. Chapters two to six are empirical research chapters based on glasshouse experiments. Chapter two reports the magnitude and locality of Si induction in plants (*Cucumis sativus*) in response to attack by a polyphagous chewing insect (*Helicoverpa armigera*). Chapter three assesses the impacts of Si on the direct and indirect defences of *Phaseolus vulgaris* plants against a cell-feeding mite, *Tetranychus urticae*. Chapter four addresses how Si fertilisation impacts the anti-predator defences of *H. armigera* larvae and plant compensation in response to insect

herbivory. Chapter five examines the impacts Si fertilisation on plant-mediated interspecific interactions between a chewing (*H. armigera*) and a sap-feeding (*Rhopalosiphum padi*) insect when sharing the host plant, *Brachypodium distachyon*. Chapter six examines whether feeding on Si-supplemented plants hinders cryptic colouration of *H. armigera* larvae by inhibiting carotenoid sequestration in the haemolymph.

Insect attack increased Si accumulation in *C. sativus* plants both locally in attacked tissues by at least 17% and systemically in intact (i.e. non-attacked) tissues by 10-19%. Induction of Si defences, in addition to constitutive Si defences, further decreased the performance of *H. armigera* larvae. Si supplementation of *P. vulgaris* plants suppressed the population growth and damage potential of *T. urticae*. Si supplementation also altered the emission of herbivore-induced plant volatiles (HIPVs) following *T. urticae* attack, which promoted the attraction of the predatory mite, *Phytoseiulus persimilis*, to Si-supplemented plants. *Helicoverpa armigera* larvae feeding on Si-supplemented *B. distachyon* plants had low integument resistance and high phenoloxidase activity (+71%) in the haemolymph compared to that of larvae feeding on Si-free plants. Furthermore, plants showed better compensation for herbivory when they had access to Si supply. The diminished performance of *H. armigera* larvae on Si-supplemented plants enhanced colonisation by *R. padi* when sharing the host plants contemporaneously. Feeding on Si-supplemented plants disabled green cryptic colouration of *H. armigera* larvae by decreasing the sequestration of lutein (−53%) and total carotenoids (−44%) in the haemolymph.

Taken together, this work underpins that Si fertilisation can augment direct and indirect plant defences against pest herbivores and could be a sustainable management strategy for chewing and cell-feeding herbivores. Si could also play a part in pest biocontrol by promoting natural enemy attraction and undermining the anti-predator defences of insect herbivores. In contrast, Si fertilisation could provide a competitive advantage to sap-feeding insects by diminishing the performance of co-occurring chewing insects. Potential areas of future research and the limitations of the current work are discussed.

Preface

This thesis comprises original work conducted by myself with guidance from my supervisors: Scott N. Johnson (primary supervisor) and Ben D. Moore. I conceptualised the research project and established the experimental design together with my supervisory panel. I have collected, analysed and interpreted all the data in discussion with my supervisory panel. I have written the thesis and all publications therein with guidance from my supervisory panel.

This thesis is a standard thesis and not a thesis by publication. However, it consists of five stand-alone experimental chapters that have been written in a format appropriate for specific peer-reviewed journals. Chapters have either been published (chapters 2 to 5) or are under review (chapter 6) in peer-reviewed journals. Each research chapter (chapters 2 to 6) is self-contained and thus some repetition in terms of information and methodology will be present.

Chapter 1

General Introduction

1.1 Herbivorous pests and plant defence

Almost half of the total described insect species (i.e. half a million) are considered herbivorous Schoonhoven et al. (2005). Plants and herbivorous insects are thought to be coevolved (Ehrlich & Raven, 1964). The evolutionary arms race between plants and herbivorous insects for hundreds of millions of years has resulted in myriads of defence strategies in plants and adaptations in herbivores (Carmona et al., 2011; Gatehouse, 2002; Schuman & Baldwin, 2016). Plant defences are often categorized as physical and chemical defences, both of which can be expressed constitutively or induced upon herbivore attack (Aljbory & Chen, 2018; Gatehouse, 2002; Karban, 2020; War et al., 2012).

Physical defence includes plant anatomical traits such as leaf trichomes, waxy cuticles, silicified tissues, and lignified cell walls, and these traits can form one of the first lines of defence against feeding and oviposition by herbivorous pests (Gatehouse, 2002; Schuman & Baldwin, 2016; War et al., 2012). Chemical defence comprises an arsenal of secondary metabolites (e.g. alkaloids, carotenoids, phenolics, terpenoids), some of which can be toxic or repellent for insect pests, some can function as antioxidants (e.g. carotenoids), and some can facilitate the attraction of carnivorous natural enemies of pest herbivores (Heath et al., 2012; Mithöfer & Boland, 2012; War et al., 2012). Specifically, when challenged by pest herbivores, plants can release distinct blends of volatile organic compounds, including terpenoids, which are known as herbivore-induced plant volatiles (HIPVs) (Dicke, 2009).

These volatile compounds can serve as chemical cues for foraging natural enemies (i.e. predators and parasitoids) of herbivores and attract them to defend plants as 'bodyguards', a phenomenon termed indirect plant defence (Dicke, 2009; Dicke & Baldwin, 2010). Another defence strategy in plants is tolerance of herbivory, which refers to the ability of plants to withstand or recover from pest feeding damage. Plants can exploit different physiological mechanisms to tolerate herbivory, including increased rates of photosynthesis, increased branching and tillering, and resource translocation (i.e. non-structural carbohydrates) from storage organs such as roots to shoots for compensatory growth (Peterson et al., 2017; Tiffin, 2001).

In general, host plant defence can determine the performance and fitness of insect herbivores feeding on them (Gatehouse, 2002; Schoonhoven et al., 2005; War et al., 2012). Furthermore, the quality and defensive traits of host plants can impact the anti-predator defences of insect herbivores against their natural enemies, including morphological (e.g. cryptic colouration, integument resistance), behavioural (e.g. headrearing, biting, regurgitating), chemical (e.g. glucosinolate sequestration), and immune (e.g. encapsulation and melanisation) defences (Greeney et al., 2012; Gross, 1993; Lampert, 2012; Winde & Wittstock, 2011).

1.2 Silicon in plants and its roles in anti-herbivore defences

Silicon (Si) is a beneficial mineral nutrient for plants. Although not considered essential for plant growth, Si provides multiple benefits to plants, particularly in alleviating biotic (e.g. pathogens, insects) and abiotic (e.g. salinity, drought, heavy metal toxicity) stresses (Epstein, 1999; Frew et al., 2018; Reynolds et al., 2016). Considering the roles of Si in

stress alleviation analogous to organic secondary metabolites, Epstein described Si as an 'inorganic secondary nutrient' for plants (Epstein, 2009).

1.2.1 Silicon uptake and accumulation in plants

Si comprises almost 31% of the soil constituents (Epstein, 1999). Thus, Si is ubiquitous in the soil, although mostly present in the form of insoluble silicates or oxides. Consequently, some soils can be deficient in bioavailable Si as plant roots can take up Si only as undissociated orthosilicic acid (H_4SiO_4) from the soil solution (Epstein, 1994; Ma & Yamaji, 2006). Silicic acid is further translocated from root to shoot by transpiration stream via xylem and subsequently polymerized to form solid amorphous silica gel ($SiO_2 \cdot nH_2O$), or opal, and irreversibly deposited in cell walls, cell lumens and spaces between cells (Ma & Yamaji, 2006; Mandlik et al., 2020). Plants can deposit Si in shoots, inflorescences and roots; Si deposition is usually higher in transpirational organs (e.g. leaves) compared to storage organs (e.g. roots) (Mandlik et al., 2020). Two transporter proteins, Lsi1 and Lsi2, involved in Si uptake and translocation in plants were first identified in rice (Ma et al., 2006; Ma et al., 2007a), and similar transporters were further reported in other plants, including wheat, barley, maize, pumpkin, cucumber, and soybean (Ma & Yamaji, 2015). Lsi1 is an influx, channel-type transporter that enables passive movement of Si from the external solution to plant cells via the plasma membrane. In contrast, Lsi2 is an efflux transporter that actively transports Si out of plant cells (Ma & Yamaji, 2015).

Plant species vary greatly in their ability to accumulate Si, which can be partially explained by variations in the presence and allocation of Si transporters and their regulation in plants (Ma & Yamaji, 2015; Mandlik et al., 2020). Plants are often classified based on their ability

to accumulate Si in tissues on a dry weight basis as Si-accumulators (> 1.5%), intermediate accumulators (0.5-1.5%) and non-accumulators (< 0.5%) (Bakhat et al., 2018). In general, non-vascular primitive plants accumulate much higher Si than vascular plants (Hodson et al., 2005). Among flowering plants, monocot orders Poales and Arecales accumulate substantial amounts of Si compared to dicots (Hodson et al., 2005). Specifically, grasses (Poaceae), including some cereal crops (e.g. rice, wheat, barley), can accumulate up to 10% Si of their dry weight which is often higher than the concentrations of other essential macronutrients in plants such as phosphorus (P), calcium (Ca), magnesium (Mg), and sulphur (S) (Epstein, 1994; Epstein, 2009; Ma & Yamaji, 2006). Although most dicots are poor Si accumulators (< 0.1%), some dicot families (e.g. Cucurbitaceae, Urticaceae, Fabaceae) can accumulate ecologically significant amounts of Si in tissues (~ 1% of total dry weight) (Hodson et al., 2005).

1.2.2 Silicon as a physical defence against herbivores

Si deposition in plant tissues makes them tougher and abrasive, forming an effective physical barrier against chewing herbivores (Massey & Hartley, 2009). Indeed, Si deposition in the epidermis of leaves can form a thick Si-cuticle double layer (e.g. 2.5 µm thick layers in rice leaves) (Yoshida et al., 1962), which is harder to shear and masticate for chewing insects (Johnson et al., 2019b). Notably, herbivory can induce increased Si accumulation in plants (Johnson et al., 2020b; Massey et al., 2007), although the magnitude and locality of such induction are not well understood for herbivorous insects (Hartley et al., 2016). Feeding on Si-supplemented plants can wear down the mandibles of chewing insects and can reduce the palatability and digestibility of tissues to insect herbivores (Frew et al., 2018; Kvedaras et al., 2009; Massey & Hartley, 2009). However, it remains unclear

whether malnutrition in insects when feeding on Si-supplemented plants compromises their anti-predator defences (i.e. morphological, behavioural, or immune defences) against their natural enemies, given that malnutrition may constrain resources for insect growth and defences.

While the effects of Si against chewing insects are well-documented (Frew et al., 2018; Johnson et al., 2021a; Reynolds et al., 2016), the impacts of Si against sap-feeding insects such as aphids remain contentious, with studies reporting negative (Dias et al., 2014), positive (Johnson et al., 2017), or neutral (Massey et al., 2007; Rowe et al., 2020) effects of Si on aphid biology or population growth. Given that aphids feed on phloem sap using probing stylets, they may actively avoid Si barriers in tissues and may suffer less from digestion inefficiency compared to chewing herbivores (Johnson et al., 2021a; Rowe et al., 2020). Furthermore, there is a dearth of research on the effects of Si against cell-content feeding herbivores such as spider mites (Table 1-1), some of which are key pests (e.g. *Tetranychus urticae*) of many economically important crops (Jeppson et al., 1975). Considering the differential effects of Si against chewing versus sap-feeding herbivores, Si fertilisation of plants can impact plant-mediated interspecific interactions (e.g. competition for resources) between chewing and sap-feeding insects when sharing a host plant. However, this has not yet been investigated.

1.2.3 Silicon can influence plant chemical defences

Plant Si defences can negatively impact the biology of arthropod herbivores in multiple ways (Table 1-2). Si in solid form or as opal is chemically unreactive and thus does not directly influence plant biochemistry or the metabolome (Epstein, 1999). However, soluble

Si (i.e. silicic acid) can act as ligand for organic metabolites and impact plant direct chemical defences (Epstein, 2009). Previous studies have shown that Si can trigger the expression of plant defence-related genes (Yang et al., 2018; Ye et al., 2013), often leading to the enhanced production of defensive enzymes (Kim et al., 2014; Ye et al., 2013). Moreover, Si can impact plant phytohormone signalling (Hall et al., 2019; Waterman et al., 2020), for example, the jasmonic acid (JA) pathway that regulates the downstream synthesis of defensive chemicals against biotic stresses, including HIPVs (Ponzio et al., 2013). Furthermore, Si has been shown to influence indirect chemical defence in plants by altering HIPV emission upon herbivore attack and thereby attracting increased numbers of natural enemies to plants (Kvedaras et al., 2010; Liu et al., 2017). However, this phenomenon is not well explored for different feeding guilds of herbivores (Table 1-1), and the mechanisms behind such altered volatile emissions are not yet clear.

Table 1-1 Effects of Si on plant defences against herbivores from different feeding guilds.

Effect of Si on plant defence	**Herbivore feeding guild**		
	Chewing	Sap-feeding	Cell-content-feeding
Constitutive direct defence	Demonstrated	Inconclusive	Understudied
Induced direct defence	Demonstrated	Inconclusive	Understudied
Induced indirect defence	Understudied	Understudied	Not studied yet

Table 1-2 Negative effects of Si against herbivorous arthropods. Si supplementation of plants can affect arthropod herbivores in multiple ways.

Negative effect of Si against herbivores	**Reference**
Reduced survival rate/ increased mortality	Nagaratna et al. (2022), Pereira et al. (2021), Leroy et al. (2022), Ferreira et al. (2011), Han et al. (2016)
Mandibular wear	Massey and Hartley (2009), Waterman et al. (2021), Acevedo et al. (2021)
Reduced growth rate/ weight gain	Massey et al. (2006), Hall et al. (2020a), Johnson et al. (2020b), Biru et al. (2021), Sidhu et al. (2013)
Increased development time	Han et al. (2015), Leroy et al. (2022), Abbasi et al. (2022), Yang et al. (2017)
Gut damage	Andama et al. (2020)
Increased abrasiveness/ reduced palatability and digestibility of leaf tissues	Massey and Hartley (2009), Han et al. (2015), Massey et al. (2006)
Reduced penetration success of borers and increased penetration time	Nikpay et al. (2015), Hosseini et al. (2012), Kvedaras et al. (2007), Sidhu et al. (2013)
Reduced consumption/ damage potential	Nascimento et al. (2018), Waterman et al. (2021), Moise et al. (2019b), Frew et al. (2017a)
Reduced oviposition	Abbasi et al. (2022), Peixoto et al. (2011), Lin et al. (2022), Ribeiro et al. (2021)
Reduced population growth/ colonisation success	Nikpay and Soleyman-Nejadian (2014), Peixoto et al. (2011), Christalcatalani et al. (2017), Alyousuf et al. (2022), Yang et al. (2017)

1.3 Study system

1.3.1 Plants

1.3.1.1 Cucumber

Cucumber, *Cucumis sativus* (Cucurbitaceae), is a vine type annual vegetable crop native to Asia and popularly grown worldwide for its pepo type of fruits. Cucumber plants are hosts for several pest herbivores including lepidopteran larvae (e.g. *Diaphania indica*, *Helicoverpa* spp.), cucumber beetles, squash bugs, aphids, thrips, miners, whiteflies, and spider mites (Ghule et al., 2014; Kaur et al., 2010). Cucumber is a moderate Si accumulator that usually accumulates 1-1.5% Si of its dry biomass (Bakhat et al., 2018). The beneficial roles of Si in withstanding fungal diseases in cucumber, such as powdery mildew and root rot, are well known (Chérif et al., 1994; Chérif et al., 1992). A few previous studies have reported Si to increase cucumber defences against herbivorous insects and mites such as *Helicoverpa punctigera* (Frew et al., 2019), banded cucumber beetles (*Diabrotica balteata*), whiteflies (*Bemisia tabaci*) (Callis-Duehl et al., 2017), and spider mites (*Tetranychus urticae*) (Harizanova et al., 2019).

1.3.1.2 French bean

French bean, *Phaseolus vulgaris* (Fabaceae), is an economically important herbaceous annual crop native to Central and South America and is cultivated worldwide for edible pods. French bean plants are susceptible to numerous pest herbivores across their life cycle, including seedling attackers (e.g. cutworms, crickets), leaf feeders (e.g. aphids, hoppers, spider mites), and pod feeders (e.g. pod borers, weevils) (Cardona, 1989). Among French bean pests, spider mites are considered highly damaging, and plants are reported to often exploit HIPVs to attract carnivorous predatory mites (Dicke et al., 1990). French bean, like

other legumes, is a low Si-accumulating plant (< 1% of dry biomass) (Hodson et al., 2005); however, Si supply can enhance its resistance to insect pests such as the fall armyworm, *Spodoptera frugiperda* (Rodrigues et al., 2018).

1.3.1.3 Purple false brome

Purple false brome, *Brachypodium distachyon* (Poaceae), is a monocot temperate grass (C_3 plant) native to the Mediterranean region and has a worldwide distribution (Scholthof et al., 2018). Although not directly important in agriculture, it is extensively used as a model for monocot plants in plant biology research because of its small and tractable genome (~ 272 Mb, five chromosomes), genetic transformability, short height, fast life cycle (seed-to-seed), and simple growth requirements (Girin et al., 2014; Scholthof et al., 2018). Furthermore, its close phylogenetic relationships with some economically important Triticeae crops such as wheat and barley have made it a suitable model for cereals in studying insect-plant interactions (Girin et al., 2014). Like other grasses, *B. distachyon* is a high Si accumulator, typically accumulating more than 2% Si of their shoot dry biomass (Hall et al., 2020b).

1.3.2 Pest herbivores

1.3.2.1 Cotton bollworm

The cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae), is one of the most devastating global pests with a diverse host range of hundreds of cultivated and wild plants, including important agricultural and horticultural crops such as cotton, tomato, potato, tobacco, sunflower, soybean, cucumber, and corn (Gomes et al., 2017; Jones et al., 2019). A female *H. armigera* moth can lay hundreds of eggs (up to 900) either singly or in clusters

on different parts of plants. At 25°C, egg hatching usually takes 3-4 days and the larval and pupal periods vary from 21-25 days and 14-18 days, respectively (Gomes et al., 2017). Larvae typically moult 6-7 times (5-6 larval instars); 80-90% of damage to plants is caused by later larval instars (3^{rd} to 6^{th}) (Gomes et al., 2017). This pest species is hard to control because of its wide host range, high reproduction rate, facultative diapause, long migration potential, and ability to develop resistance to conventional insecticides (e.g. carbamates, endosulfan, pyrethroids) and transgenic crops (e.g. Bt crops) (Ahmad et al., 1997; Ahmad et al., 2019; Jones et al., 2019).

1.3.2.2 Two-spotted spider mite

The two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae), is a cosmopolitan arthropod pest that feeds on more than a thousand host plants (Grbić et al., 2011). It is a major pest of many high-value crops, such as tomatoes, strawberries, grapes, peppers, beans, cucumbers, and citrus, in both fields and greenhouses (Grbić et al., 2011; Jeppson et al., 1975). *Tetranychus urticae* undergoes four life stages: egg, larva, nymph (protonymph and deutonymph), and adult (Shih et al., 1976). It shows haplodiploid sex determination, whereby males are haploids and females are diploids (Grbić et al., 2011; Shih et al., 1976). Larvae, nymphs, and adults of *T. urticae* suck out cell contents from leaf epidermal or mesophyll cells by inserting their sharp stylet-like mouthparts, preferably on the undersides of leaves (Bensoussan et al., 2016). Its feeding causes chlorotic spots on leaves and collapses the mesophyll layer. *Tetranychus urticae* is thought to be the most resistant pest species against pesticides, considering its resistance to the highest number of pesticide groups (Van Leeuwen et al., 2010).

1.3.2.3 Bird cherry-oat aphid

The bird cherry-oat aphid, *Rhopalosiphum padi* (Hemiptera: Aphididae), is considered a major pest of cereals worldwide (Finlay & Luck, 2011; Savaris et al., 2013). Nymphs and adults of *R. padi* feed on phloem sap using their piercing-sucking mouthparts, causing leaf senescence and stunting and reducing tillering and plant growth (Finlay & Luck, 2011; Guerrieri & Digilio, 2008). Aphids excrete sugar-rich liquid (honeydew) when feeding, which can promote secondary fungal growth and thus reduce plant photosynthesis by blocking sunlight (Guerrieri & Digilio, 2008). In addition to direct feeding damage, *R. padi* causes serious economic losses by transferring yellow dwarf viruses, the causal agents for detrimental cereal diseases (Finlay & Luck, 2011). Adult females of *R. padi* can reproduce both sexually and asexually, although in the absence of their primary tree hosts, parthenogenetic viviparous (i.e. live birth) reproduction occurs continuously on secondary cereal hosts (Finlay & Luck, 2011). Parthenogenesis enables *R. padi* to rapidly turn over its generations and thus produce large numbers of offspring.

1.3.3 Predator

1.3.3.1 Persimilis mite

The predatory mite, *Phytoseiulus persimilis* (Acarina: Phytoseiidae), is a specialist predator that voraciously feeds on all stages of spider mites (subfamily Tetranychinae), such as *T. urticae* (Barber et al., 2003; Opit et al., 2004). *Phytoseiulus persimilis* is widely used in *T. urticae* management in greenhouses. It has four life stages: egg, larva, nymph (protonymph and deutonymph), and adult (Laing, 1968). Predation begins from nymphal stages as larvae do not feed (Laing, 1968). Nymphs usually feed on the developmental stages of spider mites, while *P. persimilis* adults feed on all host stages, each destroying

5-20 prey per day (Barber et al., 2003; Laing, 1968). *Phytoseiulus persimilis* reproduces sexually and can complete generations faster than its hosts under favourable temperatures and humidity (Laing, 1968). This species is eyeless and uses olfactory sensilla located at the tip of its first pair of legs to exploit chemical cues (HIPVs) from plants infested with spider mites (van Wijk et al., 2006).

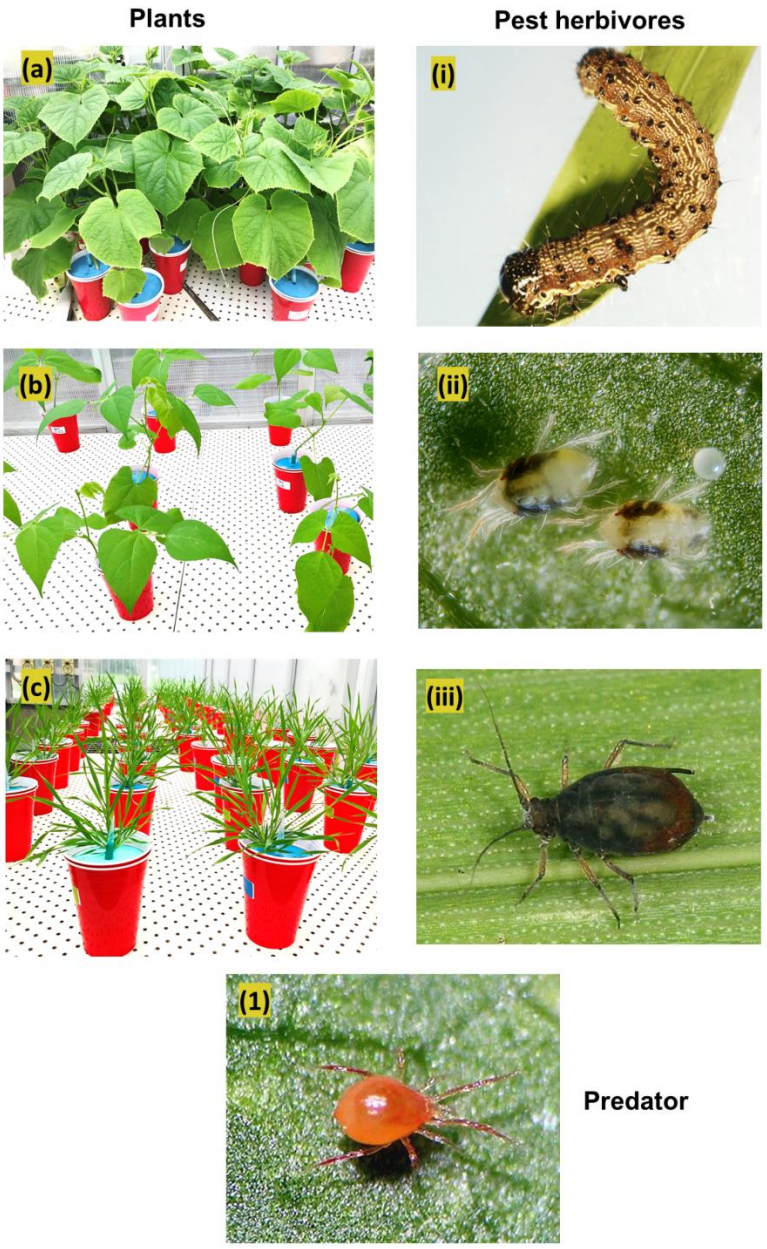

Figure 1-1 Study system used across different experimental chapters. Plant species: (a) cucumber, (b) French bean, and (c) purple false brome; pest herbivores: (i) *Helicoverpa armigera*, (ii) *Tetranychus urticae*, and (iii) *Rhopalosiphum padi*; predator: (1) *Phytoseiulus persimilis*. Experimental chapters investigate specific research questions utilising appropriate combinations of plants and animals as follows: chapter 2 (a-i), chapter 3 (b-ii-1), chapters 4 and 6 (c-i), chapter 5 (c-i-iii). Photos of all animals except *H. armigera* are taken from the internet.

1.4 Thesis overview

This thesis reports some novel aspects of plant Si defences against insect and mite herbivores. The overarching objective of this work was to explore how Si impacts the direct and indirect defences of plants against pest herbivores and to assess the plant-mediated effects of Si on the anti-predator defences of insects and interspecific interactions between insect herbivores. To accomplish this, the impacts of Si supplementation were assessed across different Si-accumulating plants (i.e. high, moderate, and low Si-accumulators) against herbivorous pests belonging to different feeding guilds (i.e. chewers, cell-content feeders, and sap-feeders) with particular emphasis on the global insect herbivore, *Helicoverpa armigera*.

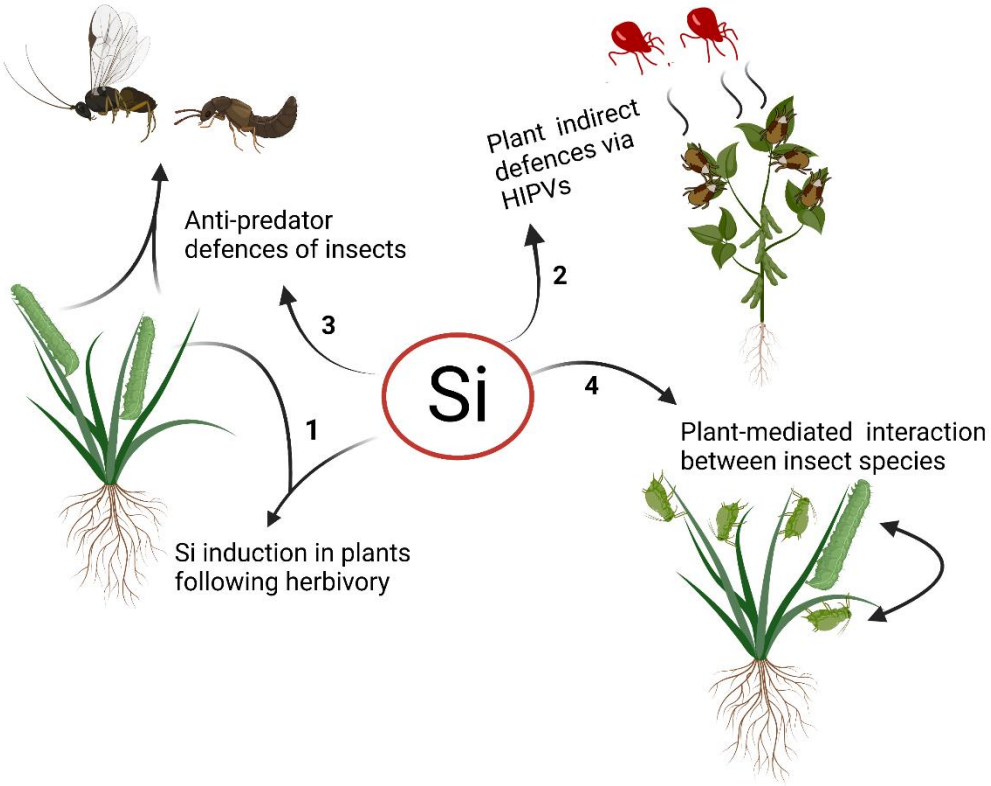

Figure 1-2 The central focus of this work was to assess some novel aspects of plant Si defences against insect and mite herbivores. Specifically, this work examined the impacts of Si on plant direct (1) and indirect (2) defences against herbivores, anti-predator defences of insect herbivores (3), and plant-mediated indirect interactions between insect species (4).

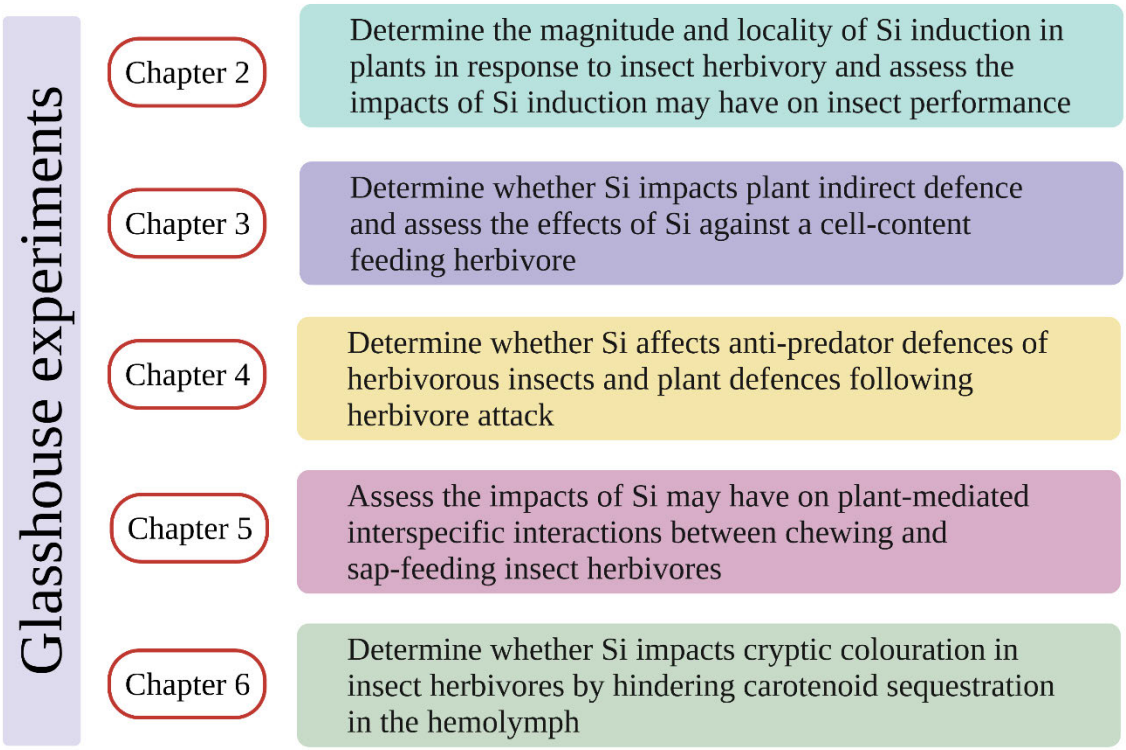

Figure 1-3 Main aims for each of the thesis chapters. All experiments were performed in a glasshouse under controlled temperature and relative humidity conditions.

1.5 Thesis outline

Chapter 1 introduces the general aspects of plant defence against pest herbivores, the mechanisms of Si uptake and accumulation in plants, and the roles of Si in plant physical and chemical defences against herbivorous insect pests. This chapter also introduces the study systems used across the experiments.

Chapter 2 investigates the magnitude and locality of Si induction in an intermediate Si-accumulating plant (*Cucumis sativus*) by a polyphagous chewing insect (*Helicoverpa armigera*) and assesses how Si supplementation impacts the relative growth and consumption rates of *H. armigera* when feeding on detached leaves (*ex situ*) in Petri dishes

and intact leaves (*in situ*) of plants. This chapter entitled 'Novel evidence for systemic induction of silicon defences in cucumber following attack by a global insect herbivore' (Tarikul Islam, Ben D. Moore, and Scott N. Johnson) was published in *Ecological Entomology*, vol. 45: 1373-1381, on 03 August 2020.

Chapter 3 investigates how Si supplementation of French bean, a low Si-accumulating plant, impacts egg-laying, population growth, and damage potential of a cell-content feeding mite herbivore (*Tetranychus urticae*). Furthermore, this chapter examines the effects of Si on plant indirect defence by testing the preference of the predatory mite, *Phytoseiulus persimilis*, for volatiles from Si-supplemented and Si-free plants either with or without *T. urticae* infestation. This chapter entitled 'Silicon suppresses a ubiquitous mite herbivore and promotes natural enemy attraction by altering plant volatile blends' (Tarikul Islam, Ben D. Moore, and Scott N. Johnson) was published in *Journal of Pest Science*, vol. 95: 423-434, on 03 May 2021.

Chapter 4 investigates the impacts of Si fertilisation of *Brachypodium distachyon*, a high Si-accumulator, on the anti-predator defences of the global insect pest, *Helicoverpa armigera*, integrating morphological, behavioural, and immune defence parameters. This chapter also examines how Si supply impacts plant constitutive and induced trichome production and plant compensatory growth following herbivory. This chapter entitled 'Silicon fertilisation affects morphological and immune defences of an insect pest and enhances plant compensatory growth' (Tarikul Islam, Ben D. Moore, and Scott N. Johnson) was published in *Journal of Pest Science* (https://doi.org/10.1007/s10340-022-01478-4), on 13 January 2022.

Chapter 5 examines the impacts of Si on plant-mediated interspecific interactions between a chewing (*Helicoverpa armigera*) and a sap-feeding (*Rhopalosiphum padi*) insect herbivore when contemporaneously sharing a host plant (*Brachypodium distachyon*). This chapter also assesses the performance of insects when feeding separately on plants and the preference of insects for Si-supplemented and Si-free plants using dual-choice tests. This chapter entitled 'Plant silicon defences reduce the performance of a chewing insect herbivore which benefits a contemporaneous sap-feeding insect' (Tarikul Islam, Ben D. Moore, and Scott N. Johnson) was published in *Ecological Entomology* (https://doi.org/10.1111/een.13183), on 22 July 2022.

Chapter 6 investigates the plant-mediated effects of Si on carotenoid sequestration in the haemolymph of *Helicoverpa armigera* larvae and their cryptic colouration. This chapter also assesses the effects of Si and insect attack, and their interactions, on carotenoid and chlorophyll levels in the model grass, *Brachypodium distachyon*. This chapter entitled 'Plant silicon disables cryptic colouration of an insect herbivore by reducing their ability to sequester carotenoids' (Tarikul Islam, Sidra Anwar, Chris Cazzonelli, Ben D. Moore, and Scott N. Johnson) is currently under review in a peer-reviewed journal.

Chapter 7 synthesises the key findings in chapters 2-6 and discusses the overall significance and broader implications of this research in the context of ecology and applied entomology. This chapter also presents the caveats on this work and the direction for further research.

Chapter 2

Novel evidence for systemic induction of silicon defences in cucumber following attack by a global insect herbivore

---

Published as Islam et al. (2020), *Ecological Entomology*, 45, 1373-1381

2.1 Abstract

Silicon (Si) is a beneficial plant nutrient that has been reported to ameliorate many abiotic and biotic stresses in plants, including insect herbivory. Insect herbivory has been shown to induce Si accumulation in plants, although the magnitude and nature of induction remain largely ambiguous. In particular, it is unclear whether herbivore induction of Si defences is confined to attacked tissues (local) or occurs elsewhere in the plant (systemic). We grew cucumber, *Cucumis sativus* L. plants (var. Burpless F1 and Beit Alpha), an intermediate Si accumulator, hydroponically under Si-supplemented or Si-free conditions and measured the level of Si induction caused by a polyphagous chewing insect, the cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae). We also examined the impacts of Si on insect performance by conducting *in vitro* feeding assays on excised leaves (*ex situ*) and intact leaves of plants (*in situ*). Herbivory significantly increased Si accumulation both locally in attacked leaves (21% increase in Beit Alpha and 17% in Burpless F1) and systemically in non-attacked leaves (19% increase in Beit Alpha and 10% in Burpless F1). Si supplementation significantly increased % foliar Si and C:N ratio, while significantly decreasing larval relative consumption (RC) and relative growth rate (RGR) in the *in situ* assays. In *ex situ* assays, however, Si only reduced larval RGR when fed on Beit Alpha plants. Our results confirm that Si-based defences can operate in moderate Si-accumulating

plants and provide evidence for the first time that insect herbivory induces systemic Si accumulation equivalently between plant varieties.

2.2 Introduction

Plants have developed a repertoire of physical and chemical defences to combat insect herbivores (Carmona et al., 2011; Gatehouse, 2002; Mithöfer & Boland, 2012; Schuman & Baldwin, 2016). Broadly, plant defences are classified as constitutive (i.e. defences that are continuously expressed) and induced (i.e. defences that are activated when challenged by herbivores) defences. Induction of plant defences is often more efficient than constitutive defence (Cipollini et al., 2003; Karban, 2020; Preisser et al., 2007), particularly when defences are metabolically costly and herbivore attack is sporadic and can be anticipated due to previous herbivory (Aljbory & Chen, 2018; Arimura et al., 2005; Karban, 2011; Karban et al., 1999; Karban & Myers, 1989; Kessler, 2015). Herbivores can induce defences locally in attacked tissues only or systemically in un-attacked tissues beyond local responses (Wu & Baldwin, 2010). Both types of induction include triggering of plant signalling molecules by herbivores, including peptides, reactive oxygen species (ROS) and jasmonic acid (JA) (Kant et al., 2015; Karban & Myers, 1989). Particularly, JA and its derivatives (e.g. methyl jasmonate) have been linked to the elicitation of systemic plant defences under insect attack (Kant et al., 2015). Physical defences such as silicon (Si) deposition (silicification) in plant tissues are mostly regarded as constitutive defences. However, Si accumulation in tissues can be induced quantitatively (Hartley et al., 2016; Massey et al., 2007; McNaughton & Tarrants, 1983). Specifically, herbivore feeding and artificial damage have been shown to induce Si uptake and accumulation in plants (Hartley et al., 2015; Massey et al., 2007; McLarnon et al., 2017). However, the magnitude and

locality (whether local or systemic) of Si induction after insect attack remain elusive and understudied (Hartley et al., 2016).

Plants take up Si as orthosilicic acid, $Si(OH)_4$, using their roots, sometimes actively by specific influx and efflux transporters as, for example, in some monocots (e.g. rice, sorghum, wheat) and dicots (e.g. cucumber) species (Liang et al., 2005; Ma & Yamaji, 2008, 2015) and sometimes passively *via* the transpiration stream, such as in oats (Jones & Handreck, 1965). Subsequently, Si is transported through the xylem to the shoot and deposited in and around plant cells or as cell wall components, sometimes as discrete opaline phytoliths (SiO_2) (Ma & Yamaji, 2006, 2008). Plants show inter- and intra-specific variations in Si uptake and deposition, accumulating 0.1-10% Si of their dry weight (Hodson et al., 2005; Ma et al., 2003). Silicification of plant tissues alleviates a range of abiotic and biotic stresses, including insect herbivory, through diverse mechanisms (Frew et al., 2018; Reynolds et al., 2016). For example, phytoliths in the leaf epidermis, spines and trichomes make plant tissues tougher and more abrasive, reducing their palatability and digestibility to herbivores (Massey et al., 2006; Reynolds et al., 2009). Silicified tissues can also wear down insect mouthparts marginally (Kvedaras et al., 2009) or substantially (Massey & Hartley, 2009) and preclude nutrient absorption in their guts, which in turn can retard their growth (Frew et al., 2018; Massey & Hartley, 2009). Previous studies have reported the impacts of Si on insect growth and consumption rates based on both *in situ* feeding assays on plants (Massey et al., 2006; Moise et al., 2019a; Ryalls et al., 2017) and *ex situ* assays with excised leaves (Callis-Duehl et al., 2017; Hall et al., 2020b; Moraes et al., 2004). Given the inducibility of Si under herbivory, *ex situ* assays might undermine the actual effects of Si-based defences.

Recent evidence suggests that JA signalling induces Si uptake when plants experience herbivore attack (Hall et al., 2020b; Hall et al., 2019). While a few studies have shown Si induction in plants under insect herbivory, all of them have focused on hyper-accumulating grasses (Hall et al., 2019; Johnson et al., 2019a; Massey et al., 2007), except one study on soybean (Johnson et al., 2020b). As such, we have little data on the extent of Si induction by insect herbivores across different Si accumulating species and no data across plant genotypes within a species (Hartley et al., 2016). Given that plant genotypes differ in Si accumulation rates (Ma et al., 2003; Ma et al., 2007b), insect feeding could affect the magnitude of Si induction differently across genotypes.

We used cucumber, *Cucumis sativus* L., an intermediate accumulator of Si (0.5-1.5% of dry weight) (Hodson et al., 2005), and the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), a global pest of many economically important crops including cucumber (Kulkarni et al., 2009), to determine whether herbivory induces Si uptake. If so, does it induce Si accumulation locally in the attacked leaves only or systemically in all leaves? Using two cucumber varieties, Burpless F1 and Beit Alpha, we also examined whether varietal differences (genotypes) within the species affect Si induction and subsequent Si defences against herbivores. Specifically, we aimed to measure the effects of insect herbivory on the level of Si induction in cucumber varieties and to assess the impacts of Si supplementation on insect growth and leaf consumption. We hypothesised that herbivory would induce Si accumulation systemically in cucumber leaves and that the magnitude of induction would be different between plant varieties. We also hypothesised that larval leaf consumption and growth rate would be negatively

affected by Si supplementation and that insect feeding assay methods (*ex situ* vs *in situ*) would influence the efficacy of Si defences.

2.3 Materials and methods

2.3.1 Plant material and growth conditions

Cucumbers of two varieties, Burpless F1 and Beit Alpha (Mr. Fothergill's Seeds, NSW, Australia), were grown hydroponically in a naturally lit glasshouse at 25/18°C day/night temperatures with 60 ± 5% humidity. These parthenocarpic varieties were selected because they are popularly cultivated hydroponically under protected conditions (Burt, 2007; Shaw et al., 2004) and have been reported to exploit Si defences to withstand biotic stresses, including insect herbivory (Callis-Duehl et al., 2017; Nuñez-Palenius et al., 2006). Seeds were sterilised in a solution of 1% bleach and 0.1% Triton X-100 for an hour and subsequently washed in water before being sown into wet perlite medium in germination trays (38 cm × 24.5 cm × 11.8 cm). After two weeks, uniform seedlings with two embryonic leaves were transferred from perlite to non-aerated hydroponic vessels, each consisting of two nested disposable plastic cups (480 ml) with a foam disc tailored to fit at the top as per Hall et al. (2020b). The discs were incised with a cork borer to accommodate a cucumber seedling in each. In total, 200 plants (100 of each variety) were grown hydroponically for six weeks under the same glasshouse conditions. The hydroponic nutrient solution for cucumber was prepared as per Hochmuth (2001), which was devoid of any Si. To prepare Si-supplemented (+Si) solution, liquid potassium silicate (K_2SiO_3) (21% K_2O and 32% SiO_2, Agsil32, PQ Australia, SA, Australia) was added at a concentration of 2 mM (SiO_2 equivalent) as polymerisation starts to occur above this level (Bakhat et al., 2018). Furthermore, HCl was added to maintain pH 6.0 for optimal plant

growth (Hochmuth, 2015). In non-Si solutions (– Si), KCl was added to match the extra K^+ provided in the +Si solution, and the same pH was maintained using HCl. Each plant received approximately 330 ml fresh nutrient solution (either +Si or –Si), and the solution was changed twice a week for the first four weeks and thrice a week afterwards.

2.3.2 Experimental design

The experiment comprised a full 2 × 2 factorial design with 50 replicates per treatment, incorporating plant variety (Burpless F1 and Beit Alpha) and Si supplementation (+Si and –Si) as factors. Insect herbivory was added as a third factor (presence and absence) only for the *in situ* feeding assays (see the feeding assay section) to evaluate the effects of herbivory on plant Si accumulation. Fifty plants of each variety were randomly assigned to the +Si treatment, and the other 50 plants were assigned to –Si. Plants were randomly distributed within the glasshouse and rotated weekly to minimise any chamber or edge effects.

2.3.3 Insect feeding assays and plant harvesting

Third instar *H. armigera* larvae supplied by CSIRO Agriculture & Food, Narrabri, NSW, Australia, were reared on an artificial diet (modified from Teakle and Jensen (1985)) before being used for feeding assays conducted in Petri dishes (*ex situ* assays) and on plants (*in situ* assays). Larvae were synchronised based on their weight. Larvae weigh from18 to 22 mg and 10 to 15 mg were used randomly for *ex situ* and *in situ* feeding assays, respectively. Furthermore, the relative consumption (RC) and relative growth rate (RGR) of larvae were calculated following Massey and Hartley (2009). Relative growth rate estimates the change in larval fresh mass relative to initial mass and was calculated as mass gained (mg)/initial

mass (mg)/time (days). Relative consumption estimates the mass of ingested leaf material relative to larval mean body mass over the experimental period and was calculated as ingested food (mg dry mass)/mean larval body mass (mg fresh mass).

For the *ex situ* feeding assays, late third instar larvae (N = 20 larvae and plants) were starved for 24 hrs, weighed and each provided with a cucumber leaf (third fully expanded leaf) of known weight in a Petri dish. Leaves were detached from plants just before harvesting. Larvae were allowed to feed for 24 hrs and then starved for another day to allow all frass to be expelled before being re-weighed. The remaining leaf material was oven-dried at 60°C for one week and weighed. The initial leaf fresh mass was converted to dry mass from the leaf water content of the same plant. The leaf water content was calculated by weighing three fresh leaves from the harvested plants and re-weighing them after oven-drying for one week at 60°C. Harvested plants (20 +Si and 20 –Si plants of each variety) were similarly oven-dried, and the shoot and root biomass was recorded. Dried insect-fed leaves were ball-milled to a fine powder for Si, carbon (C) and nitrogen (N) analysis.

For the *in situ* feeding assays, early third instar larvae (N = 15 larvae and plants) were starved for 24 hrs, weighed and placed singly on the third fully expanded leaves of five-week-old plants, and the leaves were caged with fine nylon netting bags (16.5 cm × 12.5 cm). Equivalent leaves of 15 +Si and 15 –Si plants of each variety were bagged without insects as a control. Larvae were allowed to feed for seven days and then starved for a day before being re-weighed. Insect-fed leaves were detached from plants, weighed and scanned on a flatbed scanner (CanoScan LiDE210). The scanned leaves were analysed using ImageJ (National Institutes of Health, Maryland, USA; Version 1.52) to estimate the

area of consumed portions and the whole leaves (Johnson et al., 2016; Rasband, 1997). To measure whether herbivory induced Si accumulation, bagged leaves with or without insects (corresponding to local accumulation) were sampled separately from the rest of the unbagged leaves (corresponding to systemic accumulation), and all were oven-dried at 60°C for one week. Insect-fed leaves were weighed, and subsequently, all sampled leaves were ball-milled to a fine powder for Si analysis. The dry mass of the consumed leaf area was calculated from the dry mass and area of the whole leaf (Fig. S2-1).

2.3.4 Plant elemental analyses

Si concentrations in leaves were determined by analysing ca. 80 mg finely ground materials per sample in an X-ray fluorescence spectrometer (Epsilon $3^{\times}$; PANalytical, EA Almelo, The Netherlands) following the methods described in Reidinger et al. (2012). Measurements were calibrated against a standard of known Si concentration (Citrus leaves, 0.5% Si). Leaf C and N concentrations were measured by analysing ca. 6 mg ground materials per sample (N = 10 plants) using an elemental analyser (Carlo Erba CE 1110) that detects N_2 and CO_2 by thermal conductivity and mass spectrometry.

2.3.5 Statistical analyses

All analyses were performed in R, version 3.6. Data were tested for confirmation of normality of residuals and homogeneity of variances by using QQ-plot and residual plot functions from the 'car' package (Crawley, 2012). Plant biomass, foliar C, foliar N, C:N and insect RGR and RC (for both *in situ* and *ex situ* feeding assays) were analysed using two-way analysis of variance (ANOVA) tests, comparing the individual effects of Si-supplementation and variety as factors as well as their interaction. Leaf Si concentrations

in case of *in situ* feeding assays (both local and systemic accumulation) were analysed using two-way ANOVA tests, incorporating the individual and interactive effects of variety and insect as factors. Si supplementation was excluded from factors, as –Si plants had Si concentrations below detection limits (<0.3%). Linear regressions were used to explore relationships between foliar Si and foliar C concentrations.

2.4 Results

2.4.1 Feeding assays

Si supplementation in interaction with plant variety significantly reduced the RGR of *H. armigera* larvae (Fig. 2-1a; Table 2-1) in the *ex situ* feeding assays. However, RC was not affected by either Si supplementation or plant variety (Fig. 2-1b; Table 2-1). In the *in situ* feeding assays, Si supplementation significantly reduced larval RGR and RC (Fig. 2-2a and 2-2b; Table 2-1). Neither variety nor the interaction between Si and variety had a significant effect, indicating that Si uniformly affected RGR and RC in this assay (Table 2-1).

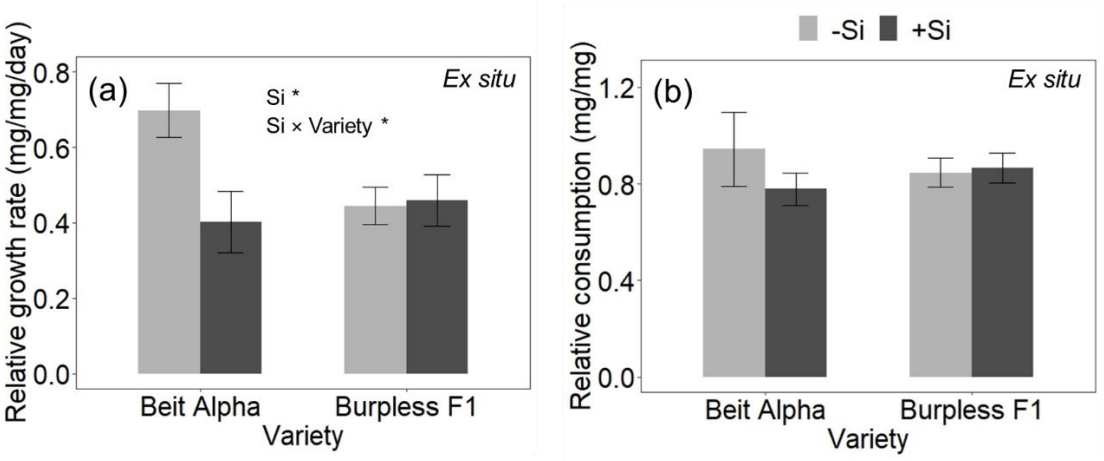

Figure 2-1 Effects of Si and plant variety on (a) relative growth rate (mg/mg/day) of *H. armigera* larvae and (b) relative consumption (mg/mg) in the *ex situ* feeding assays (*N* = 20 larvae and plants). Mean ± SE shown. Asterisks indicate the level of significance (*$p < 0.05$).

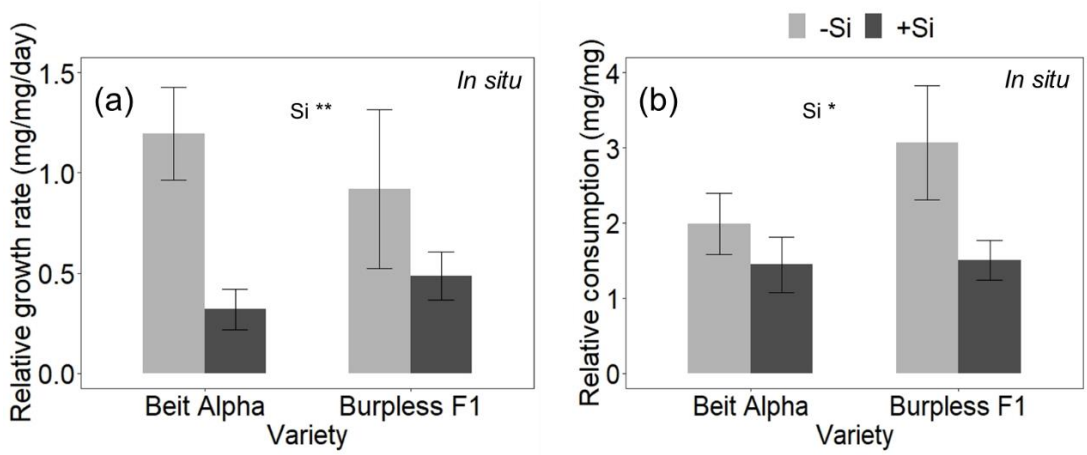

Figure 2-2 Effects of Si and plant variety on (a) relative growth rate (mg/mg/day) of *H. armigera* larvae and (b) relative consumption (mg/mg) in the *in situ* feeding assays (*N* = 15 larvae and plants). Mean ± SE shown. Asterisks indicate the level of significance (*$p < 0.05$, **$p < 0.01$).

2.4.2 Plant Si accumulation

Insect feeding significantly increased local Si accumulation by 21% in Beit Alpha and 17% in Burpless F1 (Fig. 2-3a; Table 2-1). Likewise, systemic Si accumulation in cucumber leaves (i.e. leaves of insect-attacked plants that were not fed upon by larvae) was increased by 19% in Beit Alpha and 10% in Burpless F1 (Fig. 2-3b; Table 2-1). Si accumulation was significantly affected by variety, whereby Burpless F1 accumulated higher Si than Beit Alpha both locally and systemically. (Fig. 2-3; Table 2-1).

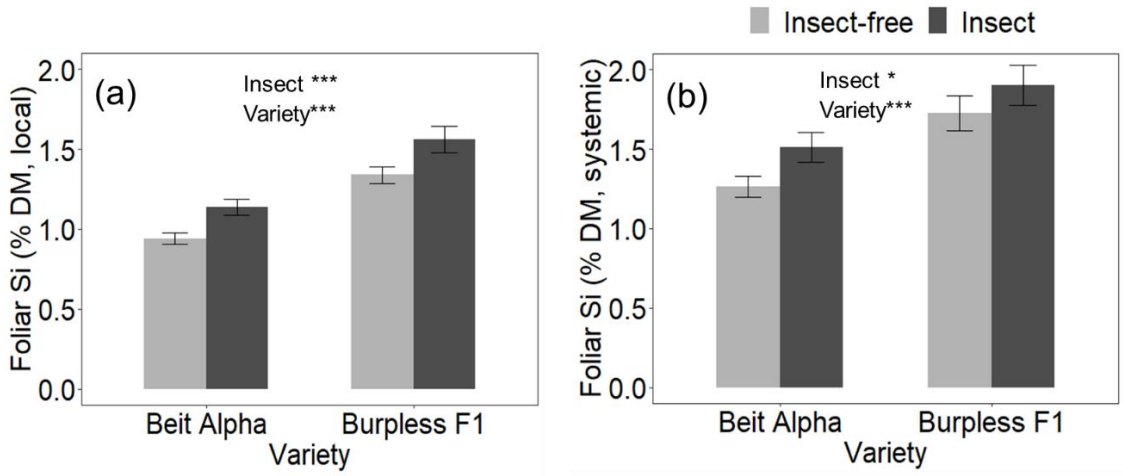

Figure 2-3 Effects of plant variety and insect herbivory on foliar Si accumulation (% dry matter) in Si-supplemented plants (a) locally in attacked leaves and (b) systemically in non-attacked leaves (N = 15 larvae and plants). Mean ± SE shown. Asterisks indicate the level of significance (*P < 0.05, ***P < 0.001).

2.4.3 Plant biomass and foliar elements

Si supplementation in interaction with plant variety significantly increased shoot (+30%) and root biomass (+32%) in Burpless F1 but had no significant effects on Beit Alpha (Table 2-2). In terms of leaf elements, Si supplementation significantly decreased % foliar C and % foliar N while significantly increasing the C:N ratio (Table 2-2). In both varieties, % foliar C was strongly negatively correlated with % foliar Si (R^2 = 0.70, p = 0.002 for Burpless F1 and R^2 = 0.67, p = 0.003 for Beit Alpha) (Fig. 2-4).

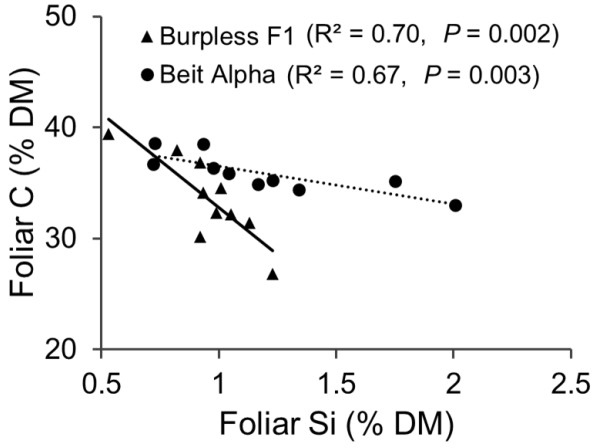

Figure 2-4 Linear regression models showing the correlation between foliar Si and foliar C concentrations in Si-supplemented plants. The solid line with triangular data points represents the independent regression model for Burpless F1, and the dotted line with circular data points represents Beit Alpha plants (N = 10).

Table 2-1 Summary of ANOVA results: a-b) impacts of variety and Si on insect RGR and RC in the *ex situ* and *in situ* feeding assays and c) impacts of variety and insect herbivory on local and systemic Si accumulation in plants. Statistically significant effects are highlighted in bold ($p < 0.05$).

	Fig.	*df*	Variety		Si		Variety × Si	
			F	*p*	*F*	*p*	*F*	*p*
a) *Ex situ* feeding assays								
RGR	1a	1,76	1.97	0.164	4.13	**0.046**	5.09	**0.027**
RC	1b	1,76	0.003	0.957	0.61	0.436	0.95	0.332
b) *In situ* feeding assays								
RGR	2a	1,56	0.05	0.825	7.25	**0.009**	0.84	0.364
RC	2b	1,56	1.36	0.248	4.71	**0.034**	1.10	0.298
	Fig.	*df*	Variety		Insect		Variety × Insect	
			F	*p*	*F*	*p*	*F*	*p*
c) Si accumulation in plants								
Foliar Si (local)	3a	1,56	49.72	**<0.001**	12.98	**<0.001**	0.05	0.831
Foliar Si (systemic)	3b	1,56	17.86	**<0.001**	4.34	**0.042**	0.13	0.722

Table 2-2 Effects of Si and plant variety on plant biomass, % foliar C, % foliar N, and C:N ratio. Mean ± SE shown for plant responses. Statistically significant effects are highlighted in bold ($p < 0.05$).

	Beit Alpha		Burpless F1	
Plant response	–Si	+Si	–Si	+Si
Shoot biomass (g)	6.09 ± 0.36	6.00 ± 0.28	4.14 ± 0.10	5.38 ± 0.26
Root biomass (g)	1.08 ± 0.09	1.08 ± 0.07	1.04 ± 0.05	1.37 ± 0.08
% Foliar C	38.58 ± 0.83	35.87 ± 0.55	37.67 ± 0.84	33.57 ± 1.21
% Foliar N	5.31 ± 0.12	4.84 ± 0.19	5.53 ± 0.19	4.43 ± 0.29
C:N ratio	7.27 ± 0.11	7.50 ± 0.30	6.86 ± 0.24	7.76 ± 0.37

ANOVA outcomes

	df	Variety		Si		Variety × Si	
Variable		*F*	*p*	*F*	*p*	*F*	*p*
Shoot biomass	1,76	22.97	**<0.001**	4.70	**0.033**	6.19	**0.015**
Root biomass	1,76	3.01	0.087	5.35	**0.023**	5.03	**0.028**
% Foliar C	1,36	3.26	0.079	14.61	**<0.001**	0.61	0.439
% Foliar N	1,36	0.22	0.645	14.52	**<0.001**	2.36	0.133
C:N ratio	1,36	0.07	0.787	4.41	**0.043**	1.54	0.222

2.5 Discussion

For the first time, we demonstrated systemic Si induction in cucumber leaves following insect attack. We found that *H. armigera* induced local and systemic Si accumulation consistently in both plant varieties. Si induction further led to better herbivore defences in plants, negatively affecting insect growth and consumption rates.

We found that feeding by a larva on a single leaf for seven days induced both local and systemic Si accumulation in both varieties. Cucumber plants can take up Si both actively and passively (Liang et al., 2005), which is unlikely to be true for many other dicots that depend mostly on passive mechanisms (Hodson et al., 2005). Considering this, Si induction in response to insect herbivory might be driven by two mechanisms in cucumber. First, insect feeding removed photosynthetic leaf tissues and disrupted leaf integrity. This could cause uncontrolled evaporative loss of water through the cut edges and abraded cuticle of the damaged leaves (Ostlie & Pedigo, 1984; Schoonhoven, 1990). For instance, feeding by *Helicoverpa zea* was found to increase transpiration by 20-90% from soybean leaflets (Aldea et al., 2005). Such enhanced transpiration rates might contribute to passive Si uptake through the transpiration stream (Faisal et al., 2012). Second, cucumber plants might actively take up more Si to resist subsequent damage. Two putative transporter proteins, *CsLsi1* and *CsLsi2*, have recently been linked to active Si uptake in cucumber (Wang et al., 2015). *CsLsi1* is an influx transporter that facilitates Si movement across the plasma membrane, translocating Si from the external solution to the plant cells (Sun et al., 2017). *CsLsi2* is an efflux transporter that actively transports Si out of plant cells (Sun et al., 2018). Insect attack has been shown to alter the expression of both transporters in rice, causing active Si uptake in plants (Ye et al., 2013). We suggest that systemic Si

accumulation in cucumber leaves was controlled actively, whereas local accumulation corresponded to either passive or both mechanisms.

We found that plant variety significantly affected Si accumulation; Burpless F1 accumulated higher Si than Beit Alpha. Previous studies have reported that plant varieties can vary substantially in Si accumulation under both stressed and unstressed conditions (Deren, 2001; Deren et al., 1994; Ma et al., 2007b). These variations could be caused by anatomical differences among varieties, for example, differences in stomatal density, size and conductance, or by genetically determined physiological differences, the latter being the most influential (Ma et al., 2003; Ma et al., 2007b; McLarnon et al., 2017). Even so, we found increases in Si induction between cucumber varieties to be proportionately similar following insect attack.

Our results show that Si supplementation negatively affected larval RGR and RC in the *in situ* assays. These results support an increasing body of literature suggesting that Si is an effective defence against insect herbivores (Alhousari & Greger, 2018; Liang et al., 2015; Massey & Hartley, 2009; Reynolds et al., 2009). However, most studies on anti-herbivore Si defences have focused on grasses (Hartley et al., 2016), as they accumulate high concentrations of Si, sometimes over 10% of their dry biomass (hyper-accumulation) (Hodson et al., 2005). Given that Si is not only a physical defence against herbivores but can also influence chemical defences in plants and enhance plant tolerance to herbivory (Johnson et al., 2019a), Si supplementation is receiving growing recognition in moderate- and non-accumulating plant species (Cooke & Leishman, 2011; Frew et al., 2019; Johnson et al., 2020b). Only two studies, so far, have investigated the impacts of Si against cucumber insect pests. Frew et al. (2019) found reduced growth rates of *Helicoverpa*

punctigera when fed Si-treated cucumber plants. Likewise, Callis-Duehl et al. (2017) reported Si-fertilised cucumber plants to be less visited and less eaten by banded cucurbit beetles (*Diabrotica balteata*) and whiteflies (*Bemisia tabaci*).

Interestingly, we found that Si significantly reduced RC by *H. armigera* larvae in the *in situ* feeding assays but not in the *ex situ* assays. This underpins that induction of Si defences under insect attack leads to better herbivore defences in plants. Such reduced consumption of silicified leaves could be because Si deposition under the leaf epidermis forms Si-cuticle double layers, which are thicker and therefore harder to shear for larvae (Yoshida et al., 1962). This mechanism has been well documented to stop fungal penetration, for example, by *Pythium ultimum,* in cucumber (Chérif et al., 1992). Besides, Si might enhance antioxidant defence enzyme activity including polyphenol oxidases, peroxidases and catalases under *in situ* conditions, which could prevent insect feeding (Chérif et al., 1994; Ye et al., 2013). The differences in feeding duration between the *ex situ* (24 hrs) and *in situ* (7 days) assays might also influence insect performance. Although RGR and RC are relative estimates per unit of time (days), it is plausible that starved caterpillars might have fed indiscriminately on both +Si and −Si plants in the initial hours. Consequently, the effects of Si might be more evident on larvae after continuous feeding for a few days.

Although it is unclear why Si increased plant biomass in the variety Burpless F1 but not in Beit Alpha, such differences in varietal performance have been previously reported (Deren, 2001; Mohaghegh et al., 2010). One plausible reason behind this could be that Si can differentially affect the nutrition dynamics and photosynthetic rates of plant varieties (Deren, 2001; Detmann et al., 2012). We found that foliar C and N concentrations were decreased by Si, resulting in an increase in the C:N ratio. These changes in foliar nutrient

concentrations could be the result of stoichiometric dilution caused by Si supplementation (Neu et al., 2017). This might influence plant biomass, as Si has been suggested to act as a metabolically cheap, structural substitute for carbon, liberating more carbon for plant growth (Cooke & Leishman, 2011). We found a strong negative correlation between foliar C and Si concentrations, which is at least compatible with this (Neu et al., 2017; Quigley et al., 2020). However, as we could not detect Si concentration in –Si plants, it is difficult to conclude how changes in nutritional physiology contributed to altered growth of +Si and −Si plants between cucumber varieties.

In conclusion, our results demonstrate that Si is an inducible defence in cucumber and that induction is both local and systemic. Silicification of plant tissues provided defence against herbivores in both cucumber varieties. The fitness benefits (reproductive success) for plants coming from such Si-based defences still need to be investigated, as defences usually come with a fitness cost (Cipollini et al., 2018). Johnson and Hartley (2018), however, speculated that Si defences could be acquired more economically than carbon (phenolic) defences. Recently, Si fertilisation has also been reported to enhance the marketable fruit yield and fruit quality (firmness) of cucumber (Abd-Alkarim et al., 2017). Our results suggest that Si defences are beneficial to moderate Si-accumulating plant species and that systemic induction may be achieved efficiently.

Chapter 3

Silicon suppresses a ubiquitous mite herbivore and promotes natural enemy attraction by altering plant volatile blends

Published as Islam et al. (2022c), *Journal of Pest Science*, 95, 423-434

3.1 Abstract

Silicon (Si) accumulation in plants is widely recognised as an effective physical defence against chewing herbivores. However, its effects on some feeding guilds such as cell-content feeders are understudied despite being severe economic pests (e.g. *Tetranychus urticae*). Moreover, most studies focus on the direct impacts of Si, but there is growing evidence that Si also impacts indirect defence. We examined the effects of Si on French bean, *Phaseolus vulgaris*, defences against a ubiquitous mite herbivore, the two-spotted spider mite, *T. urticae*. We grew plants hydroponically with (+Si) or without (–Si) silicon, assessed *T. urticae* performance and tested the preference of the predatory mite, *Phytoseiulus persimilis*, for volatiles from *T. urticae*-infested (+M) or uninfested (–M) plants. The provision of Si to plants suppressed *T. urticae* egg-laying, population growth and leaflet damage, and partially ameliorated *T. urticae*-induced reductions in stomatal conductance and net photosynthesis. Furthermore, *T. urticae* infestation increased foliar Si accumulation. Predatory mites were more attracted (64%) to volatiles from +Si plants experiencing herbivory than to –Si plants. The relative emissions (%) of volatile compounds viz. *E*-2-hexenyl benzoate, hexanal, *E*-*trans*-β-ocimene, D-limonene, β-caryophyllene and methyl salicylate were elevated from +Si +M plants, while the relative emissions of 3-hexanol, *trans*-calamenene, *o*-xylene and *o*-cymene were lowered

compared to –Si +M plants. Our results show, for the first time, that Si defences are inducible and effective even in low Si-accumulating plants against *T. urticae* and suggest that Si could play a role in pest biocontrol.

3.2 Introduction

Silicon (Si) is increasingly recognised as a highly effective plant physical defence against arthropod herbivores (Reynolds et al., 2009), particularly those that chew plant tissue, including borers (Hou & Han, 2010), folivores (Massey et al., 2006) and root herbivores (Frew et al., 2017b). Plants take up Si via their roots as aqueous orthosilicic acid, $Si(OH)_4$, and deposit it in and around cells, often as discrete phytoliths of amorphous silica (SiO_2) (Ma & Yamaji, 2006). Silicification makes plant tissues abrasive and tougher, reducing their masticability and digestibility to herbivores and causing mouthparts to wear while feeding (Massey & Hartley, 2009). Besides this, Si can influence inducible direct chemical defences in plants via the jasmonic acid (JA) signalling pathway (Hall et al., 2019; Kim et al., 2014). For example, Si can increase the induced activity of defence enzymes, including peroxidase and polyphenol oxidase, in rice under wounding stress (Kim et al., 2014) and herbivore attack (Ye et al., 2013). Reciprocally, herbivory can induce Si accumulation locally in attacked tissues and systemically (Islam et al., 2020). However, research on Si effects on plant defence has largely focused on herbivores of two feeding guilds, chewing and piercing-sucking, and has primarily measured direct defence responses (Leroy et al., 2019; Reynolds et al., 2016). In particular, there is a dearth of literature on the effects of Si on cell content-feeding herbivores such as the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae), a ubiquitous chelicerate pest of both greenhouse and field crops (Grbić et al., 2011). Moreover, Si has been shown to impact indirect defence (i.e. those

involving natural enemies of herbivores) but the mechanisms are poorly understood (Leroy et al., 2019; Reynolds et al., 2016).

Plants release distinct volatile blends upon herbivore attack, known as herbivore-induced plant volatiles (HIPVs) (Dicke, 2009). This phenomenon is referred to as indirect plant defence since HIPVs can attract natural enemies, for instance, predators and parasitoids of the herbivore (Colazza et al., 2004; Dicke et al., 1990). Si-augmented indirect plant defence was first reported by Kvedaras et al. (2010). They observed a higher attraction rate of the predatory beetle, *Dicranolaius bellulus*, to Si-supplemented cucumber plants under *Helicoverpa armigera* attack and found high predation rates of *H. armigera* eggs from Si-supplemented plants when placed into a lucerne stand. The authors hypothesised that Si might alter HIPV emission to recruit more natural enemies but did not characterise or quantify those volatile compounds. Subsequently, Si has been shown to promote the attraction of two parasitic wasps, *Trathala flavo-orbitalis* and *Microplitis mediator*, to leaffolder-infested rice plants (Liu et al., 2017). More recently, the aphid parasitoid, *Lysiphlebus testaceipes*, was reported to be more attracted to volatiles from aphid-infested, +Si wheat plants compared to aphid-infested, −Si plants (de Oliveira et al., 2020). Both studies have described quantitative changes in HIPV emission under Si supplementation as a mechanism for enhanced natural enemy attraction. Conversely, Connick (2011) reported that despite qualitative and quantitative changes in HIPVs following Si supplementation, the green lacewing, *Mallada signata*, was attracted equally to both +Si and −Si grapevine plants infested with grapevine moth larvae. Given this paucity of literature and divergent findings, the impacts of Si on plant indirect defence need to be

investigated further if this promising pest management approach is to be exploited for different feeding guilds of herbivores (Leroy et al., 2019; Reynolds et al., 2016).

Tetranychus urticae is a global pest of more than 1400 plant species and 140 different plant families, causing enormous losses to many economically important crops, including tomatoes, strawberries, grapes, citrus, peppers, cucumbers and beans (Grbić et al., 2011; Jeppson et al., 1975). Both adults and immature stages (larvae and nymphs) of *T. urticae* feed by inserting their stylet-like mouthparts, preferably into the lower (abaxial) leaf surface (Bensoussan et al., 2016). They suck out cell contents from mesophyll cells, causing chlorotic spots on the upper (adaxial) leaf surface (Park & Lee, 2002). Their feeding also reduces stomatal conductance and net photosynthesis in damaged and adjacent undamaged cells (i.e. physiological injury to plants) (de Freitas Bueno et al., 2009; Park & Lee, 2002). *Tetranychus urticae* biological features, including high fecundity, short generation time, and haplodiploid sex-determination, cause fast population growth (Grbić et al., 2011). Moreover, *T. urticae* is infamous for being resistant to acaricides (Van Leeuwen et al., 2010) and being able to suppress plant defences (Alba et al., 2015). In light of this, sustainable management of *T. urticae* requires maximising host plant defence (Agut et al., 2018) and efficient use of biocontrol agents, particularly the specialised predatory mite, *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae), which feeds on all *T. urticae* stages (Barber et al., 2003; Opit et al., 2004).

We examined if, and how, Si impacts direct and indirect defences in French bean, *Phaseolus vulgaris*, an important legume crop (Fabaceae), against *T. urticae*. We grew plants hydroponically to create Si-supplemented (+Si) and Si-free (−Si) conditions. Our

objectives were to investigate the impacts of Si on (i) the population growth and damage potential (physical and physiological) of *T. urticae* and thereby plant direct defence; and (ii) the behavioural response of *P. persimilis* via any potential changes in plant volatile emissions (indirect defence). We opted for *P. persimilis* because this predator is blind and exclusively relies on plant chemical cues (HIPVs) for foraging (Dicke et al., 1990). We hypothesised that Si would reduce the performance of, and damage by, *T. urticae* and, based upon previous observations in other systems, promote the attraction of predatory mites by altering the composition of HIPVs.

3.3 Materials and methods

3.3.1 Plant growth conditions

Plants were grown in two batches in a naturally lit glasshouse at 22/18°C day/night temperatures and 60% (± 5%) relative humidity. Plants grown in the first batch (N = 128) were used for assessing *T. urticae* performance (N = 40) and for measuring plant gas exchange rates (N = 48) and biomass (N = 40). The second batch of plants (N = 80) was used to examine indirect defence (i.e. olfactometer bioassays and volatile collection) and leaf elemental analyses. Dwarf French bean, *Phaseolus vulgaris* L., seeds (Mr. Fothergill's Seeds, NSW, Australia) were surface sterilised in 1% sodium hypochlorite (NaOCl) for one minute and subsequently washed in water. Seeds were then placed on moist tissue paper in Petri dishes for germination. After 48 hours, germinated seeds were transferred to wet perlite medium and grown for an additional week. Seedlings were then transplanted to non-aerated hydroponic vessels, each comprised of two nested disposable plastic cups (480 ml) with a fitted foam disc at the top as per Hall et al. (2020b). Foam discs were pierced with a cork borer to accommodate a seedling in each vessel. Plants were provided

with 330 ml of fresh nutrient solution twice a week. The nutrient solution contained 2 mM KNO_3, 4.15 mM $Ca(NO_3)_2.4H_2O$, 4.15 mM $MgSO_4$, 2 mM KH_2PO_4, 150 µM NaFe(III)EDTA, 40.5 µM H_3BO_3, 11.5 µM $MnCl_2.4H_2O$, 0.75 µM $CuCl_2.2H_2O$, 0.50 µM $Na_2MoO_4.2H_2O$, and 1.35 µM $ZnSO_4.7H_2O$. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

3.3.2 Experimental design

Silicon treatment (+Si) was applied by adding liquid potassium silicate (K_2SiO_3) (21% K_2O and 32% SiO_2, Agsil32, PQ Australia, SA, Australia) to the nutrient solution to achieve a 2 mM concentration (SiO_2 equivalent) as silicic acid polymerises beyond this level (Ma & Yamaji, 2006). The extra K^+ ions provided in the +Si treatment were balanced in the silicon-free control treatment (–Si) by adding KCl (ACS reagent, Sigma-Aldrich, St. Louis, MO, USA). Both +Si and –Si solutions were adjusted to pH 6.0 with HCl. Cups were randomly rotated within the glasshouse every week to minimise any position bias.

3.3.3 Rearing of **Tetranychus urticae** *and the predatory mite*

The *Tetranychus urticae* Koch stock population was reared on lima bean (*Phaseolus lunatus*) plants grown hydroponically in –Si solution. To generate same-aged adults of *T. urticae*, 20 adult females were transferred to the youngest trifoliate leaves of bean plants and allowed to lay eggs for 24 hr. A thin layer of lanolin (Sigma-Aldrich, MO, USA) was applied around the petiole of these leaves using a needleless syringe to prevent *T. urticae* from escaping (Fig. S3-1) (Ximénez-Embún et al., 2017). Adults were then removed, and eggs and subsequent nymphs were monitored everyday. Adult females emerged on the same day were considered same-aged. The predatory mite, *Phytoseiulus persimilis*, colony

was supplied by Biological Services (Loxton, SA, Australia) and was maintained on *T. urticae*-infested bean leaves from –Si plants. Both mite populations were reared in a glasshouse chamber with natural lighting at 22/18°C day/night temperatures and 60% (± 5%) humidity.

3.3.4 Performance of Tetranychus urticae *and leaflet damage*

The performance of *T. urticae* was measured by estimating egg-laying, population growth and physical damage on leaflets at two-time points, 6 and 14 days after inoculation (DAI) (N = 10 plants of each Si treatment at each time point). Plants (20 +Si and 20 –Si), after three weeks of growth, were inoculated with 10 same-aged, adult *T. urticae* females on the central leaflet of the youngest, fully expanded leaf. Mites were confined on leaflets using the lanolin barrier. The inoculation density was chosen based on our preliminary observations that a leaflet infested with 10 *T. urticae* females can sustain optimal population growth over 14 days. A thin layer of lanolin (Sigma-Aldrich, MO, USA) was applied around the petiole of these leaflets using a needleless syringe to prevent *T. urticae* from escaping. At each time point, 20 infested leaflets (10 from each Si treatment) were harvested and examined under a stereomicroscope (Olympus, SZX7) to count the number of eggs and mobile stage spider mites (larvae, nymphs, and adults). Leaflets were subsequently scanned at a resolution of 300 dpi in a flatbed scanner (CanoScan LiDE210) and analysed with GIMP 2.10 (GNU image manipulation program) to estimate the damaged area as per Cazaux et al. (2014). Briefly, each scanned leaflet image was provided with a new layer and overlaid with a grid of 0.25 mm x 0.25 mm divisions. The chlorotic spots were then marked with red dots using the pencil tool, where each dot size was defined in pixels. The total number of pixels of the dotted area was calculated from the histogram

tool and finally converted to the damaged area (mm^2) based on the numerical relationship between pixels and the size of the grid division.

3.3.5 Gas exchange measurements

Given that *T. urticae* feeding causes physiological injury (i.e. by limiting gas exchange) to leaves beyond physical damage (Reddall et al., 2004), we measured leaflet net photosynthesis and stomatal conductance at two time points, 6 and 14 DAI, using a portable photosynthesis system (LI6400XT, Li-Cor, Lincoln, NE, USA). Plants (12 +Si and 12 –Si, distinct from those used for assessing *T. urticae* performance) were inoculated after three weeks of growth with 10 *T. urticae* females on the central leaflet of the youngest, fully expanded leaf. *Tetranychus urticae* females were confined on each leaflet using a lanolin barrier as described previously. The equivalent leaflets of 24 uninfested plants (12 of each Si treatment) were also provided with a lanolin barrier. Gas exchange measurements were taken from 12 infested leaflets (6 of each Si treatment) at each time point after carefully removing all *T. urticae* stages, exuviae and webs using a fine paintbrush. Likewise, we measured gas exchange rates in 12 uninfested plants (6 of each Si treatment) at each time point after brushing the equivalent leaflets with a paintbrush. All measurements were taken between 10:00 am and 2:00 pm local time from 6 cm^2 leaflet area with the following conditions: LED light source set at 1500 µmol quanta m^{-2} s^{-1}, 400 µmol mol^{-1} CO_2 concentration, 500 µmol s^{-1} air flow rate, 22°C block temperature, and 1.2-1.8 kPa leaf vapour-pressure deficit. Before taking measurements from each leaflet, the gas exchange rates were allowed to stabilise for two minutes. Five readings were then taken sequentially at two-second intervals, and the average value was counted.

3.3.6 Olfactometer assays

We used a Y-tube olfactometer with constant airflow to test the preference of *P. persimilis* following the protocol of Sabelis and Van de Baan (1983). Briefly, the olfactometer consisted of two choice arms (8 cm each) and a base arm (13 cm) of the same internal diameter (2 cm). A Y-shaped metal wire was placed in the centre of the Y-tube, parallel to the tube walls to make a walking path for the predatory mite. The Y-tube was placed over a black cotton cloth with a cold light source at the top. Each choice arm of the Y-tube was connected by odourless Teflon tubing to a glass chamber (3 L) containing a treatment plant (either +Si or –Si) (see illustration in Fig. 3-1).

In total, 48 plants (24 +Si and 24 –Si) were used for olfactometer bioassays. Plants were randomly assigned for inoculation with *T. urticae* after 3-4 weeks of growth. Forty adult *T. urticae* females were transferred randomly onto the youngest, fully expanded tri-foliate leaves to create *T. urticae*-infested plants (+M), and they were allowed to feed for six days. Previous studies have shown that exposing plants to 30-40 adult *T. urticae* females for six days can enhance HIPV emission and promote predatory mite attraction (Nachappa et al., 2006; Schausberger et al., 2012). As such, plants were tested in three combinations at 6 DAI: (i) Si-supplemented, *T. urticae*-infested (+Si +M) vs. Si-free, *T. urticae*-infested (–Si +M) to examine if Si promotes the attraction of *P. persimilis* upon spider mite herbivory, (ii) Si-supplemented, uninfested (+Si –M) vs. Si-free, *T. urticae*-infested (–Si +M) to examine if Si reduces predators' foraging efficiency by attracting them to uninfested plants, (iii) Si-supplemented, uninfested (+Si –M) vs. Si-free, uninfested (–Si –M) to investigate if Si attracts *P. persimilis* in the absence of herbivory. For each comparison, eight plant pairs were used with 15 predatory mites tested per plant pair. Plants

were transferred from hydroponic cups to glass cups (size 400 ml) with matching nutrient solutions before placing them in glass chambers to avoid any volatiles from plastics. Each glass cup was then covered with aluminium foil. Pressurised air was filtered through activated carbon and then humidified by passing through deionised water before splitting and admitting into glass chambers containing treatment plants. The airflow rate coming from each odour chamber to each Y-tube choice arm was maintained at 100 ml/min using separate flow meters (Fig. 3-1).

Predatory mites were starved for 2-3 hrs before bioassays. Each predatory mite was released singly on the wire at the base arm and was observed for a maximum of 5 min. Mites reaching the end of any choice arm were treated as choice responses. Mites that fell from the wire or did not make a choice within the observed time were omitted from the analysis. Out of 120 predatory mites tested per comparison, the numbers of omitted predatory mites were 18, 19 and 24 for +Si +M vs. –Si +M, +Si –M vs. –Si +M, and +Si –M vs. –Si –M, respectively. Each predatory mite was used only once. After testing seven to eight predatory mites, the odour sources were altered by interchanging Teflon tubing between the choice arms to minimise any position biases of the predators. Furthermore, both the Y-tube and the wire were cleaned with 100% ethanol to avoid any influence of previously used predatory mites. Bioassays were conducted in an air-conditioned room at 23 ± 1°C within a few consecutive days.

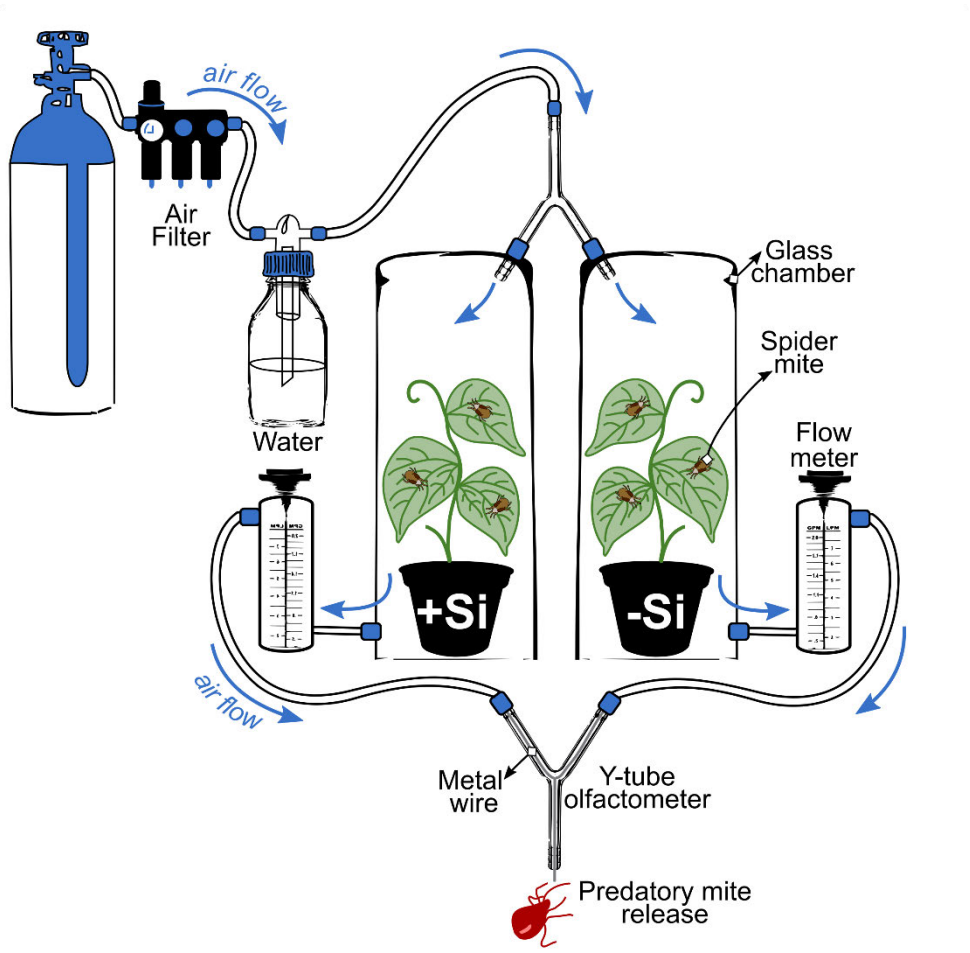

Figure 3-1 Schematic outline of the Y-tube olfactometer set-up. Predatory mites were released singly on the metal wire, and each predatory mite was observed for a maximum of 5 min.

3.3.7 Volatile collection, extraction, and quantification

We collected volatiles from 20 +M plants (10 of each Si treatment) at 6 DAI along with 12 –M plants (6 of each Si treatment) following a push-pull sampling method (Tholl et al., 2006). *Tetranychus urticae*-infested plants (+M) were generated as described previously in the olfactometer bioassays section. Volatiles were collected simultaneously from two plants (one +Si and another –Si, with or without *T. urticae*) for 3 hr using Porapak-Q sorbent tubes (6 mm x 70 mm, 78/39 mg polymer with glass wool separators). Plants were placed in separate glass chambers as described in the previous section, and a constant flow of filtered, humidified air at 300 ml/min was maintained into each chamber. After purging the system for 30 min, a sorbent tube was attached to the air outlet of each glass chamber by Teflon tubing. The airflow rates coming from the plant sources to sorbent tubes were maintained at 200 ml/min using flow meters. An air pump (AirLite, SKC Inc., USA) was connected to the sorbent tubes to pull air with volatile blends across the adsorbent polymer. The fresh weight of plant shoots was recorded immediately after volatile collection, and adsorbent tubes were kept at –20°C until further analysis.

Volatiles were extracted from the adsorbent polymer by adding 600 µl HPLC grade dichloromethane (DCM) with 5 µl nonyl acetate (100 ng/µl) as an internal standard. A gas chromatography-mass spectrometry (GC-MS) system (7890A, Agilent Technologies, Santa Clara, CA, USA) equipped with a BP1 column (30 m × 0.53 mm × 0.5 µm, SGE Analytical Science) was used for volatile analysis following the protocols described in Wei et al. (2006). The initial oven temperature was set at 30°C for 2 min and then increased by a ramp of 5°C/min to 200°C, followed by an increasing rate of 20°C/min to 280°C. The extract was injected in splitless mode at 250°C injector temperature with a constant flow

of hydrogen at 1 ml/sec as a carrier. Volatile compounds were identified from the NIST02 library (Scientific Instrument Services, Inc., Ringoes, NJ, USA) and the Terpenoids library (MassFinder 4 software, Hochmuth Scientific Consulting, Hamburg, Germany) databases by comparing the mass spectra. The relative emissions (%) of compounds in a blend were calculated by dividing the peak area of each compound by the total peak area of the volatile blend (Wei et al., 2006). The amounts of volatile compounds (ng/hr/plant) were calculated by comparing the peak area of each compound with the internal standard (Table S3-1, supplementary data).

3.3.8 Plant biomass and leaf elemental analyses

Forty uninfested plants (N = 20 of each Si treatment) from the first batch were grown for seven weeks until they started bending. Plants were then harvested and oven-dried at 60°C for one week before recording the dry shoot and root biomass. Leaf elemental analyses were performed with the second batch of plants to elucidate the effects of maximum *T. urticae* infestation on Si induction. Twenty-eight +Si plants (14 +M and 14 –M) and 28 –Si plants (14 +M and 14 –M) were used for Si quantification. Plants were oven-dried at 60°C, and the youngest, fully expanded tri-foliate leaves were ball-milled to a fine powder. Foliar Si concentrations (% dry mass) were measured by analysing approximately 80 mg ball-milled samples in an X-ray fluorescence spectrometer (Epsilon 3^x; PANalytical, EA Almelo, The Netherlands) following the protocol of Reidinger et al. (2012). Measurements were calibrated against a certified plant material of known Si concentration (NCS ZC73018 Citrus leaves, China National Institute for Iron and Steel). Si concentrations in −Si plants were below the machine detection limit (< 0.3%) and hence excluded from the analysis.

Tetranychus urticae females prefer leaves with high foliar N concentrations and low C:N ratios (Hoffland et al., 2000). Given that there could be a trade-off between foliar Si and C (Islam et al., 2020), we measured the concentrations (% dry mass) of foliar C and N in 10 +Si and 10 –Si plants in the absence of herbivory. For this, the ball-milled leaf materials used for Si quantification were analysed (6-7 mg per sample) in a CHNS elemental analyser (Elementar vario EL cube, Analysensysteme GmbH, Hanau, Germany) based on the Dumas method with a combustion temperature of 950°C.

3.3.9 Statistical analysis

All analyses were performed in R, version 3.5.1. Gas exchange parameters were analysed using two-way analysis of variance (ANOVA) tests, comparing the individual and interactive effects of Si and *T. urticae*. The number of *T. urticae* eggs, mobile stage mites, and leaflet damaged area were analysed using two-way ANOVA tests, using Si and time point as fixed factors. Tukey's HSD tests were used to determine differences between groups when interaction effects in ANOVA were significant. The choices of predatory mites in olfactometer bioassays were analysed using chi-squared goodness-of-fit tests. The relative emissions (%) of volatile compounds were arcsine-transformed, and the amounts of compounds (ng/hr/plant) were log(x+1) transformed to meet the assumption of homoscedasticity. Each compound was further compared using Student's *t*-tests between +Si +M and –Si +M plants and +Si –M and –Si –M plants. The mean values and confidence intervals (CIs) of the transformed data were converted to original values for presentation. Principal component analysis (PCA) was used to further analyse the relative emissions of compounds. Shoot fresh weight was compared between +Si +M and –Si +M and +Si –M

and –Si –M plants using Student's *t*-tests. All other parameters were analysed using one-way ANOVA.

3.4 Results

3.4.1 Population growth of Tetranychus urticae *and leaflet damage*

Si significantly reduced egg-laying (21%) by *T. urticae* females at 6 DAI but did not affect the number of mobile stage spider mites (9.8 ± 0.13 on −Si plants vs. 9.9 ± 0.10 on +Si plants) as there was no egg hatching to that point in time (Fig. 3-2a and 3-2b; Table 3-1). Subsequently, both the number of eggs and *T. urticae* population were 34% and 39% lower, respectively, on +Si plants compared to –Si plants at 14 DAI (Fig. 3-2a and 3-2b; Table 3-1). The physical damage on leaflets due to *T. urticae* feeding was not affected by Si treatment at 6 DAI (Fig. 3-3; Table 3-1). However, the leaflet damaged area was lower on +Si plants at 14 DAI, indicating less feeding injury due to the low *T. urticae* population (Fig. 3-3; Table 3-1).

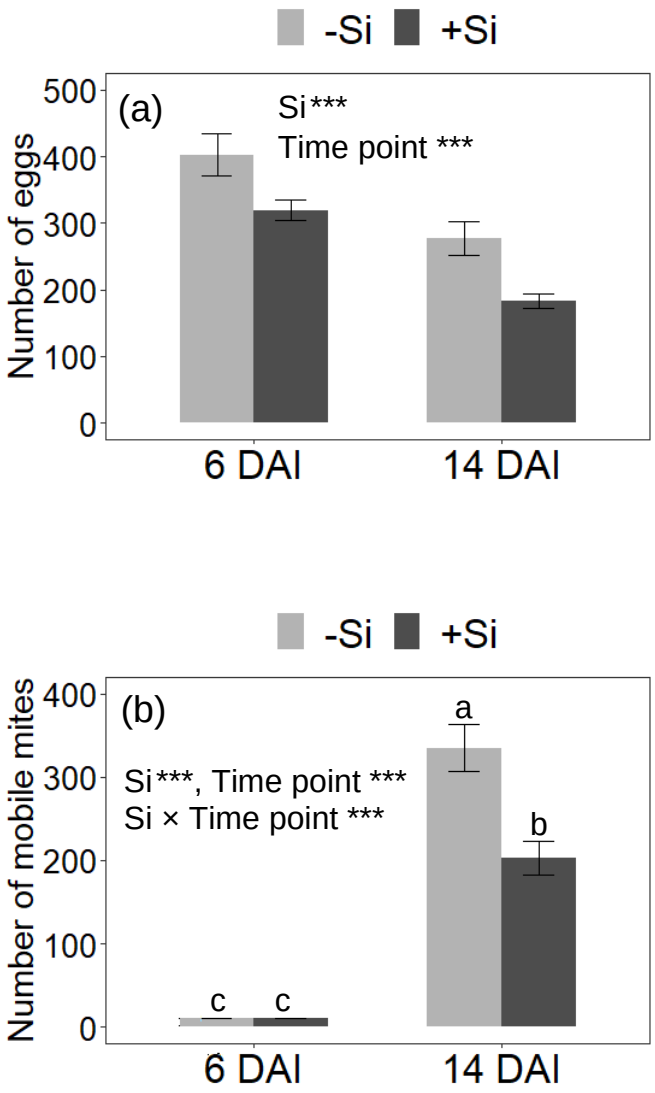

Figure 3-2 Effects of Si and time point on (a) number of eggs laid by *T. urticae* females, and (b) number of mobile stage mites (N = 10 plants) at 6 DAI and 14 DAI. Mean ± SE shown. Asterisks indicate the level of statistical significance (***$p < 0.001$). Different lowercase letters on bars indicate statistically significant differences between groups.

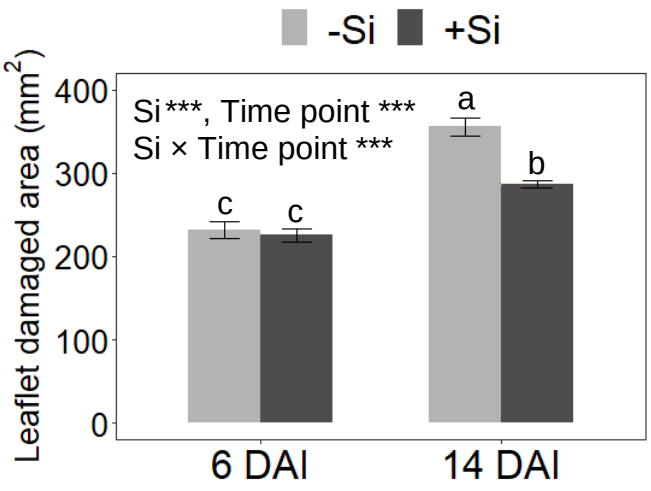

Figure 3-3 Effects of Si and time point on leaflet damage by *T. urticae* (*N* = 10 plants). Mean ± SE shown. Asterisks indicate the level of statistical significance (***$p < 0.001$). Different lowercase letters on bars indicate statistically significant differences between groups.

Table 3-1 Two-way ANOVA table showing the effects of Si and time point on the number of eggs, mobile stage *T. urticae* and leaflet damaged area.

	Fig.	*df*	Si		Time point		Si × Time point	
			F	*p*	*F*	*p*	*F*	*p*
Eggs	2a	1,36	15.53	**<0.001**	33.85	**<0.001**	0.06	0.809
Mobile mites	2b	1,36	14.23	**<0.001**	219.07	**<0.001**	14.27	**<0.001**
Damaged area	3	1,36	18.53	**<0.001**	114.75	**<0.001**	13.16	**<0.001**

Statistically significant effects are highlighted in bold ($p < 0.05$).

3.4.2 Leaflet photosynthesis and stomatal conductance

Tetranychus urticae feeding reduced net photosynthesis and stomatal conductance regardless of Si treatment (Tables 3-2 and 3-3). The leaflet net photosynthesis following *T. urticae* infestation was 42% and 62% higher in +Si plants at 6 DAI and 14 DAI, respectively, compared to –Si plants (Tables 3-2 and 3-3). Likewise, the stomatal conductance of +Si +M plants was higher compared to –Si +M plants at 6 DAI. Nevertheless, the gas exchange rates of –Si and +Si plants were statistically similar in the absence of *T. urticae* infestation (Tables 3-2 and 3-3).

Table 3-2 Effects of Si and *T. urticae* inoculation on leaflet net photosynthesis (μmol CO_2 $m^{-2}s^{-1}$) and stomatal conductance (mol H_2O $m^{-2}s^{-1}$) at 6 and 14 days after *T. urticae* inoculation (DAI). Mean ± SE shown.

Parameter		Uninfested		Infested	
	DAI	−Si	+Si	−Si	+Si
Net photosynthesis	6	12.86 ± 0.15 [a]	12.98 ± 0.23 [a]	5.46 ± 0.10 [c]	7.75 ± 0.24 [b]
	14	13.15 ± 0.22 [a]	12.87 ± 0.49 [a]	3.38 ± 0.08 [c]	5.48 ± 0.12 [b]
Stomatal conductance	6	0.15 ± 0.004 [a]	0.15 ± 0.003 [a]	0.06 ± 0.002 [c]	0.08 ± 0.002 [b]
	14	0.16 ± 0.004 [a]	0.15 ± 0.005 [a]	0.03 ± 0.002 [b]	0.05 ± 0.003 [b]

Different lowercase letters indicate statistically significant differences between groups.

Table 3-3 Two-way ANOVA table showing the effects of Si and *T. urticae* inoculation on leaflet gas exchange parameters at 6 and 14 DAI.

Parameter	DAI	*df*	Si		*T. urticae*		Si × *T. urticae*	
			F	*p*	*F*	*p*	*F*	*p*
Net photosynthesis	6	1,20	39.87	**<0.001**	1089.55	**<0.001**	32.15	**<0.001**
	14	1,20	10.75	**0.004**	954.50	**<0.001**	18.48	**<0.001**
Stomatal conductance	6	1,20	11.25	**0.003**	845.0	**<0.001**	11.25	**0.003**
	14	1,20	1.64	0.215	865.64	**<0.001**	6.55	**0.019**

Statistically significant effects are highlighted in bold ($p < 0.05$).

3.4.3 Behavioural response of predatory mites

Phytoseiulus persimilis mites were significantly more attracted to volatiles from +Si +M plants (64% out of 102 responsive mites) compared to volatiles from −Si +M plants (Fig. 3-4; $\chi^2 = 7.69$, $p = 0.006$). However, predatory mites were significantly more attracted (70% out of 101 responsive mites) to volatiles from −Si +M plants when tested against volatiles from +Si –M plants ($\chi^2 = 16.64$, $p < 0.001$). Predatory mites ($N = 96$) did not discriminate between volatiles from +Si and –Si plants in the absence of *T. urticae* infestation (Fig. 3-4; $\chi^2 = 0.167$, $P = 0.683$).

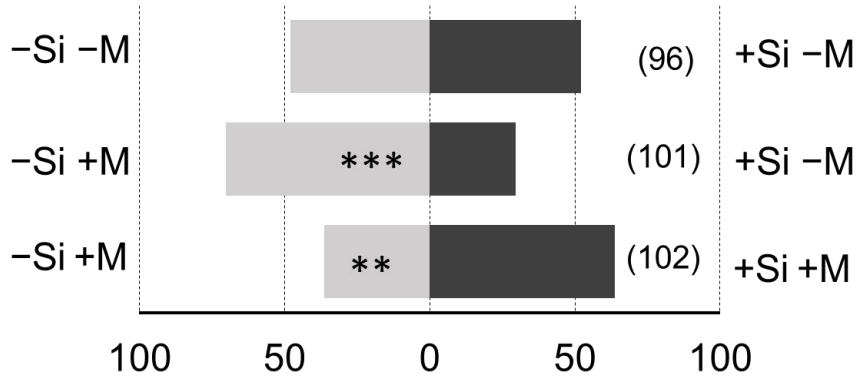

Figure 3-4 Preferences of *P. persimilis* mites for volatiles from different treatment plants in Y-tube olfactometer bioassays. Values are the percentages of predatory mites responding to specific volatile sources. The number in parentheses indicates the number of responsive mites out of 120 predatory mites tested for each comparison. Asterisks indicate significant differences from a 50:50 distribution (χ^2 goodness-of-fit test, $^{**}p < 0.01$, $^{***}p < 0.001$).

3.4.4 Volatile emission by plants

In total, 17 volatile compounds were detected in the headspace of plants across four treatments (+Si +M, –Si +M, +Si –M, and –Si –M), including four green leaf volatiles and seven terpenoids. Overall, Si-supplementation did not cause the synthesis of any novel volatiles; rather, it altered the proportional composition of volatile blends (Fig. 3-5). *Tetranychus urticae* infestation, however, affected the number of compounds emitted by plants. Both +Si and –Si plants emitted 17 compounds upon spider mite herbivory and eight compounds in the absence of herbivory. The relative emissions (%) of *E*-2-hexenyl benzoate, hexanal, *E*-*trans*-β-ocimene, D-limonene, β-caryophyllene, and methyl salicylate by +Si +M plants were significantly higher (*p* values: 0.004, 0.004, 0.007, 0.007, 0.033, and 0.002, respectively), while the relative emissions of 3-hexanol, *trans*-

calamenene, *o*-xylene, and *o*-cymene were significantly lower (*p* values: 0.020, 0.002, 0.017, and 0.007, respectively) compared to –Si +M plants (Fig. 3-5; Table S3-2). Likewise, the amounts (ng/hr/plant) of hexanal, *E*-*trans*-β-ocimene, D-limonene, and methyl salicylate (*p* values: 0.025, 0.007, 0.007, and 0.029, respectively) in the volatile blends released from +Si +M plants were significantly elevated compared to –Si +M plants, whereas the amounts of 3-hexanol, 2-hexanol, *trans*-calamenene, *o*-xylene, and *o*-cymene were significantly lowered (*p* values: < 0.001, 0.018, < 0.001, 0.010, and 0.035, respectively) (Tables 3-S1 and 3-S3). In the absence of herbivory, the percent relative emissions of eucalyptol, *o*-xylene, 1-methylcyclopentanol, and dodecane by +Si –M plants were significantly lower (*p* values: 0.009, 0.014, 0.008, and 0.003, respectively), whereas the emission of 3-hexanol ($p = 0.014$) was higher as compared to –Si –M plants. There were no significant differences in the total emission of volatiles (Fig. S3-2; Table S3-3) or shoot fresh weight (Table S3-4 and S3-5) between +Si +M and –Si +M and +Si –M and –Si –M plants. The PCA analysis of volatile compounds resulted in four principal components. The first and second principal components explained 57.3% and 13.1% of the total variance, respectively (Fig. 3-6). The PCA biplot shows that *T. urticae* infestation mostly influenced the first principal component (Fig. 3-6).

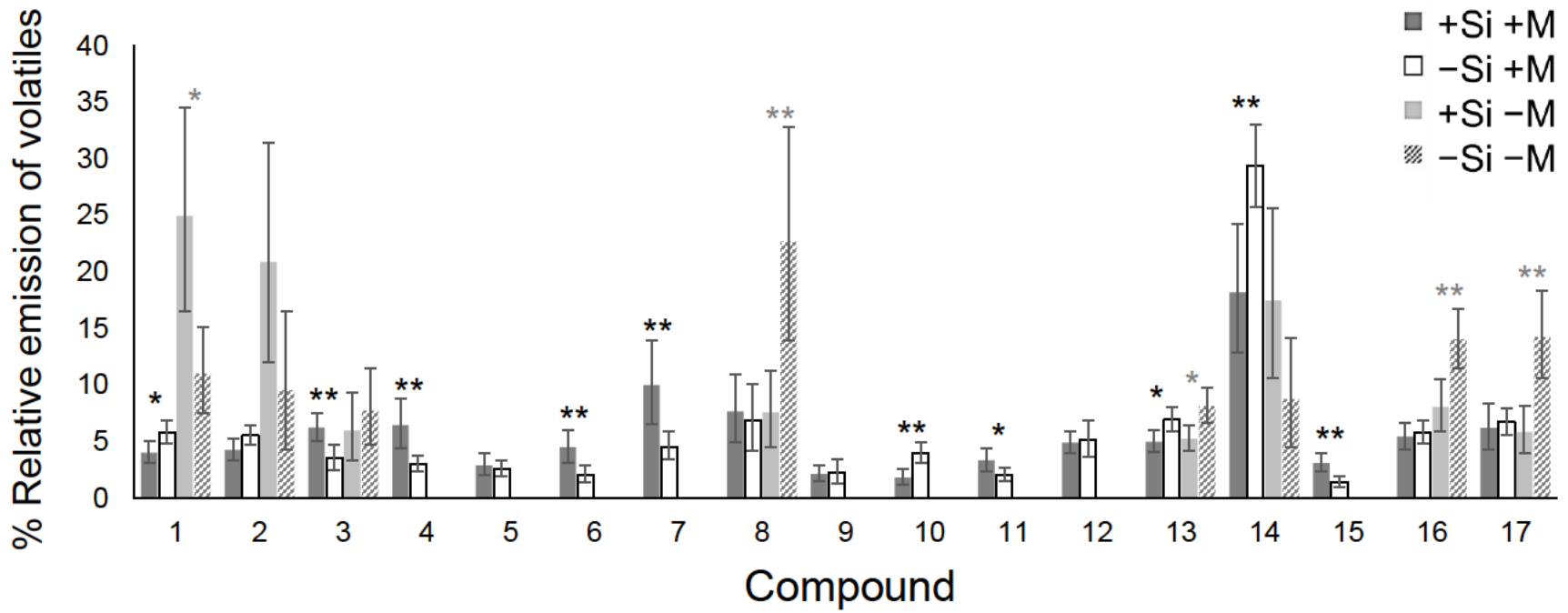

Figure 3-5 The relative emissions (%) of volatile compounds by different treatment plants. Mean ± 95% confidence interval shown. Black asterisks (*) indicate significant differences between +Si +M and –Si +M plants and grey asterisks (*) indicate significant differences between +Si –M and –Si –M plants (Student's *t*-tests, for both comparisons *$p < 0.05$, **$p < 0.01$). Compound numbers: (1) 3-Hexanol, (2) 2-Hexanol, (3) *E*-2-Hexenyl benzoate, (4) Hexanal, (5) (*E*)-4,8-Dimethyl-1,3,7-nonatriene, (6) *E-trans*-β-Ocimene, (7) D-Limonene, (8) Eucalyptol, (9) Aromandendrene, (10) *trans*-Calamenene, (11) β-Caryophyllene, (12) Indole, (13) *o*-Xylene, (14) *o*-Cymene, (15) Methyl salicylate, (16) 1-Methylcyclopentanol, (17) Dodecane.

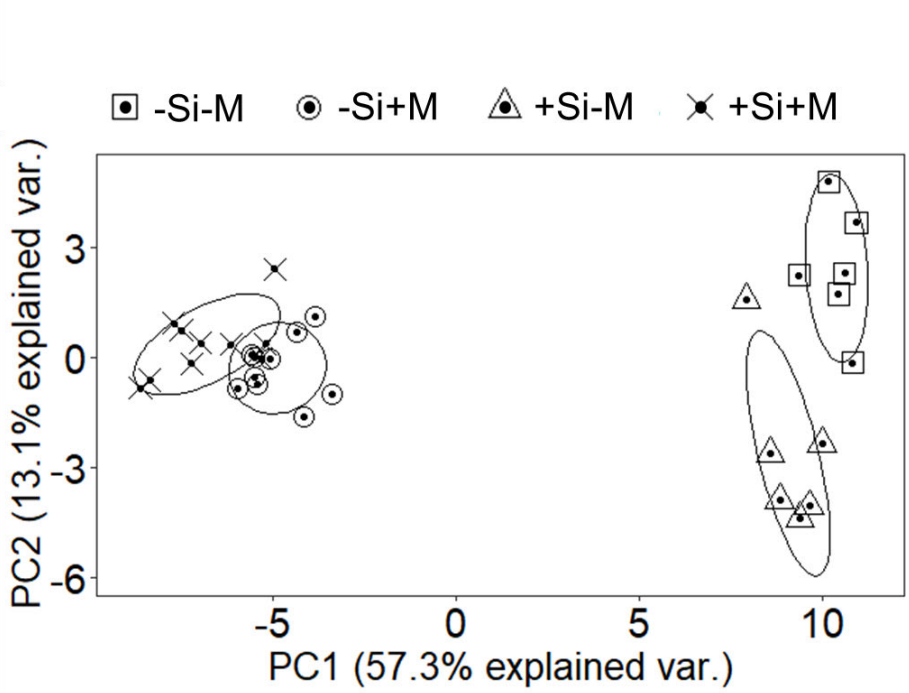

Figure 3-6 Principal component analysis of the volatile compounds emitted by different treatment plants. Grouping of samples according to the first two principal components with confidence regions (ellipses).

3.4.5 Plant biomass and leaf elemental analyses

Si significantly increased shoot dry biomass by 19%, although it had no effect on root dry biomass (Table 3-4a). Moreover, Si significantly decreased % foliar C but had no effects on % foliar N and the C:N ratio (Table 3-4a). *Tetranychus urticae* infestation significantly increased foliar Si concentrations by 68% in French bean leaves (Table 3-4b).

Table 3-4 Group means (± SE) and ANOVA results showing (a) effects of Si on plant biomass, % foliar N, % foliar C, and C:N ratio and (b) effects of *T. urticae* on % foliar Si accumulation in +Si plants.

(a) Parameter	−Si	+Si	ANOVA		
			df	*F*	*p*
Shoot dry biomass (g)	3.74 ± 0.12	4.44 ± 0.15	1,38	13.46	**<0.001**
Root dry biomass (g)	0.60 ± 0.02	0.59 ± 0.03	1,38	0.07	0.794
% Foliar N	3.58 ± 0.21	3.84 ± 0.25	1,18	0.67	0.425
% Foliar C	42.29 ± 0.35	39.54 ± 0.72	1,18	11.88	**0.003**
C:N ratio	12.18 ± 0.68	10.92 ± 1.14	1,18	0.90	0.355

(b) Parameter	+Si plants		ANOVA		
	Uninfested	Infested	*df*	*F*	*p*
% Foliar Si	0.60 ± 0.014	1.01 ± 0.059	1,26	46.24	**<0.001**

Statistically significant effects are highlighted in bold ($p < 0.05$).

3.5 Discussion

To our knowledge, this is the first report of Si induction and Si-augmented indirect plant defence in response to *T. urticae* infestation. We found that Si reduced egg-laying and population growth of *T. urticae* and thereafter leaflet damage while ameliorating stomatal conductance and net photosynthesis following *T. urticae* feeding. Besides, *T. urticae* infestation increased foliar Si accumulation in French bean. Notably, *P. persimilis* mites were more attracted to plants experiencing *T. urticae* infestation if those plants had access to Si, an outcome resulting from altered HIPV composition.

Our results show a 34% reduction in *T. urticae* oviposition and a 39% reduction in its population growth over two weeks due to Si supplementation. Such a reduced oviposition rate suggests lower suitability of +Si plants as hosts for *T. urticae* (Yano et al., 1998). Moreover, the efficacy of Si in suppressing the *T. urticae* population is equivalent to some other direct control strategies, including the use of botanical extracts and entomopathogens. For example, Miresmailli and Isman (2006) found 52% reductions in the *T. urticae* population on greenhouse tomato following the application of rosemary oil-based acaricide. In line with this, Dara et al. (2018) found that the application of botanical acaricides and entomopathogens caused 23-42% reductions in the *T. urticae* population on field-grown strawberries. While the use of synthetic acaricides could potentially cause rapid knockdown of *T. urticae*, there are high risks of acaricide resistance, pest resurgence and toxicity to beneficial organisms (Döker & Kazak, 2019; Patil et al., 2018; Van Leeuwen et al., 2010).

Only a handful of studies have examined the effects of hydroponically or soil-applied Si against *T. urticae*. Harizanova et al. (2019) reported a lower rate of *T. urticae* population growth on hydroponically grown cucumber (a moderate Si-accumulator) with Si supplementation. Interestingly, Gatarayiha et al. (2010) found that soil application of Si did not cause mortality of *T. urticae* but enhanced their susceptibility to the entomopathogenic fungus, *Beauveria bassiana*. Our results show, for the first time, that Si can suppress the population growth of *T. urticae* in a low Si-accumulating plant species. One underlying mechanism behind this could be the enhanced triggering of plant chemical defence by *T. urticae* under Si supplementation. For instance, Si has been linked to the increased activity of two defence enzymes, syringaldazine peroxidase and guaiacol peroxidase, in cucumber upon *T. urticae* attack (Harizanova et al., 2019). Strikingly, *T. urticae* secretes saliva while feeding that contains effector proteins (Villarroel et al., 2016). These effector molecules can interfere with the plants' recognition of herbivory and sabotage host defences (effector-triggered susceptibility) (Villarroel et al., 2016). For example, spider mites can suppress jasmonic acid and salicylic acid (SA) production in tomato, independent of any hormonal crosstalk (Alba et al., 2015). Interestingly, Si deposited in the apoplast has been suggested to hinder the flow of effector molecules and act as a barrier to the effector recognition process by plant cells (Coskun et al., 2019). It is, therefore, possible that Si hindered defence suppression by *T. urticae*, triggering the induction of plant chemical defences that restricted *T. urticae* population growth.

We found that the physical damage on leaflets of +Si and −Si plants was similar after six days of *T. urticae* exposure. The reason could be that *T. urticae* feeding liquefied cell content stealthily, somewhat similarly to aphids, by puncturing mesophyll cells with a

retractable stylet without damaging the epidermis (Bensoussan et al., 2016). As such, *T. urticae* might largely avoid the effects of different Si structures, including silica bodies (phytoliths), during probing and feeding, which can preclude wearing of their mouthparts and digestion inefficiency. These mechanisms have been proposed to negate the effects of Si on aphids (Massey et al., 2006; Rowe et al., 2020). However, despite similar visual damage, we found that leaflets of +Si plants had higher stomatal conductance and net photosynthetic rate compared to −Si plants, corroborating the only past study in cucumber (Harizanova et al., 2019). The putative reason could be that *T. urticae* feeding caused less physiological injury to leaflets of +Si plants regardless of physical damage. *Tetranychus urticae* can reduce photosynthesis by sucking out contents from spongy and palisade parenchyma cells, thereby directly reducing leaf chlorophyll contents (Park & Lee, 2002). Besides, dehydration and destruction of mesophyll tissue disrupt the turgidity of stomatal guard cells, triggering stomatal closure and inhibiting gas exchange (Park & Lee, 2002; Reddall et al., 2004). This could also lead to lower stomatal conductance and photosynthetic rate of the infested leaflets. Si deposition in the cell wall of the stomatal apparatus has been shown to prevent aberrant movements of stomata in rice leaves (Ueno & Agarie, 2015). We speculate that silicified stomata might show some resistance to closure triggered by *T. urticae* feeding injury, which in turn ameliorated stomatal conductance. This might further increase net photosynthesis, as it is largely dependent on stomatal conductance when leaf conductance is lower (Reddall et al., 2004).

Even though French bean was previously considered to be a Si excluder (Takahashi et al., 1990), we found increased foliar Si accumulation following *T. urticae* infestation. This underpins the induction of Si defence upon spider mite herbivory. Plants can take up Si

actively by using transporter proteins and/or passively via the transpiration stream (Ma & Yamaji, 2006). While *T. urticae* injury reduces stomatal transpiration, it has been found to increase nighttime cuticular transpiration by 3.5 times in peppermint, *Mentha piperita* (De Angelis et al., 1982). Additionally, *T. urticae* feeding causes many penetration holes in leaves, which can cause uncontrolled evaporation of water, countering reduced stomatal transpiration (Reddall et al., 2004). However, given the relative importance of stomatal transpiration, we suggest that Si induction could be an active process in French bean experiencing *T. urticae* infestation. Two putative influx transporter genes, *GmNIP2-1* and *GmNIP2-2*, have been identified in soybean (another legume) and suggested to regulate active Si uptake (Deshmukh et al., 2013).

Our data suggest that Si caused a shift in the proportional composition of HIPVs upon *T. urticae* infestation, which in turn promoted predator attraction, validating previous studies (de Oliveira et al., 2020; Liu et al., 2017). Interestingly, *P. persimilis* did not show any preference for volatiles from +Si plants in the absence of spider mite herbivory despite quantitative changes in the volatile blends. This is particularly important because attracting natural enemies in the absence of a pest population, for example, by using synthetic HIPV-based attractants, can be counterproductive and compromise biocontrol (Kaplan, 2012). Our results show that following *T. urticae* infestation, the proportional emissions of six different compounds were higher from +Si plants than –Si plants. Among these, *E-trans*-β-ocimene, β-caryophyllene and methyl salicylate are well-recognised attractants for herbivore natural enemies (Krugner et al., 2014; Rasmann et al., 2005; Van Poecke et al., 2001), including *P. persimilis* (De Boer & Dicke, 2004; Schausberger et al., 2012). However, natural enemy attraction has been suggested as a function of overall HIPV

quality (composition) rather than the occurrence of any specific volatile compounds in the blend (van Wijk et al., 2008). Besides, *P. persimilis* perceives the volatile blend as a 'whole' instead of a repertoire of strictly elemental objects (van Wijk et al., 2010). Thus, we presume that the altered ratio of compounds in the volatile blends was the key to enhanced predator attraction. Although the underlying mechanisms and reasons for such altered emissions are not clear, we suggest two possible explanations for this. First, Si hinders plant defence suppression by *T. urticae*, amplifying the induction of chemical defence. Given the phytohormonal regulation of HIPV emission (Ponzio et al., 2013), Si might prevent the suppression of JA and SA by *T. urticae*, facilitating interruption-free HIPV production that promotes more attraction of natural enemies. Second, stressed plants could use Si for different functions (as more Si is available because of induction) including structural support and growth at a metabolically cheaper cost (Cooke & Leishman, 2011), thereby liberating resources to fine-tune volatile defences. Moreover, direct defence benefits provided by Si could help plants invest more in indirect defence.

Overall, our findings reveal that Si directly suppresses *T. urticae* oviposition and population growth and reduces their damage potential to French bean. Likewise, Si promotes natural enemy attraction by shifting the composition of HIPVs produced in response to *T. urticae* infestation, which can play a part in top-down control. This suggests that Si may be an alternative or supplement to commercial volatile-based attractants for natural enemies, which could be a promising avenue of pest management. However, natural enemy attraction does not necessarily guarantee enhanced biocontrol (Kaplan, 2012); thus, it needs to be carefully investigated under realistic ecological settings before making any recommendation. Nonetheless, our study establishes that Si defences are inducible and

functional in low Si-accumulating plants and that Si underpins both direct and indirect plant defences against cell-content feeding herbivores.

Chapter 4

Silicon fertilisation affects morphological and immune defences of an insect pest and enhances plant compensatory growth

---

Published as Islam et al. (2022b), *Journal of Pest Science*

4.1 Abstract

Herbivorous insects have evolved various anti-predator defences, including morphological, behavioural, and immune defences, which can make biocontrol of herbivorous pests challenging. Silicon (Si) accumulation in plants is a potent physical defence against mandibulate insects. However, it remains uncertain how Si affects the anti-predator defences of insect herbivores and plant defences following herbivory. We grew the model grass, *Brachypodium distachyon*, hydroponically with (+Si) or without (–Si) Si and investigated the plant-mediated effects of Si on the anti-predator defences of the cotton bollworm, *Helicoverpa armigera*, integrating morphological (i.e. integument resistance and thickness), behavioural, and immune defences. We also examined the effects of Si on plant compensatory growth and leaf trichome production. Larval growth, leaf consumption, and integument resistance to puncture (i.e. signifying larval resistance to parasitoid attack) were lower when feeding on +Si plants compared to when feeding on –Si plants. Larval integument thickness, defensive behaviours, haemocyte density, and lysozyme-like activity in the haemolymph were unaffected by Si. Larvae fed on +Si plants had higher haemolymph phenoloxidase (PO) and total-PO activities than larvae fed on –Si plants, although this did not enhance the melanisation response of larvae. Furthermore, Si supplies increased plant compensation for herbivory and constitutive trichome production,

whereas herbivory induced trichome production only on –Si plants. We provide the first evidence for plant-mediated effects of Si on anti-predator defences of an insect herbivore. We suggest that the lower integument resistance of larvae when feeding on Si-supplemented plants could contribute to their vulnerability to natural enemies and that high PO activity may impose fitness costs (e.g. delayed development).

4.2 Introduction

The nutritional quality and physical and chemical defences of plants can substantially impact the performance and fitness of herbivorous insects (i.e. bottom-up effects) (Singer & Stireman, 2005; Vidal & Murphy, 2018). Changes in performance and fitness can impact the anti-predator defences of insect herbivores, which in turn can affect their vulnerability to natural enemies such as predators and parasitoids (top-down forces) (Forkner & Hunter, 2000; Pekas & Wäckers, 2020). For example, feeding on high-quality host plants can enhance insect immune defences such as encapsulation and melanisation of invaders (i.e. parasitoid eggs) (Diamond & Kingsolver, 2011; Gherlenda et al., 2016). Moreover, insect herbivores can exploit plant defence chemicals (e.g. secondary metabolites) to self-medicate (Garvey et al., 2021) or to defend against their enemies via sequestration (Winde & Wittstock, 2011).

Among insect pests, lepidopteran larvae encounter extensive top-down pressures (Bernays, 1997) and are often managed with biological control (Stiling & Cornelissen, 2005). As counteradaptations, larvae have evolved a range of anti-predator defences including morphological, behavioural, chemical, and immune defences (Greeney et al., 2012; Sugiura, 2020). For instance, thicker integuments or larger body spines can function as

morphological defences against parasitoids (Gross, 1993). Moreover, larvae can show evasive behaviours including thrashing, twisting, or dropping to avoid enemies, and aggressive behaviours including headrearing, regurgitating, or biting while encountering enemies (Greeney et al., 2012; Gross, 1993). However, after successful attacks by pathogens or parasitoids, larvae mostly rely on immune defence driven by cellular and humoral mechanisms. Haemocytes, the circulating haemolymph cells in larvae, can cause phagocytosis of microorganisms and encapsulation of foreign entities such as parasitoid eggs or larvae (Lavine & Strand, 2002). Humoral responses include the actions of phenoloxidase (PO), a key enzyme that regulates melanisation of invaders and wound healing (Eleftherianos & Revenis, 2011), and lysozyme activity that degrades bacteria and fungi (Moreno-García et al., 2013).

Plant defence against herbivores comprises resistance (e.g. physical and chemical barriers to herbivory) and tolerance to attack, including compensatory growth in response to herbivory (Núñez-Farfán et al., 2007). Both physical and chemical defences in plants can be expressed constitutively or induced when challenged by herbivores (Gatehouse, 2002). Silicon (Si) accumulation in plants is recognized as an effective physical defence against chewing herbivores (Massey & Hartley, 2009; Reynolds et al., 2016), particularly in grasses including cereal crops, as they possess limited secondary metabolite defences and can accumulate relatively higher amounts (up to 10% of dry weight) of Si (Moore & Johnson, 2016; Vicari & Bazely, 1993). Plants deposit silica (SiO_2) in tissues, including physical defence structures (e.g. trichomes), following active uptake or passive absorption of aqueous orthosilicic acid via roots and transportation via xylem (Ma & Yamaji, 2006; Mandlik et al., 2020).

Silicification of plant tissues makes them tougher and abrasive and hence less masticable and digestible for chewing herbivores (Massey & Hartley, 2009). Silicified tissues can wear down the mandibles of larvae when feeding (Massey & Hartley, 2009) and silicified trichomes can cause gut damage when passing through the digestive tract (Andama et al., 2020). Chewing insects generally feed less on silicified plants and have retarded growth because of reduced nutrient assimilation (Massey & Hartley, 2009). However, it remains uncertain whether malnourishment caused by siliceous plants compromises the anti-predator defences of insects. This question is of interest, as Si accumulation by plants has recently been shown to promote the attraction of natural enemies of herbivores (Islam et al., 2022c; Liu et al., 2017) and impact tri-trophic interactions by changing host insects' body size (Hall et al., 2021). Moreover, research on anti-herbivore Si defences has mostly focused on plant resistance, with the role of Si in plant compensation or tolerance relatively neglected. Furthermore, herbivory often induces increased Si accumulation in plants (Islam et al., 2020; Massey et al., 2007); however, how Si induction impacts the production of other inducible defence structures such as trichomes remains understudied, although there are reports that Si can enhance constitutive trichome production (Biru et al., 2021; Johnson et al., 2021a).

To the best of our knowledge, the plant-mediated effects of Si on anti-predator defences integrating morphological, behavioural, and immune defences of herbivorous insects have yet to be investigated. We grew the model grass, *Brachypodium distachyon*, hydroponically with (+Si) or without (−Si) Si and investigated the impacts of Si supplementation on anti-predator defences of the cotton bollworm, *Helicoverpa armigera*, and plant compensation and trichome production in response to herbivory. Specifically,

our objectives were (i) to elucidate the effects of Si on larval growth and feeding and consequently on larval morphological (integument resistance and thickness), behavioural (in the absence or presence of a stimulus), and immune (under immunologically naïve and challenged conditions) defences; and (ii) to determine the impacts of Si supplementation on plant compensation for herbivory and constitutive and induced trichome production. *Brachypodium distachyon* is a temperate grass with a genetically tractable genome, short life cycle, and phylogenetic connection to important cereal crops (Scholthof et al., 2018). *Helicoverpa armigera* is a global pest of many high-value agricultural and horticultural crops, costing over US$7 billion annually due to crop losses and management expenses (Jones et al., 2019). We hypothesized that Si negatively affects larval feeding and growth and thus attenuates the anti-predator defences of larvae by limiting physiological resources.

4.3 Materials and methods

4.3.1 Plants and insect herbivores

Brachypodium distachyon (accession Bd21-3) seeds were obtained from the French National Research Institute for Agriculture, Food and Environment (INRAE, Paris, France). Seeds were softened by soaking in water for 2 hrs and dehusked manually using forceps. Seeds were then sterilised in a solution of 0.9% NaOCl and 0.1% Triton-X for 30 min and subsequently washed in water several times. Sterilised seeds were sown in wet perlite in germination trays and kept refrigerated at 4°C for 7 days for cold stratification and further grown for 12 days in a naturally lit glasshouse at 22/18°C day/night temperatures on a cycle of 14 L:10 D and 50% (± 6%) relative humidity. Uniform seedlings were transplanted to non-aerated hydroponic vessels, each comprised of two nested disposable plastic cups (480 ml) with a fitted foam disc at the top as per Hall et al. (2020b).

Foam discs were slot cut to accommodate a seedling in each vessel. Cups were filled weekly with 370 ml of freshly prepared nutrient solution with (+Si) or without (−Si) Si supplementation as per Hall et al. (2020b).

The hydroponic nutrient solution contained 1 mM KNO_3, 1 mM $Ca(NO_3)_2.4H_2O$, 1 mM KH_2PO_4, 0.6 mM $MgSO_4$, 0.1 mM NaCl, 15 μM H_3BO_3, 0.5 μM $MnCl_2.4H_2O$, 0.7 μM $ZnSO_4.7H_2O$, 0.8 μM $Na_2MoO_4.2H_2O$, 0.8 μM $CuSO_4.5H_2O$, and 0.12 mM NaFe(III)EDTA. Non-aerated hydroponics have been used extensively for growing *B. distachyon* (Biru et al., 2021; de la Peña et al., 2019; Johnson et al., 2021b; Jung et al., 2014; Jung et al., 2015; Wang et al., 2019). Given that *B. distachyon* plants have short stature and fresh nutrient solutions were replenished weekly, plant roots received sufficient oxygen in hydroponics for growth and metabolism. Plants (N = 150) were grown in the same glasshouse environment, and cups were rotated weekly to avoid any position bias. *Helicoverpa armigera* (Lepidoptera: Noctuidae) larvae were supplied by CSIRO Agriculture and Food, Narrabri, NSW, Australia and were reared on an artificial diet modified from Teakle and Jensen (1985) before being inoculated onto plants.

4.3.2 Experimental design and treatments

Liquid potassium silicate (K_2SiO_3) (21% K_2O and 32% SiO_2, Agsil32, PQ Australia, SA, Australia) was added to the nutrient solution to create the +Si treatment. The concentration of silicic acid was maintained at 2 mM (SiO_2 equivalent) in +Si solution as polymerisation occurs above this concentration (Ma & Yamaji, 2006). The added K^+ ions in the +Si treatment were balanced in the control treatment (–Si) by adding KCl (Sigma-Aldrich, MO, USA). Both +Si and –Si solutions were adjusted to pH 5.5 with HCl. The addition of

chloride did not produce any chloride toxicity in plants (Biru et al., 2021; Hall et al., 2020b; Johnson et al., 2021b).

Plants were assigned to herbivore treatments (insect or no insect) after being grown hydroponically for five weeks. Eighty plants (40 +Si and 40 –Si) were randomly exposed to a second instar *H. armigera* larva for seven days. Larvae were starved for 24 hrs in Petri dishes before being weighed and placed onto plants. The rest of the plants (N = 70) were kept insect-free. All plants were kept caged for seven days in transparent acrylic cylinders (see Johnson et al. (2020b) for cage specifications). Larvae were starved for 24 hrs in Petri dishes following removal from plants to allow all frass to be expelled and subsequently weighed to estimate the relative growth rates (RGRs) of larvae. Insect RGR is a combined estimate for plant antixenotic (i.e. effects on growth due to starvation) and antibiotic effects and was calculated as larval mass gain relative to initial body mass per unit of time [mass gained (mg)/initial mass (mg)/time (days)] (Massey & Hartley, 2009). To determine larval instar, we randomly selected 40 larvae (20 fed on +Si plants and 20 fed on –Si plants) and measured their head capsule widths (mm) in the widest region using the micrometre mounted on a stereo microscope (Olympus, SZX10) (Mohammadi et al., 2010).

We collected larval frass randomly from 40 hydroponic vessels (20 holding +Si plants and 20 holding –Si plants) and matching Petri dishes following second starvation and oven-dried it at 60°C for three days before weighing (i.e. as a proxy for leaf consumption by larvae (Murray et al., 2013). Furthermore, 40 larvae (20 fed on +Si plants and 20 fed on –Si plants) were used for assessing behavioural and morphological defences, and the remaining 40 larvae were used for measuring immune defences. Thirty insect-fed plants

(15 of each Si treatment) were randomly harvested immediately after insect herbivory, as were 30 insect-free plants (15 of each Si treatment). Plants were oven-dried at 60°C for seven days before measuring shoot dry biomass to assess insect damage on +Si and –Si plants. Eighty other plants (N = 20 of each treatment) were grown for an additional two weeks to elucidate the effects of Si supplementation on plant compensation for herbivory and leaf trichome density.

4.3.3 Measurements of larval behavioural and morphological defences

We measured the escape response of larvae (i.e. flee) in the absence of any stimulus as per Vogelweith et al. (2014). Each larva (N = 20 of each Si treatment) was placed singly on a gridded plastic sheet (86 × 112 cm) and acclimated for 20 s under a glass jar. Following jar removal, the number of squares crossed by a larva within 60 s (minimum time to exit the sheet as observed in the preliminary experiment) was recorded. As no stimulus was involved, some larvae (i.e. seven fed on +Si plants and four fed on −Si plants) did not make any movements and were omitted from the analysis. Following flee measurements, each larva was placed on white filter paper, acclimated for 30 s under a glass jar and then gently pinched three times at 20 s intervals using bracket-placing tweezers at the abdominal end to imitate predator attack (Cornell et al., 1987). The defensive behaviours of larvae were video recorded (Logitech c270 webcam), and three distinct larval behaviours were characterized and logged for each larva: (a) ‘headrearing’ as characterized by the backward movement of the head and the anterior body portion to the posterior part, (b) ‘thrashing’ as characterized by the side-to-side swing movement of the head and the anterior body portion, and (c) ‘regurgitating’ on filter paper (Cornell et al., 1987).

We further measured larval integument resistance and integument thickness following the protocol of Vogelweith et al. (2014) with minor modifications. Larvae were placed at −20°C for 20 min and then thawed for 10 min before starting measurements. Integument resistance was measured using a drill press equipped with a hypodermic needle (Terumo, 0.5 × 16 mm). A precision scale (± 0.1 mg) was positioned on the drill press table, and each larva mounted on a cardboard sheet was placed over the scale. The needle was slowly lowered down by rotating the lever until it breached the dorsal integument. The scale reading (mg) when the needle breached the integument was recorded. Two measurements were taken from each larva, one on the thoracic region and another on the abdomen, and the average value was counted. Larvae were further dissected, and all internal organs and fat bodies were removed before measuring integument thickness using a thickness gauge (Teclock SM-112, Japan, precision ± 0.01 mm) (Iltis et al., 2018).

4.3.4 Measurements of larval immune defences

We measured the innate immunity of larvae (10 larvae fed on +Si plants and 10 larvae fed on −Si plants) in terms of haemocyte density, phenoloxidase (PO) activity, total-PO activity (combined estimate of PO and its precursor, prophenoloxidase), and lysozyme-like enzyme activity in the haemolymph. For this, immunologically naïve (unchallenged) larvae were chilled on ice for 20 min, a proleg was removed from each larva using a sterile micro scissor, and 3 µl of haemolymph was collected immediately using a sterile micropipette. Of this, 2 µl was flushed into a pre-chilled microcentrifuge tube containing 20 µl of cold phosphate-buffered saline (PBS, pH 7.4). Ten microliters of this haemolymph-PBS mixture was spread immediately over a Neubauer Improved Haemocytometer, and haemocytes were counted under a phase-contrast microscope at 400× magnification (Axio

Vert.A1, Zeiss, Australia). We measured the total haemocyte density and the density of individual haemocytes, classified according to Vogelweith et al. (2016) and Ribeiro and Brehélin (2006), into prohaemocytes, granulocytes, plasmatocytes, spherulocytes, and oenocytoids.

The rest of the haemolymph-PBS mixtures were stored at −20°C for the later measurements of PO and total-PO activities following the protocol of Vogelweith et al. (2011). Briefly, samples (N = 10 of each Si treatment) were thawed slowly on ice and centrifuged (3000 g, 4°C) for 15 min. Five microliters of supernatant from each sample was transferred to a microplate well containing 20 µl of PBS with either 140 µl of distilled water for measuring PO activity or 140 µl of chymotrypsin solution (Sigma-Aldrich, 0.98 mg/14 ml of distilled water) for measuring total-PO activity. Finally, 20 µl of L-DOPA solution (Sigma-Aldrich, 8 mg/2 ml of distilled water) was added as a substrate to each microplate well, and the absorbance readings were taken for samples and negative controls at 490 nm using a microplate reader (CLARIOstar Plus, BMG Labtech) for 40 min at 60 s intervals. The enzyme activity was calculated from the slope of the reaction curve (i.e. changes in optical density per min) at the linear phase and is reported as the activity per microliter of pure haemolymph.

An additional 1 µl of pure haemolymph was flushed into a pre-chilled microcentrifuge tube containing 10 µl of reaction buffer (pH 6.24, Sigma-Aldrich) and stored at −20°C for the subsequent measurements of lysozyme-like activity. Lysozyme-like activity was measured by a turbidity assay using *Micrococcus lysodeikticus* (Sigma-Aldrich) as a substrate following the method modified from Adamo et al. (2016). In short, 10 µl of haemolymph-

reaction buffer mixture from each sample was transferred to a microplate well containing 180 µl of a suspension of *M. lysodeikticus* cells (1 mg/10 ml) in reaction buffer (pH 6.24, Sigma-Aldrich). Lysozyme standards (Sigma-Aldrich) within the linear range of assays were run concurrently as positive controls along with the test samples and negative controls, and the kinetic decrease in absorbance was recorded using a microplate reader (CLARIOstar Plus, BMG Labtech) at 450 nm wavelength for 10 min at 50 s intervals. Lysozyme-like activities are presented in units, where one unit represents a 0.001 change in optical density per minute.

Furthermore, we measured the melanisation responses of larvae (10 larvae fed on +Si plants and 10 larvae fed on −Si plants) when immunologically challenged simulating solitary endoparasitoid attack (Moreno-García et al., 2013). For this, a nylon monofilament (0.2 mm diameter) was implanted 2 mm deep in the hemocoel through the dorsal abdominal part of each larva according to Gherlenda et al. (2016). After 24 hrs, we collected 3 µl of haemolymph from each larva and measured haemolymph PO, total-PO, and lysozyme-like activities as per the procedures described previously. The nylon implants were removed and photographed using a stereo microscope (Olympus, SZ61) mounted camera (Infinity*1*, Teledyne Lumenera) along with a control filament (i.e. not implanted in the hemocoel). The photos were processed in ImageJ (National Institutes of Health, Maryland; Version 1.52) and the mean grey values were estimated on a scale of 0 (light) to 255 (dark). The melanisation scores were calculated using the formula: 1 – (mean grey value of the treatment filament/mean grey value of the control filament) (Garvey et al., 2021).

4.3.5 Measurements of leaf trichome density, plant compensation, and Si concentrations

We measured the density of non-glandular trichomes (or macrohairs) on leaves of all 80 plants (N = 20 of each treatment) grown for an additional two weeks following herbivory or no herbivory. Non-glandular trichomes can defend plants against insect herbivores, often more effectively than plant secondary metabolites (Carmona et al., 2011), and herbivory can induce increased trichome density on newly emerged leaves (Dalin et al., 2008). We sampled the newly emerged, fully expanded leaves from plants and counted trichomes on abaxial and adaxial surfaces (4 mm × 4 mm area in the middle) under a stereo microscope (Olympus, SZX10). Plants were subsequently harvested and oven-dried at 60°C for 7 days and shoot and root biomass was recorded.

Plant compensation is defined as the process by which plants mitigate or nullify the damage caused by herbivory (Bardner & Fletcher, 1974), and it can be assessed by comparing the biomass of insect-fed plants with insect-free control plants (Arab & Trigo, 2011; Belsky, 1986; Gavloski & Lamb, 2000). We therefore harvested plants at two-time points for assessing plant compensation and compared at each time point the dry biomass of insect-fed −Si plants with insect-free −Si plants and the dry biomass of insect-fed +Si plants with insect-free +Si plants. The first group of plants was harvested immediately after herbivory (as mentioned in the experimental design) to measure biomass losses of −Si and +Si plants due to insect attack, and the second group of plants was harvested two weeks after herbivory to assess compensation of −Si and +Si plants for herbivory (Arab & Trigo, 2011). Furthermore, the second group of plants was used for Si quantification. The concentrations of total Si (% dry mass) in the leaves and roots of 40 +Si plants (20 insect-fed plants and

20 insect-free plants) were measured. For this, ca. 80 mg of oven-dried, ball-milled samples was analysed using an X-ray fluorescence spectrometer (Epsilon 3^{x}; PANalytical, EA Almelo, The Netherlands) according to Reidinger et al. (2012). Measurements were standardized using certified plant material of known Si concentration (NCS ZC73018 Citrus leaves, China National Institute for Iron and Steel).

4.3.6 Statistical analysis

All data were analysed in the statistical software environment R, version 3.6.1 (R Core Team, 2019). The distributions of dependent and independent variable datasets were compared using quantile-quantile plots, and homogeneity of variance was assessed using 'residuals versus fits' plots. Larval frass and morphological and behavioural defence parameters were analysed using Wilcoxon's rank-sum tests as the assumptions of normality of residuals or homoscedasticity were violated. Larval head capsule width, RGR, haemocyte density, PO, total-PO, lysozyme-like activity, and leaf and root Si concentrations were analysed using Student's *t*-tests. Haemolymph PO activity was compared with total PO for each Si treatment under similar immunological conditions using Student's *t*-tests. The effect of Si on larval integument resistance and PO activity was analysed using multiple linear regressions. The relationships between larval integument resistance and weight gain and final weight were explored using Spearman's correlations. Leaf trichome density and shoot and root biomass were analysed using two-way analysis of variance (ANOVA) tests, considering Si and insect herbivory as fixed factors. When the main or interaction effects of factors were significant in ANOVAs, differences between group means were determined by Tukey's HSD tests.

4.4 Results

4.4.1 Insect growth, feeding, and anti-predator defences

Larval RGR and frass production were significantly lower (−300% and −85%, respectively) when feeding on +Si plants compared to when feeding on –Si plants (Fig. 4-1a and 4-1b; Table 4-1). However, there was no significant difference in larval head capsule width when feeding on –Si plants (2.34 ± 0.09 mm) compared to when feeding on +Si plants (2.15 ± 0.08 mm) (mean ± SE shown) (Table 4-1), suggesting that larvae were of the same instar. None of the larval defensive behaviours (flee, headrear, thrash, and regurgitation) were affected by Si (Table S4-1). Larvae fed on +Si plants had significantly lower integument resistance than larvae fed on –Si plants (Fig. 4-2a; Table 4-1) despite no significant difference in integument thickness (Fig. 4-2b; Table 4-1). Integument resistance was strongly and positively correlated with larval weight gain ($N = 40$, $\rho = 0.83$, $p < 0.001$) and final weight ($N = 40$, $\rho = 0.98$, $p < 0.001$) (Fig. S4-1). Multiple linear regression analysis showed that Si had a significant effect on integument resistance after adjusting for the effect of larval weight gain (Table 4-3). However, Si had no significant effect on integument resistance when the effect of larval final weight was controlled (Table 4-3).

The density of individual and total haemocytes in the haemolymph was unaffected by Si (Fig. S4-2; Table S4-3). Larvae fed on +Si plants had significantly higher PO and total-PO activities in the haemolymph compared to larvae fed on –Si plants under both immunologically naïve (unchallenged) (71% and 86% increase in PO and total PO, respectively) and challenged conditions (33% increase in both PO and total PO) (Fig. 4-3a and 4-3b; Table 4-1). Accordingly, Si had a significant effect on haemolymph PO activity

after controlling for the effect of larval weight gain or final weight (Table 4-3). There were no significant differences between haemolymph PO and total PO activities in larvae fed on −Si or +Si plants under similar immunological conditions (Table S4-2). However, the melanisation responses of larvae fed on +Si or –Si plants were statistically similar (Fig. 4-4; Table 4-1). Lysozyme-like activity in the haemolymph was unaffected by Si, regardless of immunity challenge (Fig. S4-3; Table S4-3).

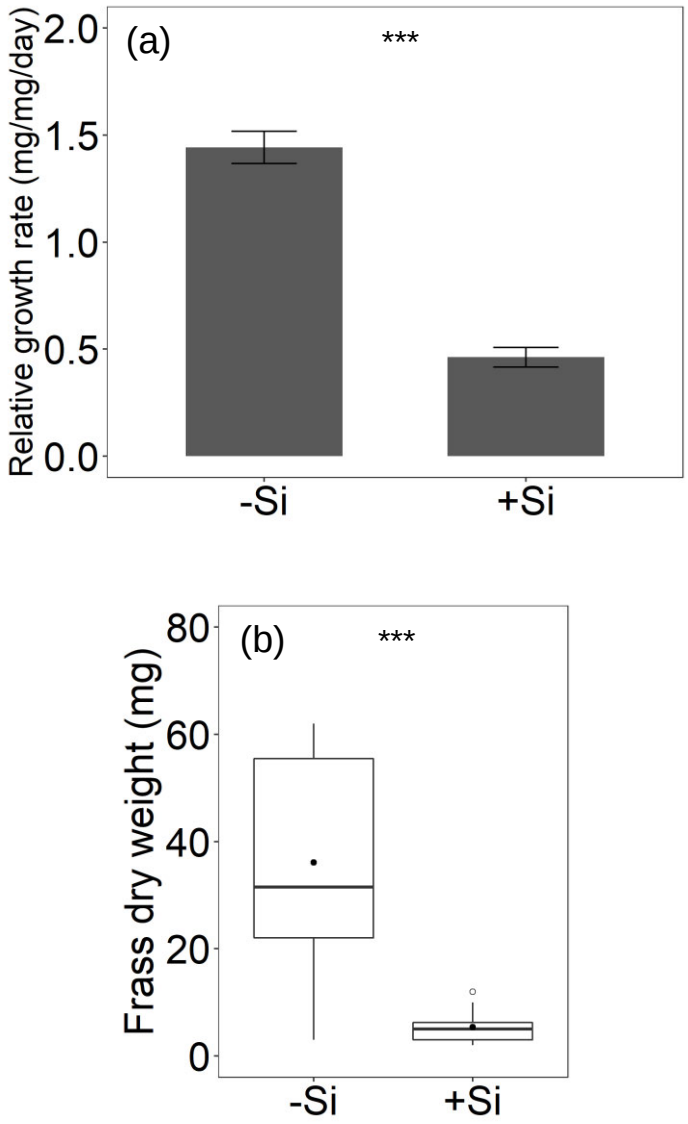

Figure 4-1 (a) Larval relative growth rate (mg/mg/day) and (b) frass dry weight (mg) on –Si and +Si plants. Mean ± SE shown (N = 40). Group means were compared using Student's *t*-tests. Asterisks indicate the level of statistical significance (*** $p < 0.001$) at 95% confidence intervals.

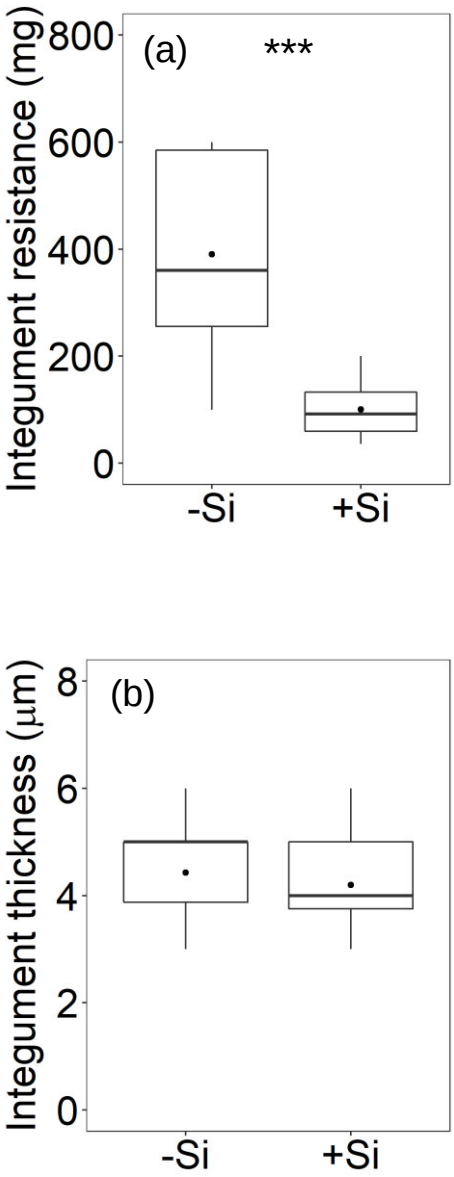

Figure 4-2 (a) Integument resistance (mg) and (b) integument thickness (μm) of larvae fed on –Si and +Si plants. The median and interquartile range (N = 20) are shown along with the mean (black circle). Differences between treatments were determined using Wilcoxon's rank-sum tests. Asterisks indicate the level of statistical significance at a 95% confidence interval (*** $p < 0.001$).

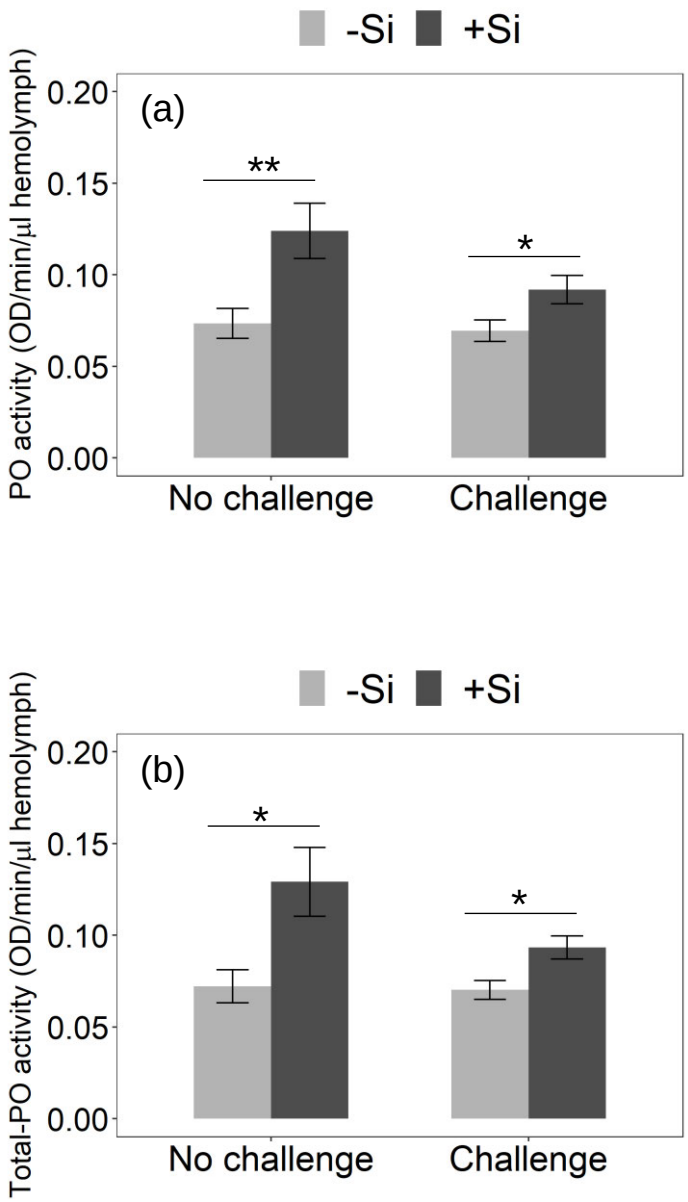

Figure 4-3 The activity of (a) phenoloxidase (PO) and (b) total-PO per microliter of larval haemolymph. Larvae were either challenged by implanting a nylon filament or kept unchallenged following feeding on –Si or +Si plants. Mean ± SE shown (N = 10). Group means were compared using Student's *t*-tests. Asterisks indicate the level of statistical significance (* $p < 0.05$, ** $p < 0.01$) at 95% confidence intervals.

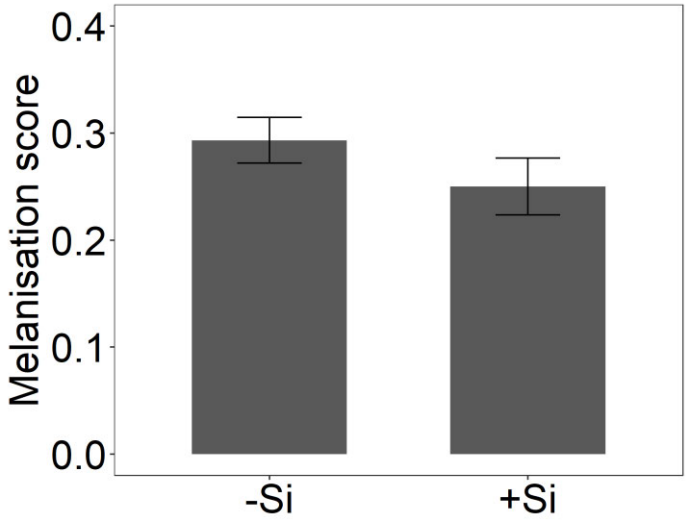

Figure 4-4 Melanisation responses of larvae fed on –Si and +Si plants. Mean ± SE shown (*N* = 10). Group means were compared using a Student's *t*-test.

Table 4-1 Summary output of Student's *t*-tests and Wilcoxon's rank-sum tests for comparing treatment (−Si and +Si) means. Statistically significant effects ($p < 0.05$) are indicated in bold.

Response variable	Fig.	Statistical analysis		
		df	Test statistic (t [a]/W [b])	*p*
Larval RGR [a]	1a	78	11.18	**<0.001**
Frass dry weight [b]	1b	-	381.5	**<0.001**
Larval head capsule width [a]		38	1.49	0.146
Larval morphological defences [b]				
Integument resistance	2a	-	385.5	**<0.001**
Integument thickness	2b	-	227.5	0.442
Immune defences of naïve (unchallenged) larvae [a]				
PO activity	3a	18	−2.94	**0.009**
Total-PO activity	3b	18	−2.74	**0.014**
Immune defences of challenged larvae [a]				
PO activity	3a	18	−2.28	**0.035**
Total-PO activity	3b	18	−2.82	**0.011**
Melanisation score	4	18	1.27	0.222
Si concentrations in plants [a]				
Leaf Si	7	38	−4.85	**<0.001**
Root Si	7	38	−0.06	0.949

[a]Analysed using a Student's *t*-test.

[b]Analysed using a Wilcoxon's rank-sum test.

4.4.2 Leaf trichomes, plant compensatory growth, and silicon concentrations

Silicon in interaction with insect herbivory significantly affected leaf trichome density, whereby +Si plants had higher (+51%) trichome density on abaxial leaf surfaces than −Si plants in the absence of insect herbivory (Fig. 4-5a; Table 4-2). However, trichome density on abaxial leaf surfaces of −Si plants increased by 46% following herbivory and became statistically similar to that of +Si plants. Likewise, trichome density on adaxial leaf surfaces of −Si plants significantly increased (+62%) following herbivory (Fig. 4-5b; Table 4-2). In terms of plant biomass, insect herbivory significantly reduced the shoot biomass of both −Si and +Si plants (−16% and −14%, respectively) harvested immediately after herbivory (Fig. S4-4; Table S4-4). There was a significant interaction between Si and herbivory on the shoot biomass of plants harvested two weeks afterwards, whereby insect-fed +Si plants produced similar shoot biomass to insect-free +Si plants (Fig. 4-6a; Table 4-2), indicating exact plant compensation with the provision of Si. However, root biomass was unaffected by Si or herbivory (Fig. 4-6b; Table 4-2). In addition, insect herbivory significantly increased leaf Si accumulation by ca. 29% but had no effect on root Si concentrations (Fig. 4-7; Table 4-1).

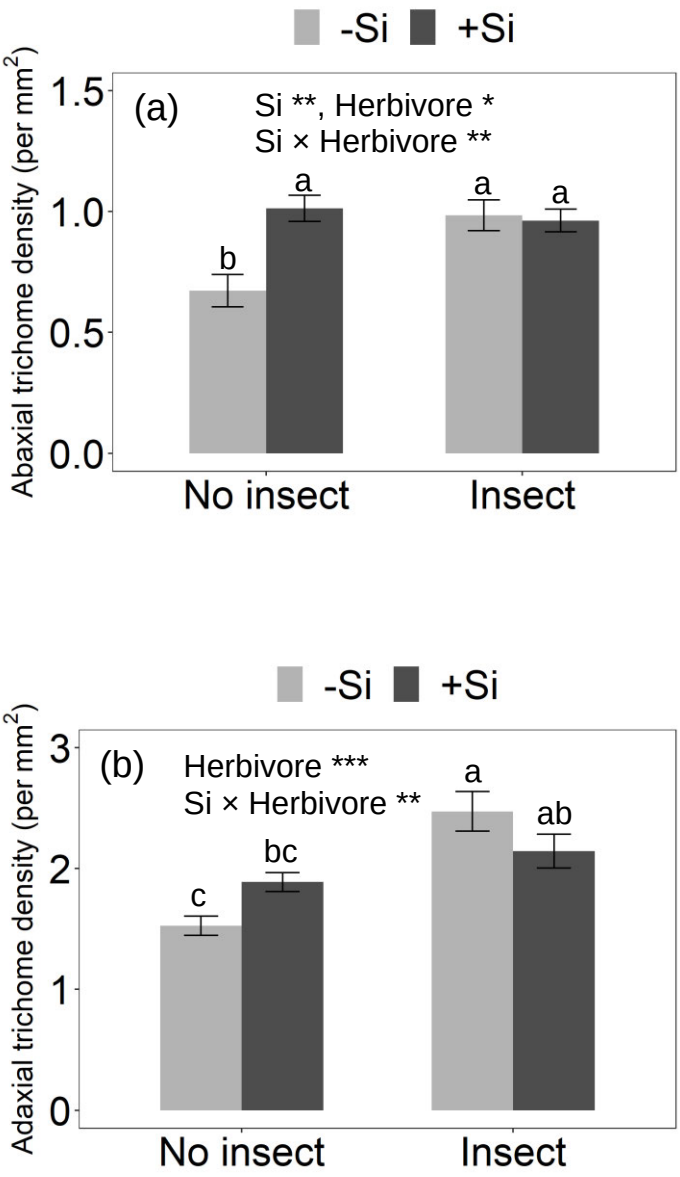

Figure 4-5 Impacts of Si and herbivory on the density of trichomes (number per mm^2) on (a) abaxial and (b) adaxial leaf surfaces. Mean ± SE shown (N = 20). Data were analysed using two-way ANOVA tests and subsequently using Tukey's HSD tests. Asterisks indicate the level of statistical significance (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Different lowercase letters indicate significant differences between means at 95% confidence intervals.

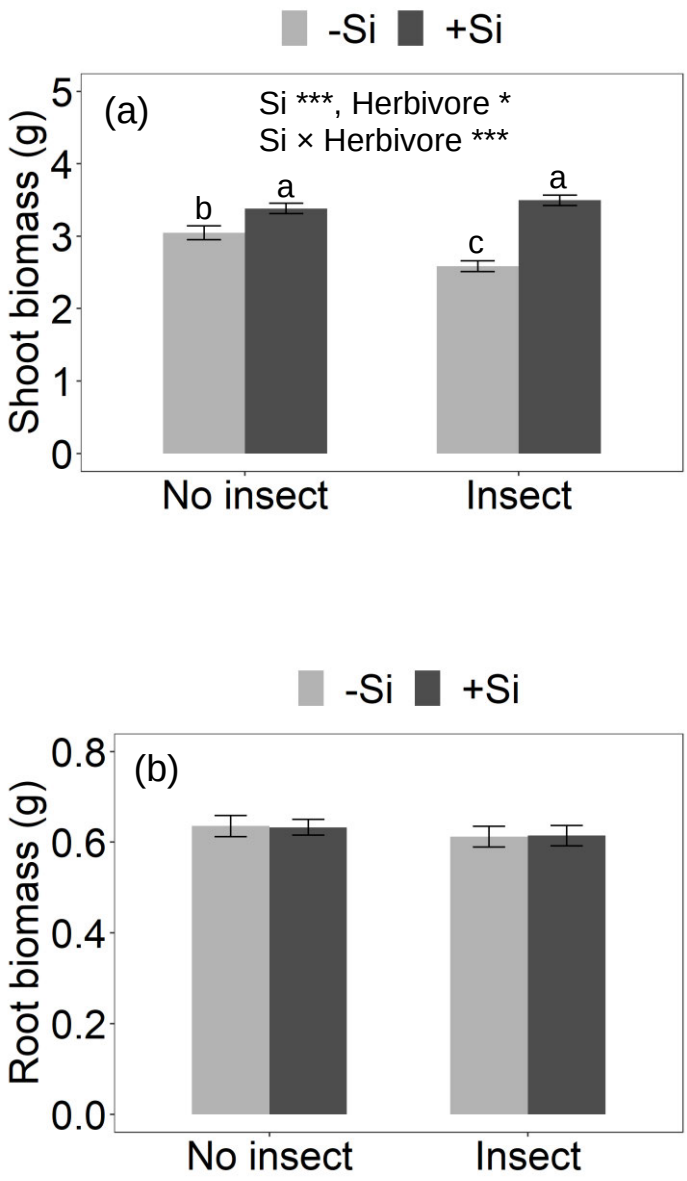

Figure 4-6 Impacts of Si and herbivory on dry (a) shoot biomass and (b) root biomass of plants. Mean ± SE shown (N = 20). Data were analysed using two-way ANOVA tests and further using Tukey's HSD tests. Asterisks indicate the level of statistical significance (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Different lowercase letters indicate significant differences between means at 95% confidence intervals.

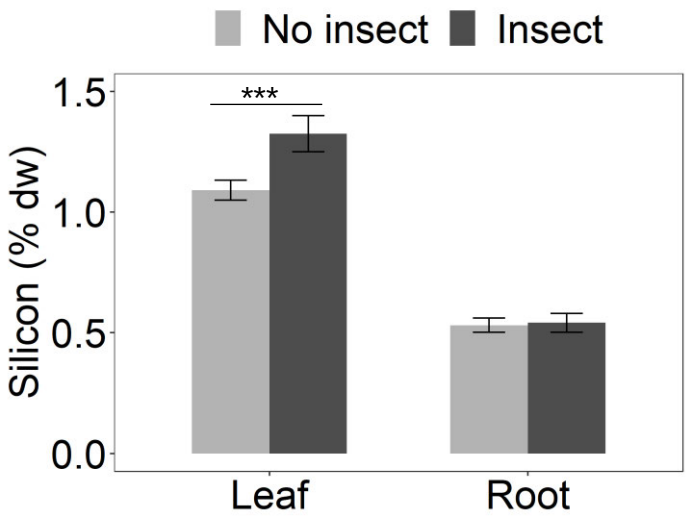

Figure 4-7 Concentrations (% dry weight) of total Si in the leaves and roots of +Si plants in the absence or presence of insect herbivory. Mean ± SE shown (N = 20). Group means were compared using Student's *t*-tests. Asterisks indicate the level of statistical significance at a 95% confidence interval (*** $p < 0.001$).

Table 4-2 Summary output of two-way ANOVA for the effects of Si and insect herbivory on leaf trichome density and plant biomass. Statistically significant effects ($p < 0.05$) are indicated in bold.

Response variable	Fig.	*df*	Si		Herbivore		Si × Herbivore	
			F	*p*	*F*	*p*	*F*	*p*
Abaxial trichome density	5a	1,76	7.49	**0.008**	5.08	**0.027**	9.69	**0.003**
Adaxial trichome density	5b	1,76	0.02	0.888	24.62	**<0.001**	8.11	**0.006**
Shoot biomass	6a	1,76	63.13	**<0.001**	5.00	**0.028**	13.42	**<0.001**
Root biomass	6b	1,76	0.001	0.982	0.95	0.333	0.01	0.908

Table 4-3 Summary output of multiple linear regressions for the effects of Si on larval integument resistance and haemolymph PO activity after controlling for the effect of larval weight gain or final weight. Statistically significant effects ($p < 0.05$) are indicated in bold.

Predictor	Integument resistance					PO activity				
	Adj. R^2	β	t	df	p	Adj. R^2	β	t	df	p
(Regression model 1)										
Weight gain	0.58	0.30	1.37	37	0.179	0.22	−0.11	−0.70	37	0.491
Si		−0.50	−2.25	37	**0.031**		0.46	2.96	37	**0.005**
(Regression model 2)										
Final weight	0.96	0.95	19.50	37	**<0.001**	0.22	−0.12	−0.80	37	0.431
Si		−0.04	−0.87	37	0.393		0.46	3.13	37	**0.003**

4.5 Discussion

We report for the first time, to our knowledge, how Si supplementation of plants impacts the morphological and behavioural defences of an insect herbivore. Silicon reduced larval RGRs and feeding, thereby lowering integument resistance which was associated with compensatory production of immunity proteins in the haemolymph. Moreover, Si supply enhanced constitutive trichome production on leaves and augmented plant compensation for herbivory whereas insect herbivory induced trichome production on plants that had no access to Si.

4.5.1 Silicon weakened larval morphological defence but did not affect defensive behaviours

Larvae fed less on +Si plants (i.e. as evident from the lower levels of frass production) and displayed retarded growth rates, which is in line with the existing literature on the effects of Si against chewing herbivores, including folivores (Biru et al., 2021; Islam et al., 2020; Johnson et al., 2020b), borers (Kvedaras et al., 2009; Nikpay et al., 2015) and root feeders (Frew et al., 2017b). The threefold decrease in larval growth rates observed here due to malnutrition could potentially impact biocontrol by natural enemies. For example, Remmel et al. (2011) estimated that a twofold rise in the linear size of folivorous insect larvae increases avian predation rates by 3.6-fold while reducing arthropod predation rates by 4.9-fold.

We found a strong positive correlation between larval final weight and integument resistance ($N = 40$, $\rho = 0.98$, $p < 0.001$). In accordance with this, Iltis et al. (2018) reported a positive correlation between larval body size and integument resistance of the

lepidopteran grape pest, *Lobesia botrana*, despite no changes in integument thickness. Although it is not evident how larger larvae showed higher integument resistance despite similar integument thickness, we speculate that there might be some variations in the integument ultrastructures of larvae fed on –Si plants compared to larvae fed on +Si plants, including the extent of sclerotization and tanning. This finding suggests that feeding on +Si plants could make larvae more vulnerable to parasitoid attack, as parasitoid oviposition is often more successful in smaller larvae with lower integument resistance (Beckage & Riddiford, 1978; Gross, 1993). We observed consistent defensive behaviours of *H. armigera* larvae, irrespective of lower leaf consumption and growth rates. This result concurs with the findings of Zhou et al. (2017), who found that evasive (escape) and aggressive (thrashing and dropping) behaviours of the oriental armyworm, *Mythimna separata*, were unaffected by body size and weight.

4.5.2 Interactions between larval morphological and immune defences

Our results indicate a potential trade-off between insect morphological and immune defences; larvae fed on +Si plants had lower integument resistance but higher haemolymph PO and total-PO activities. Such trade-offs between anti-predator defensive traits have been demonstrated in other lepidopteran (Vogelweith et al., 2014) and hymenopteran (Boevé & Schaffner, 2003) larvae, including trade-offs caused by variations in larval diet (Vogelweith et al., 2011). For instance, in several sawfly species, a lower integument resistance and propensity for bleeding have been linked to a higher haemolymph deterrence against predators such as ants and wasps (Boevé & Schaffner, 2003). Our results differ from the previous study by Frew et al. (2017b), who found no effects of Si supplementation of sugarcane on PO activity in the root-feeding grub, *Dermolepida albohirtum*.

Even though the underlying mechanisms of how Si enhanced PO activity in the haemolymph are not clear, previous research has shown that consumption of plant toxins (e.g. nicotine) or starvation could be immunotherapeutic for insects and could enhance PO activity (Garvey et al., 2021; Yang et al., 2007). We suggest that Si accumulation in plants forced larvae into starvation and consumption of silicified tissues and trichomes may have caused larval gut damage and exerted physiological stress on them, contributing to higher haemolymph PO activities. This high level of PO may impose fitness costs on larvae, as the production and maintenance of PO is very costly (González-Santoyo & Córdoba-Aguilar, 2012) and resource allocation to PO could constrain resources for other physiological functions, including larval development (Cotter et al., 2008) and the expression of sexual traits (Siva–Jothy, 2000).

4.5.3 High PO activity did not enhance melanisation

We found no differences between haemolymph PO and total-PO activities in larvae under similar immunological conditions (challenge or no challenge), which could be because naturally activated PO denotes total-PO activity in the haemolymph of larvae (Vogelweith et al., 2011). Thus, the addition of chymotrypsin as an artificial PO activator did not enhance total-PO activity. Notably, high PO activity in the haemolymph of larvae fed on +Si plants did not enhance their melanisation responses when larvae were immunologically challenged. In lepidopteran larvae, circulating haemocytes, namely, oenocytoids, produce prophenoloxidase (proPO) zymogen, which is further activated into PO following proteolytic cleavage (Kanost & Gorman, 2008). Although high PO activity often positively impacts encapsulation and melanisation (González-Santoyo & Córdoba-Aguilar, 2012; Shiao et al., 2001), some previous studies have found no such positive associations (Piñera

et al., 2013; Wilson-Rich et al., 2008). Since PO functions against pathogens and parasitoids via melanogenesis (i.e. a process whereby PO oxidises phenols to quinones, which further polymerise to melanin) (Eleftherianos & Revenis, 2011), our finding suggests that high PO activity does not necessarily imply high immune defences in larvae; rather, it could signify physiological stress (González-Santoyo & Córdoba-Aguilar, 2012).

The possible explanations for similar melanisation responses in larvae are twofold. First, melanisation depends on both PO activity and haemocyte density, as invaders need to be first coated (i.e. encapsulation) by adhesive haemocyte cells (Lavine & Strand, 2002). Given that the density of haemocytes in larvae fed on +Si or –Si plants was similar, larvae may have produced an identical encapsulation of the nylon implants. Second, both the activation of PO and the production of melanin (a nitrogen-rich compound) require substantial investments of nitrogen (González-Santoyo & Córdoba-Aguilar, 2012). Hence, high PO activity in the haemolymph can limit nitrogen for melanogenesis in larvae fed on +Si plants, especially considering the fact that Si and nitrogen accumulation in plants can interact antagonistically (Wu et al., 2017).

4.5.4 Post-attack plant defences

We found that Si increased constitutive trichome density on leaves, which substantiates previous studies on grasses (Biru et al., 2021; Johnson et al., 2021a) and other crops (Acevedo et al., 2021). Interestingly, herbivory induced trichome production only on –Si plants because +Si plants were already better defended with constitutively-produced silicified trichomes, which were found to be essential for defence against chewing herbivores in rice (Andama et al., 2020). We suggest that investment in induced trichome

defence by −Si plants might compromise, in part, their capacity for compensatory growth, as there could be a potential trade-off between plant growth and defence (Herms & Mattson, 1992; but see Koricheva, 2002). Conversely, with the provision of Si, insect-fed plants produced similar biomass to insect-free plants within two weeks of the recovery period, despite initial biomass losses, substantiating the previous finding that Si can support plant compensatory growth (Johnson et al., 2019a).

4.5.5 Conclusions

Our study establishes that Si supplementation of plants can affect the anti-predator defences of insect herbivores. Moreover, Si supply can increase constitutive trichome production on leaves and augment plant compensation for herbivory. We found a potential trade-off between larval morphological and immune defences when fed on siliceous plants; a lower integument resistance was associated with higher PO and total-PO activities in the haemolymph, although it did not enhance the melanisation response of larvae when challenged. We presume that the lower integument resistance of larvae could enhance their vulnerability to some natural enemies and that high PO activity could impose physiological costs, potentially impeding other physiological functions. However, reduced anti-predator defences of larvae do not necessarily guarantee increased biological control by natural enemies, and further research is needed to understand how changes in anti-predator defences of herbivorous insects under Si supplementation impact their susceptibility to natural enemies in realistic settings. Nonetheless, our study provides the first evidence for the plant-mediated effects of Si on anti-predator defences of an insect herbivore and presents a framework for studying the impacts of Si on natural enemies of herbivores via changes in anti-predator defences of host insects.

Chapter 5

Plant silicon defences reduce the performance of a chewing insect herbivore which benefits a contemporaneous sap-feeding insect

---

Published as (Islam et al., 2022a), *Ecological Entomology*

5.1 Abstract

Plant-mediated interactions between phytophagous insects are ubiquitous. Silicon (Si) accumulation can defend plants against chewing insect herbivores, although sap-feeders are comparatively less affected. It remains unknown how Si impacts interspecific interactions between chewing and sap-feeding insect herbivores when sharing a host plant. We grew the model grass *Brachypodium distachyon* with (+Si) or without (−Si) Si and assessed the impacts of Si supplementation on the contemporaneous performance and interguild interactions between a chewing (*Helicoverpa armigera*) and a sap-feeding (*Rhopalosiphum padi*) insect herbivore. We further performed dual-choice tests to assess insect preferences for +Si or −Si plants with or without prior insect attack. Si reduced the relative growth rate (RGR) of both separately and contemporaneously fed caterpillars (*H. armigera*). Conversely, aphid abundance was higher on +Si plants compared to −Si plants. Caterpillar RGR and aphid abundance were negatively correlated on shared host plants. Furthermore, decreased caterpillar RGR on +Si plants benefitted aphid colonisation, indicating the plant-mediated effects of Si on interspecific competition between the two insects. Attack by caterpillars induced leaf Si accumulation, regardless of aphid presence. In dual-choice tests, caterpillars preferred aphid-attacked −Si plants to aphid-attacked +Si plants, whereas aphids preferred caterpillar-attacked +Si plants to caterpillar-attacked −Si

plants. Our results provide the first evidence for plant-mediated effects of Si on interspecific competition between two insect herbivores. We suggest that the dissimilar effects of Si against different feeding guilds of herbivores may promote asymmetry in interspecific competition when sharing host plants, potentially impacting insect abundance and distribution.

5.2 Introduction

Interactions between phytophagous insect species can strongly influence their performance and fitness (Kaplan & Denno, 2007). An assessment of 193 pairwise interactions between phytophagous insects reported in previous studies revealed that interspecific competition was far more common (76%) compared to facilitation (i.e. positive interactions) (6%) and non-competitive interactions (18%) (Denno et al., 1995). Insect herbivores can compete directly for limited resources (e.g. food, shelter, reproduction sites); competition can become intense when insects co-occur spatiotemporally and their niches overlap (Denno & Kaplan, 2007; Denno et al., 1995; Kaplan & Denno, 2007). Moreover, herbivores can interact indirectly on shared hosts through induced changes in plant morphology and phenology (e.g. leaf trichome density, leaf flushing) and chemical defences (Denno & Kaplan, 2007; Denno et al., 2000; Lynch et al., 2006; Traw & Dawson, 2002). Notably, changes in plant nutrition can impact interspecific interactions between insect herbivores (Denno & Kaplan, 2007; Redman & Scriber, 2000). For example, ammonium nitrate fertilisation of *Brassica oleracea* plants was found to negate the negative effects of a leaf-chewer (*Plutella xylostella*) on the contemporaneous performance of a phloem-feeder (*Brevicoryne brassicae*) (Staley et al., 2011).

Silicon (Si) is an inorganic nutrient that can provide a range of benefits to plants, from enhanced growth to alleviation of abiotic and biotic stresses including insect herbivory (Cooke & Leishman, 2016; Epstein, 2009; Frew et al., 2018; Reynolds et al., 2009). Plant roots absorb silicon as undissociated orthosilicic acid (H_4SiO_4) from the soil solution, which is further transported through the xylem to shoots and deposited in and around cells as silica gel or phytoliths (SiO_2. nH_2O) (Epstein, 1994; Ma & Yamaji, 2006). In grasses (Poaceae), silicon can constitute up to 10% of dry weight (i.e. hyper-accumulation) and function as the primary anti-herbivore defence, as they generally possess fewer secondary metabolite defences (Moore & Johnson, 2016; Vicari & Bazely, 1993). Silicification can increase tissue hardness and abrasiveness, rendering them less palatable and digestible to mandibulate invertebrate herbivores (Massey & Hartley, 2009). Furthermore, silicified tissues can wear down mandibles of herbivores when feeding (Massey & Hartley, 2009), and silica-rich plant structures (e.g. trichomes) can lacerate insect midguts (Andama et al., 2020). Insect feeding can induce higher Si accumulation in plants (Hartley et al., 2016; Islam et al., 2020). Although Si is primarily considered a physical defence against herbivores, soluble Si can also act as a ligand of organic metabolites and influence plant chemical defences (Epstein, 2009).

Anti-herbivore effects of Si have been demonstrated extensively for chewing insect herbivores including folivores (Biru et al., 2021; Han et al., 2015; Waterman et al., 2021), stem-borers (Hosseini et al., 2012; Nikpay et al., 2015), and root-feeders (Frew et al., 2017b; Frew et al., 2016). However, the significance of Si for sap-feeding herbivores is not clear-cut. For instance, previous studies have reported positive (Johnson et al., 2017), negative (Dias et al., 2014), and neutral effects (Massey et al., 2006; Rowe et al., 2020) of

Si against aphids. Because of their haustellate mouthparts (i.e. retractable and flexible stylets), sap-feeders can largely avoid silica barriers in tissues when feeding (Massey et al., 2006). Thus, sap-feeding herbivores are generally less affected by Si compared to chewing herbivores (Johnson et al., 2021a). Moreover, Si-mediated changes in plant growth (e.g. increased biomass) and chemistry (e.g. higher amino acid concentrations) can facilitate improved performance of phloem-feeding insects such as aphids (Johnson et al., 2017). Taken together, the strong negative effects of Si against chewing herbivores and comparatively moderate to no effects against sap-feeding herbivores could impact their interguild interactions on shared host plants. However, this remains to be tested empirically.

We examined the plant-mediated effects of Si on interguild interactions between a generalist chewing insect, the cotton bollworm, *Helicoverpa armigera*, and a sap-feeding insect, the bird cherry-oat aphid, *Rhopalosiphum padi*, when contemporaneously sharing the host plants (*Brachypodium distachyon*). *Brachypodium distachyon* is a temperate grass with a small and tractable genome and close phylogenetic connections with Triticeae crops and hence is used widely as a model for monocot plants (Scholthof et al., 2018). *Helicoverpa armigera* caterpillars are polyphagous, and depending on crop plants, they can move within and between neighboring plants to find and establish appropriate feeding sites (Zalucki et al., 1986). Larval movements and distribution can be regulated by the place of oviposition and the developmental stage of plants (Pascua & Pascua, 2002). Late-instar caterpillars (third to sixth) may wander less and invest more time on feeding compared to early-instar caterpillars (Gomes et al., 2017; Johnson & Zalucki, 2007). *Rhopalosiphum padi* is dioecious; they spend winter on primary tree hosts (e.g. bird cherry) and summer

on secondary cereal hosts (e.g. oat, barley, wheat) (Finlay & Luck, 2011). On cereals and grasses, *R. padi* usually reproduces parthenogenetically and gives birth to apterous females. However, they can develop alate females and migrate to other crops or plants when plants become overcrowded or plant quality deteriorates (De Barro, 1992). Nymphs and apterous adults are sessile in nature and can stay on the same plants for their lifetime or only disperse to adjacent plants (Honěk et al., 1998).

Plant-mediated interactions between chewing and sap-feeding insect herbivores are widespread, including interactions between caterpillars and aphids (Denno & Kaplan, 2007; Denno et al., 1995; Kaplan & Denno, 2007). We studied the interaction between *H. armigera* and *R. padi* on *B. distachyon* plants as a model of such interguild interactions that may occur in the field and can be mediated by plant Si defences. The two insect species share some common grass hosts (Cunningham & Zalucki, 2014; Finlay & Luck, 2011) and thus may co-occur in the field and experience different Si levels in their host plants depending on soil Si contents, plant ability to accumulate Si and the rate of Si fertilisation in the soil (Guntzer et al., 2011; Hodson et al., 2005). Specifically, our objective was to investigate whether the negative effects of plant Si defences against *H. armigera* benefit the contemporaneous performance of *R. padi*. To examine this, we assessed the impacts of Si supplementation on the performance of *H. armigera* and *R. padi* when feeding separately and concurrently on plants. Furthermore, we conducted dual-choice tests to assess the preference of both insects for Si-supplemented or Si-free plants with or without prior insect attack. We hypothesise that Si diminishes the performance of *H. armigera*, which benefits *R. padi* colonisation on contemporaneously shared host plants.

5.3 Materials and Methods

5.3.1 Plant growth conditions

Brachypodium distachyon seeds (Bd21-3 genotype, INRA-Versailles, France) were germinated in wet perlite and subsequently grown hydroponically in a glasshouse under ambient light, 22/18°C day/night temperatures, and 50% (± 6%) relative humidity. First, seeds were softened by soaking in water for 2 hrs before being dehusked manually and sterilized in bleach. Seeds were subsequently washed in water several times, sown in wet perlite, and kept refrigerated at 4°C for 7 days for cold stratification. Following seed germination, seedlings were grown for another two weeks before being transplanted to non-aerated hydroponic containers. Each hydroponic container comprised two nested plastic cups (480 ml) and a foam disc fitted at the top of cups to hold a seedling as described in Hall et al. (2020b). Hydroponic containers were filled weekly with 370 ml of newly prepared nutrient solution either with (+Si) or without (−Si) Si supplementation. The nutrient solution contained 1 mM KNO_3, 1 mM $Ca(NO_3)_2.4H_2O$, 1 mM KH_2PO_4, 0.6 mM $MgSO_4$, 0.1 mM NaCl, 15 μM H_3BO_3, 0.5 μM $MnCl_2.4H_2O$, 0.7 μM $ZnSO_4.7H_2O$, 0.8 μM $Na_2MoO_4.2H_2O$, 0.8 μM $CuSO_4.5H_2O$, and 0.12 mM NaFe(III)EDTA. Liquid K_2SiO_3 (21% K_2O and 32% SiO_2, Agsil32, PQ Australia, SA, Australia) was added at a concentration of 2 mM SiO_2 equivalent to prepare the +Si solution. The added K^+ ions in +Si solution were balanced in −Si solution by adding KCl, and both solutions were adjusted to pH 5.5 using HCl. Plants were rotated weekly within the glasshouse to minimise any position bias.

5.3.2 Insects

Caterpillars of *Helicoverpa armigera* were provided by CSIRO Agriculture and Food, Narrabri, NSW, Australia and kept on an artificial diet adapted from Teakle and Jensen (1985) until required. *Rhopalosiphum padi* cultures were established on barley, *Hordeum vulgare*, from a single parthenogenetic female obtained from Biological Services, Loxton, SA, Australia.

5.3.3 Performance of caterpillars and aphids (no-choice tests)

Insect performance was assessed when feeding separately and concurrently on plants by estimating caterpillar relative growth rates (RGRs) and aphid abundance. RGR was estimated as mass gained (mg) relative to initial mass (mg) per unit of time (days) (Massey & Hartley, 2009). Plants were grown hydroponically for six weeks before being exposed to insects following previous studies (Biru et al., 2021; Hall et al., 2020a; Islam et al., 2022b). Six-week-old plants were selected for applying insect treatments to ensure the plants' ability to endure caterpillar damage and aphid colonisation for up to 12 days. All plants (N = 80) either with or without insect herbivores were caged in transparent acrylic cylinders with mesh apertures as detailed in (Johnson et al., 2020b). To assess the performance of caterpillars, second instar caterpillars were starved for 24 hrs and weighed before being placed individually on the second youngest fully expanded leaf of each plant (N = 10 plants of each Si treatment). Caterpillars were allowed to feed for 10 days and further starved for 24 hrs for all frass to be expelled before being reweighed to estimate the RGR. For the aphid performance assays, five teneral adult females were placed on the youngest fully expanded leaf of each plant (N = 10 plants of each Si treatment). After 12 days, the numbers of aphid nymphs and adults on plants were counted. Similarly, the

contemporaneous performance of the two insect species was assessed by placing five teneral aphid females on the youngest fully expanded leaf and a single second instar caterpillar on the second youngest fully expanded leaf of each plant (N = 10 plants of each Si treatment). After 12 days, caterpillar RGRs were estimated, and the numbers of aphid nymphs and adults were counted. We placed a single caterpillar on each plant, as caterpillars of *H. armigera* are highly cannibalistic, particularly in later instars (Tang et al., 2016), and cannibalism might obscure plant-mediated interguild interactions between herbivores.

5.3.4 Preference of caterpillars and aphids (dual-choice tests)

Following insect performance assays, the preference of aphids and caterpillars for −Si and +Si plants with or without prior insect attack was assessed by conducting dual-choice tests in rearing cages (BugDorm, 47.5 × 47.5 × 47.5 cm) (Fig. S5-1). To assess aphid preference for insect-attacked plants, six plant pairs (6 −Si and 6 +Si) were randomly chosen from caterpillar-attacked plants (+C). Each plant pair (−Si and +Si) was placed side by side in a rearing cage, and filter paper (Whatman grade 1) was placed on top of two hydroponic containers as a connection (Fig. S5-1). Six teneral adult aphids were placed on each filter paper in a circle and allowed to choose between −Si +C and +Si +C plants. After two hours, the numbers of aphids that settled on −Si and +Si plants were counted in all cages. Likewise, the preference of aphids for insect-free plants was assessed using five plant pairs (5 −Si −C vs. 5 +Si −C). To assess caterpillar preferences, aphid-attacked plant pairs (6 −Si +A and 6 +Si +A) and insect-free plant pairs (5 −Si −A and 5 +Si −A) were used similarly. For each plant pair, four fourth-instar caterpillars reared on the artificial diet were tested. Caterpillars were placed singly on filter paper and allowed 5 min to make a choice.

Caterpillars were considered to make a choice when they climbed a plant and started navigating and feeding. Each caterpillar was tested only once, and none of the caterpillars was found to switch plants within the time frame. Aphids and caterpillars that did not settle on plants within the given time were omitted from the analysis. In the aphid preference assays, 34 out of 36 tested aphids settled on either −Si +C or +Si +C plants and 26 out of 30 tested aphids settled on either −Si −C or +Si −C plants. In the caterpillar preference assays, 23 out of 24 tested caterpillars settled on either −Si +A or +Si +A plants and 18 out of 20 tested caterpillars settled on either −Si −A or +Si −A plants.

5.3.5 Plant harvesting and silicon quantification

All plants were harvested following dual-choice tests and oven-dried at 60°C for 7 days before being weighed for dry shoot and root biomass. Dried leaves of +Si plants were further ball-milled to a fine powder for Si quantification. Concentrations of total Si were measured by analysing *ca.* 80 mg ground material per sample with an X-ray fluorescence spectrometer (Epsilon 3^x; PANalytical, EA Almelo, The Netherlands) following the protocol described in Reidinger et al. (2012). Measurements were standardised against a leaf sample of known Si concentration (NCS ZC73018 Citrus leaves, China National Institute for Iron and Steel).

5.3.6 Statistical analysis

Data analysis was conducted in the statistical software environment R, version 3.6.1 (R Core Team, 2019). Insect performance parameters were compared using Welch's *t*-tests. In the case of contemporaneous feeding, Pearson's correlation was used to explore the relationship between caterpillar RGR and aphid abundance, and multiple linear regressions

were used to predict the effect of Si on the performance of one herbivore while controlling for the effect of another herbivore. Insect preferences in dual-choice tests were analysed using chi-square goodness-of-fit tests. The effects of Si and insect attack, and their interaction, on plant biomass were analysed using two-way analysis of variance (ANOVA) tests. The effect of insect attack on leaf Si concentrations was analysed using a one-way ANOVA. The assumptions of distributions and homogeneity of variance for ANOVA were confirmed using quantile-quantile plots and residuals versus fits plots, respectively. A Tukey's HSD test was used to compare treatment means when the main effect of a factor (i.e. Si or insect attack) was significant in an ANOVA.

5.4 Results

5.4.1 Insect performance and preference tests

Caterpillar RGR was significantly reduced when feeding separately (−53%) or contemporaneously with aphids (−58%) on +Si plants compared to when feeding on −Si plants (Fig. 5-1; Table 5-1). Aphids were more abundant on +Si plants compared to −Si plants irrespective of separate or contemporaneous feeding with caterpillars (Fig. 5-2a and 5-2b; Table 5-1). Total aphid abundance was strongly and negatively correlated with caterpillar RGR on contemporaneously shared host plants (Fig. 5-3), suggesting interspecific competition between the two insect species. Multiple regression analysis showed that in contemporaneous feeding by the two insect species, Si had a significant negative effect on caterpillar RGR after controlling for the effect of aphid abundance (Table 5-2). However, Si had no significant effect on aphid abundance when the effect of caterpillar RGR was controlled (Table 5-2). Both insect species negatively affected the performance of the other when the effect of Si was controlled (Table 5-2). In dual-choice

tests, caterpillars significantly preferred −Si +A plants (78% out of 23 responsive caterpillars) to +Si +A plants (Fig. 5-4; $\chi_1^2 = 7.35$, $p = 0.007$), whereas aphids preferred +Si +C plants (71% out of 34 responsive aphids) to −Si +C plants ($\chi_1^2 = 5.77$, $p = 0.016$). Neither caterpillars nor aphids discriminated, statistically speaking, between −Si and +Si plants when plants were insect-free (Fig. 5-4; $\chi_1^2 = 0.89$, $p = 0.346$ and $\chi_1^2 = 1.39$, $p = 0.239$ for caterpillars and aphids, respectively).

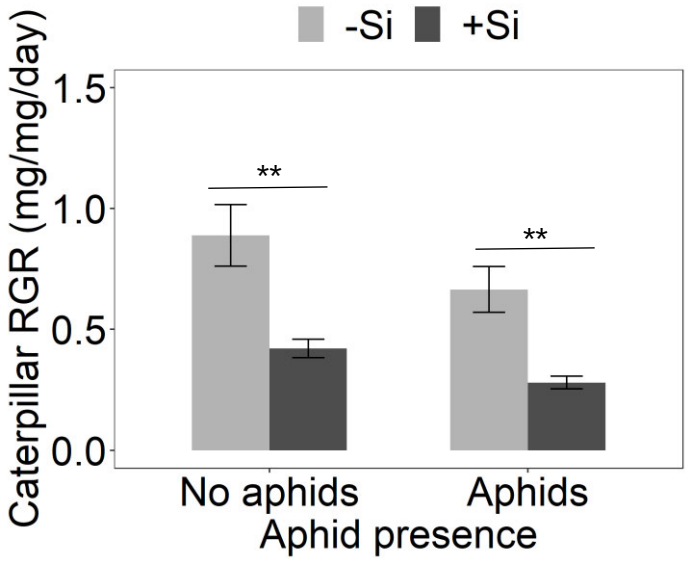

Figure 5-1 Relative growth rates (mg/mg/day) of caterpillars fed on –Si and +Si plants. Mean ± SE shown ($N = 10$). Group means were compared using Welch's *t*-tests. Asterisks indicate the level of statistical significance (** $p < 0.01$) at 95% confidence intervals.

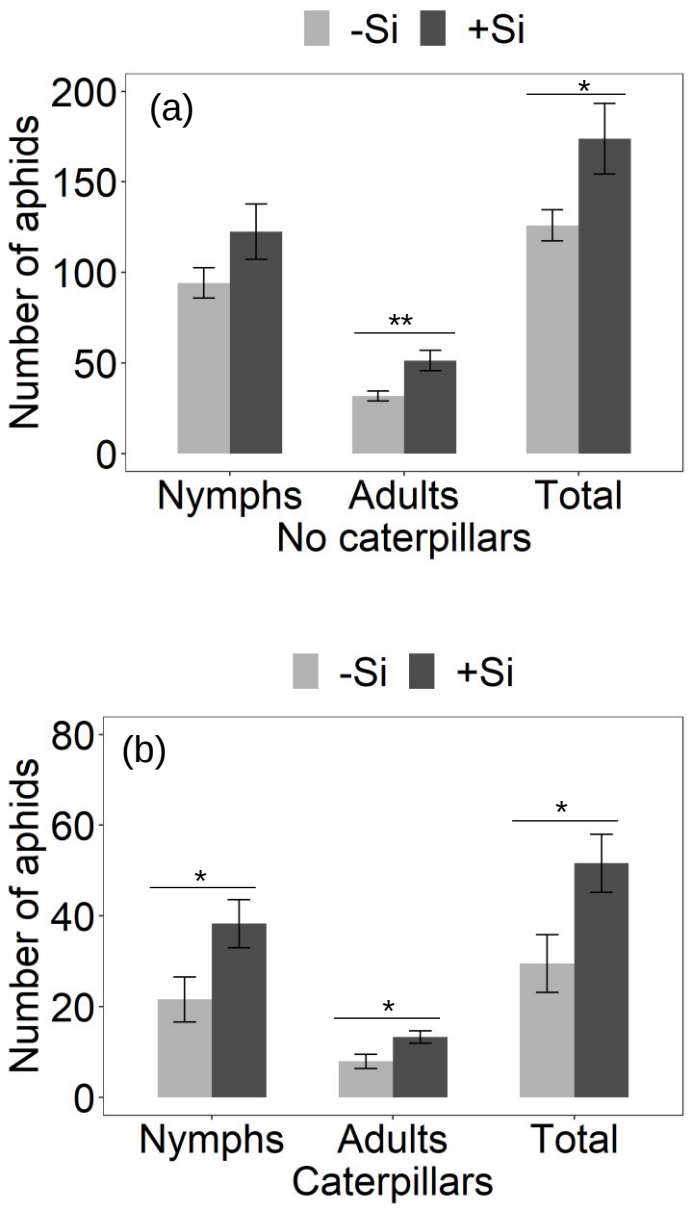

Figure 5-2 Aphid abundances on –Si and +Si plants in (a) absence of caterpillars and (b) presence of caterpillars. Mean ± SE shown (N = 10). Group means were compared using Welch's *t*-tests. Asterisks indicate the level of statistical significance (* $p < 0.05$, ** $p < 0.01$) at 95% confidence intervals.

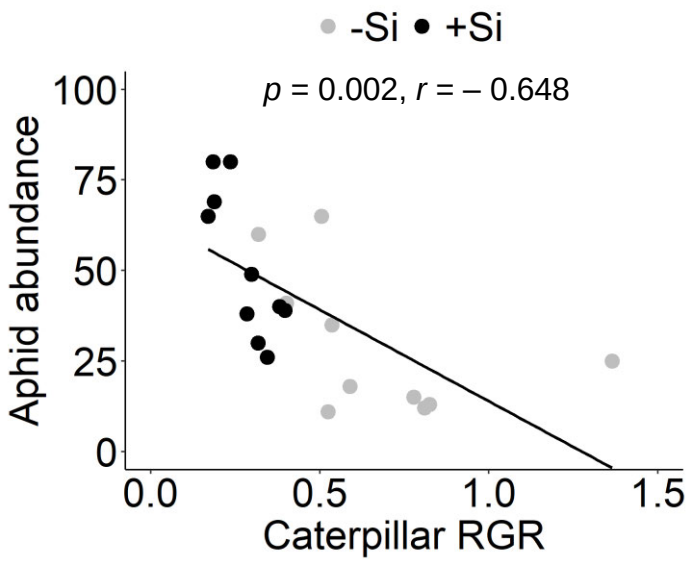

Figure 5-3 The negative correlation between caterpillar RGR and aphid abundance when sharing the host plants.

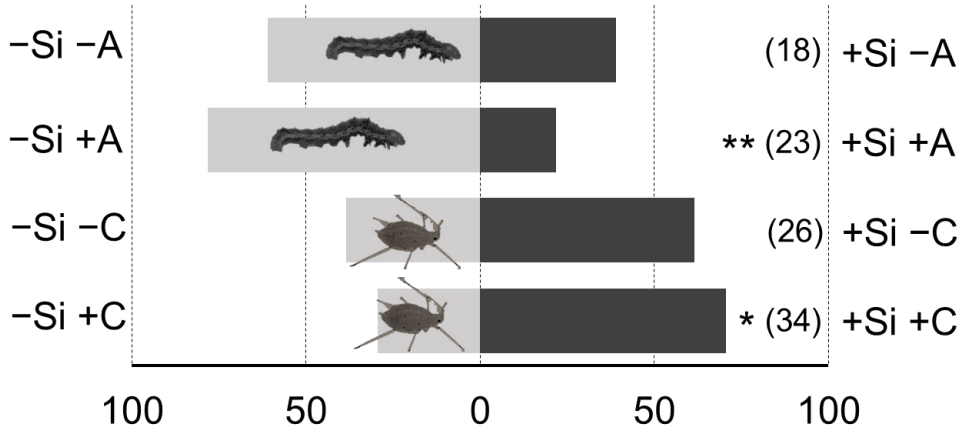

Figure 5-4 Preferences of caterpillars and aphids in dual-choice tests. Values are percentages of insects settled on treatment plants. The total number of insects settled on plants in each comparison is mentioned in parentheses. Asterisks indicate significant differences from a 50:50 distribution (χ^2 goodness-of-fit test, * $p < 0.05$, ** $p < 0.01$). Aphid-free plants (–A), aphid-attacked plants (+A), caterpillar-attacked plants (+C) and caterpillar-free plants (–C).

Table 5-1 Summary output of Welch's *t*-tests for comparing caterpillar RGRs and aphid abundances between −Si and +Si plants. Statistically significant effects ($p < 0.05$) are indicated in bold.

Response variable	Fig.	Statistical analysis		
		df	Test statistic (*t*)	*p*
Separate feeding				
Caterpillar RGR	1	10.68	3.52	**0.005**
Number of aphid nymphs	2a	13.91	−1.64	0.124
Number of aphid adults	2a	13.03	−3.11	**0.008**
Total number of aphids	2a	12.36	−2.25	**0.043**
Concurrent feeding				
Caterpillar RGR	1	10.37	3.92	**0.003**
Number of aphid nymphs	2b	17.92	−2.30	**0.033**
Number of aphid adults	2b	17.71	−2.62	**0.017**
Total number of aphids	2b	18.00	−2.45	**0.025**

Table 5-2 Summary output of multiple linear regression analysis: (a) effect of Si on caterpillar RGR after controlling for the effect of total aphid abundance and (b) effect of Si on total aphid abundance after controlling for the effect of caterpillar RGR. Statistically significant effects ($p < 0.05$) are indicated in bold.

Predictor	Adjusted R^2	β	Test statistic (t)	df	P
(a) Caterpillar RGR					
Si	0.54	−0.47	−2.63	17	**0.018**
Aphid abundance		−0.41	−2.29	17	**0.035**
(b) Aphid abundance					
Si	0.36	0.11	0.44	17	0.663
Caterpillar RGR		−0.57	−2.29	17	**0.035**

5.4.2 Plant biomass and leaf Si concentrations

Overall, Si significantly increased the shoot biomass of plants, whereas insect attack significantly decreased it (Fig. 5-5a; Table 5-3). Specifically, the shoot biomass of +Si plants was significantly higher than −Si plants when plants were fed upon by either caterpillars or caterpillars and aphids. Insect attack significantly reduced the root biomass of plants regardless of Si treatment (Fig. 5-5b; Table 5-3). The root biomass of aphid-attacked and dual herbivore-attacked plants was significantly lower than that of caterpillar-attacked plants (Fig. 5-5b). Insect attack significantly affected leaf Si concentrations, whereby caterpillar feeding alone and concurrently with aphids increased Si concentrations by 93% and 140%, respectively, compared to insect-free +Si plants (Fig. 5-6; Table 5-3). Leaf Si concentration in aphid-attacked plants was statistically similar to that in insect-free plants.

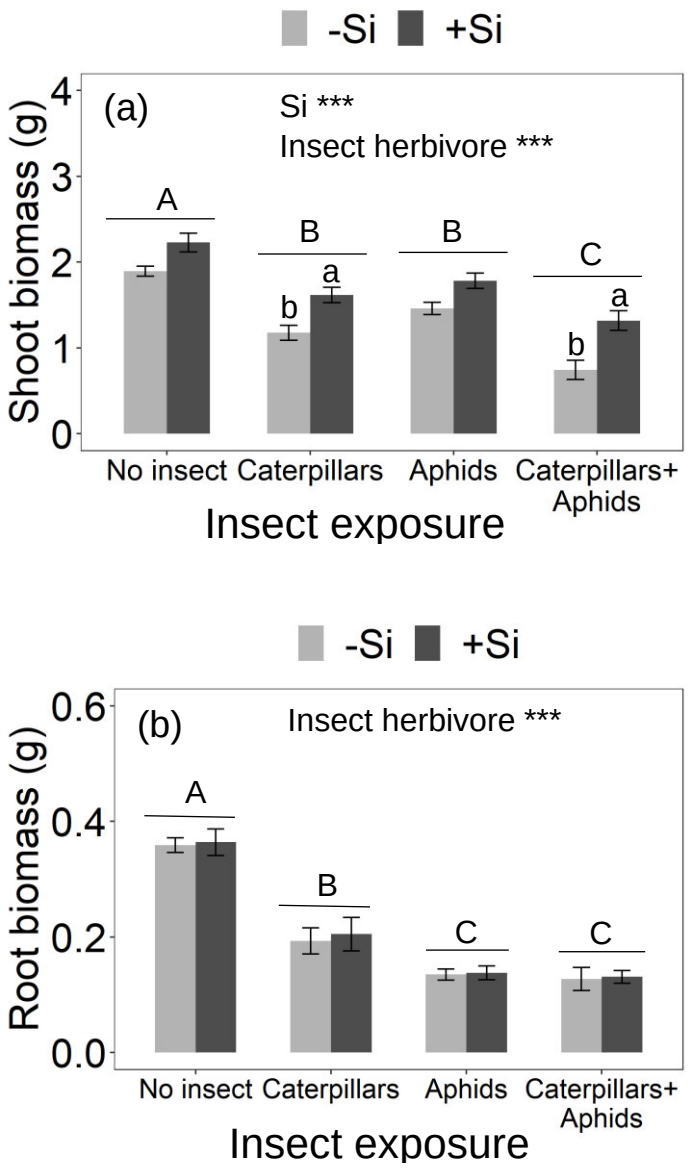

Figure 5-5 Effects of Si and insect herbivore attack on (a) shoot biomass and (b) root biomass of plants. Mean ± SE shown (N = 10). Differences between treatments for each parameter were determined using a two-way ANOVA. Asterisks indicate the level of statistical significance (*** $p < 0.001$) at 95% confidence intervals. A Tukey's HSD test was used to compare treatment means when the main effect of a factor (i.e. Si or insect herbivory) was significant. Different uppercase and lowercase letters indicate significant differences between insect treatments and Si treatments within an insect treatment, respectively.

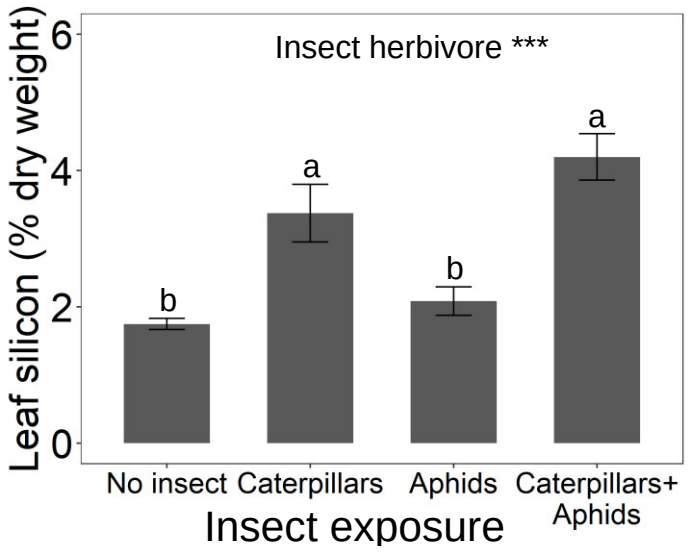

Figure 5-6 Effects of insect herbivore attack on leaf Si concentrations (% dry weight) in +Si plants. Mean ± SE shown (N = 10). Differences between treatments were compared using a one-way ANOVA and a Tukey's HSD test. Asterisks indicate the level of statistical significance (*** $p < 0.001$) at a 95% confidence interval. Different lowercase letters above bars indicate significant differences between treatments.

Table 5-3 Summary output of two-way ANOVA tests for the effects of Si and insect herbivore attack on plant shoot and root biomass and one-way ANOVA for the effect of insect herbivore attack on leaf Si concentrations. Statistically significant effects ($p < 0.05$) are indicated in bold.

Response variable	Si			Insect herbivore			Si × Insect herbivore		
	df	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
Shoot biomass	1,72	40.02	**<0.001**	3,72	42.76	**<0.001**	3,72	0.80	0.50
Root biomass	1,72	0.21	0.65	3,72	67.01	**<0.001**	3,72	0.02	0.99
Leaf Si	-	-	-	3,36	15.10	**<0.001**	-	-	-

5.5 Discussion

We report for the first time, to our knowledge, plant-mediated effects of Si on interspecific competition between a chewing and a sap-feeding insect herbivore, whereby the reduced performance of the chewing insect *H. armigera* on Si-supplemented plants benefitted the performance of the contemporaneous sap-feeding insect *R. padi*. Si fertilisation of plants decreased the RGR of *H. armigera* caterpillars irrespective of separate or concurrent feeding. Conversely, *R. padi* was more successful at colonising Si-supplemented plants compared to Si-free plants.

The strong negative correlation between caterpillar RGR and aphid abundance on contemporaneously shared host plants indicates substantial interspecific competition between the two insect species. This result is consistent with previous studies showing competitive interactions between chewing and sap-feeding insect herbivores when sharing a host plant (Inbar et al., 1999; Simelane, 2006; Staley et al., 2011; Tomlin & Sears, 1992). Si had a strong negative effect on caterpillar RGR regardless of competition or solitary feeding, supporting the extensive body of literature that Si is a potent physical defence against mandibulate insect herbivores (Frew et al., 2018; Reynolds et al., 2009; 2016). However, aphid abundance was higher on +Si plants compared to −Si plants, which differs from several previous studies. The increased performance of caterpillars and aphids on −Si and +Si plants, respectively, was consistent with their preferences for insect-fed, −Si and +Si plants in dual-choice tests. Aphids can use visual (e.g. plant size, shape and colour) and olfactory cues (e.g. herbivore-induced plant volatiles) for locating suitable host plants (i.e. +Si plants in this case, as they were less damaged) (Döring, 2014; Gish & Inbar, 2006). It is not clear how caterpillars chose their hosts, however. Given that the preference of

caterpillars was recorded after observing each for 5 min, we speculate that some caterpillars may reject their host plants following a few feeding bouts and may switch to another plant.

Although it is not evident how Si enhanced aphid abundance, Johnson et al. (2017) reported that Si increased the biomass of the legume *Medicago sativa*, which was associated with a higher abundance of the pea aphid *Acyrthosiphon pisum*. Likewise, increased vegetative growth of plants either because of mineral fertilisation (Honěk, 1991; Hosseini et al., 2010) or genotypic variation (Rousselin et al., 2018) has been linked to a higher aphid abundance. Hence, it is possible that aphid colonisation benefitted from the increased growth and biomass of +Si plants, as it might provide expanded niches for aphid colonisation. Nonetheless, when interspecific competition was involved, aphid abundance was negatively impacted by caterpillar RGR (as evident from the multiple linear regression). This suggests that aphid colonisation benefitted from the diminished performance of caterpillars on +Si plants. Reduced caterpillar RGR on +Si plants implies less feeding damage on plants, which may have facilitated aphid colonisation by enhancing access to optimal feeding and reproduction sites. This may have reduced exploitative competition between the two insect species and between aphids, contributing to aphid colonisation success. Furthermore, increased Si accumulation in plants upon caterpillar damage could reduce salicylate concentrations (Waterman et al., 2020), which may favour aphid colonisation, as the salicylate signalling pathway primarily regulates plant chemical defences against sap-feeding insects (Erb et al., 2012).

Feeding by caterpillars either separately or concurrently with aphids induced Si accumulation in plants, substantiating the results of previous studies that Si induction is

caused by chewing but not sap-feeding insect herbivores (Hartley et al., 2016; Islam et al., 2020; Johnson et al., 2020a; Johnson et al., 2020b). Furthermore, caterpillar-attacked plants (i.e. those attacked by caterpillars either with or without aphids) had higher shoot biomass when they had access to Si supply. This result concurs with the current literature that Si can promote plant growth and ameliorate herbivory stress (Bakhat et al., 2018; Epstein, 2009). Si is thought to be an energetically cheaper alternative to carbon-based structural components such as lignin (Cooke & Leishman, 2011; Epstein, 1994). Hence, Si fertilisation can liberate additional carbon for plant growth. Moreover, Si can support plant erectness and enhance light interception on leaves, which can increase the rate of photosynthesis (Epstein, 1994).

In conclusion, our results show that Si can influence plant-mediated, interspecific competition between insect herbivores when competition involves herbivores from two different feeding guilds, of which one is more susceptible to Si defences than the other. Si diminished the performance of *H. armigera* while promoting aphid colonisation. Further work on the impacts of Si on interactions between different feeding guilds of herbivores incorporating natural enemies would be valuable, as Si can impact the attraction of natural enemies (Islam et al., 2022c; Liu et al., 2017) and tri-trophic interactions (Hall et al., 2021). Nonetheless, our results suggest that Si fertilisation of plants may promote asymmetry in interspecific competition between chewing and sap-feeding herbivores by suppressing the performance of chewers, which in turn can augment the performance of sap-feeders.

Chapter 6

Plant silicon disables cryptic colouration of an insect herbivore by reducing its ability to sequester carotenoids

---

6.1 Abstract

Cryptic colouration in herbivorous insects is an effective strategy for evading their natural enemies; it generally requires sequestration of carotenoids derived from plant tissues in the insect haemolymph. Carotenoids and chlorophyll pigments can alleviate oxidative stress in plants imposed by insect attack. For grasses, silicon (Si) accumulation is the main deterrent to herbivory, but it is unknown whether Si defences inhibit carotenoid sequestration and cryptic colouration in insects and whether Si impacts pigment levels in plants upon insect attack. Using the model grass *Brachypodium distachyon*, we demonstrated that the global insect cotton bollworm (*Helicoverpa armigera*) exhibited lower relative growth rates (RGRs) and brown to black colouration when feeding on Si-supplemented (+Si) plants, whereas larvae feeding on Si-free plants (−Si) exhibited higher RGRs and green cryptic colouration. Larvae sequestered lower levels of lutein (−53%) and total carotenoids (−44%) in the haemolymph when feeding on +Si plants than when feeding on −Si plants. Regardless of plant Si status, larvae partly excreted carotenoid and chlorophyll pigments. Si reduced carotenoid and chlorophyll contents in *B. distachyon* leaves in the absence of insect attack. Insect attack subsequently induced pigment synthesis and Si accumulation in +Si plants. We provide novel evidence that Si fertilisation can disable insect cryptic colouration by inhibiting carotenoid sequestration in the haemolymph. Potential reduction

in the degree of crypsis can make insects more detectable to visually hunting predators (e.g. birds, spiders) and enhance their likelihood of being attacked and killed.

6.2 Introduction

Silicon (Si) accumulation is primarily considered a physical defence in plants and it can alleviate a range of biotic and abiotic stresses (Epstein, 2001). Grasses, such as cereals, are hyper-accumulators of Si (i.e. accumulate up to 10% of their shoot dry weight) and are predominantly reliant on Si defences to combat herbivore attack (Hodson et al., 2005; O'Reagain & Mentis, 1989; Vicari & Bazely, 1993). Si is taken up by plant roots as aqueous orthosilicic acid and is deposited as hydrated silica or phytoliths throughout the plants, including the leaf epidermis, trichomes, and spines (Ma & Yamaji, 2006). Silicification makes plant tissue abrasive and harder, making plants non-preferred hosts for chewing herbivores (Epstein, 2009; Reynolds et al., 2009). Chewing insects when feeding on siliceous plants can suffer from mandibular wear (Massey & Hartley, 2009; Waterman et al., 2021), gut damage (Adamo et al., 2016), and malnutrition (Islam et al., 2020; Johnson et al., 2021a) and can exhibit compromised anti-predator defences (Islam et al., 2022b). Si can also influence plant chemical defences, including the production of secondary metabolites and antioxidant defence enzymes (Epstein, 2009; Frew et al., 2018) and the release of herbivore-induced plant volatiles (HIPVs) (Islam et al., 2022c). Therefore, Si supplementation of plants can attract a greater number of natural enemies (e.g. predators, parasitoids) of herbivores by altering HIPV emission (Islam et al., 2022c; Liu et al., 2017).

Natural enemies of insects such as predators and parasitoids can locate prey by exploiting optical (e.g. prey colour, shape, size) and chemical cues (e.g. HIPVs, prey odours, pheromones) (Dicke, 2009; Harmon et al., 1998; Vet & Dicke, 1992; Vosteen et al., 2016).

Many vertebrate (e.g. birds, amphibians) and invertebrate (e.g. spiders, ground beetles, ants) predators primarily depend on their sight for hunting prey insects. In counteradaptation, insect herbivores often exhibit cryptic (i.e. background matching) or aposematic (i.e. warning) colouration to avoid predation (Greeney et al., 2012; Gross, 1993). Crypsis can reduce the probability of being detected and killed by predators reliant on vision, particularly when insects exhibit crypsis against a uniform background (Lyytinen, 2001).

Carotenoids are lipophilic isoprenoid molecules (C_{40}) that play essential roles in plant colouration, photosynthesis, and photoprotection (Cazzonelli, 2011). They provide unique colouration to plants (yellow to red) and together with chlorophyll (i.e. green pigments) harvest light energy for photosynthesis and safeguard plants from oxidative stress, including those caused by insect herbivory (Pérez-Gálvez et al., 2020; Schaefer & Rolshausen, 2006). Plant leaves can contain several carotenoids, of which, violaxanthin, β-carotene, lutein, and neoxanthin usually occur in the highest concentrations (Heath et al., 2012). Like plants, carotenoids play many crucial functions in insects including the cryptic colouration of insect bodies and eggs, precursors for visual pigments, induction of diapause, and protection from light and oxidative stresses (Ahmad, 1992; Heath et al., 2012). Most insects, however, cannot synthesise their own carotenoids (Heath et al., 2012).

Insect herbivores generally obtain carotenoids from diet (Heath et al., 2012), except a few insects, such as aphids, that have acquired carotenoid biosynthesis genes from microbial symbionts and thus can produce their own carotenoids (Moran & Jarvik, 2010). Lepidopteran larvae, for example, produce green cryptic colouration by sequestering

dietary carotenoids (mostly lutein and β-carotene) in the haemolymph (Hackman, 1952; Heath et al., 2012). The green haemolymph is often termed insectoverdin, which is a mixture of yellow carotenoids and blue bile pigments (e.g. pterobilins, mesobiliverdin) of larvae (Hackman, 1952). This colouration allows larvae, particularly in final instars, to blend into the green plant background and avoid predation (Czeczuga, 1986; Nguyen et al., 2019; Welch et al., 2017). The inability of larvae to sequester carotenoids or the minimum threshold of carotenoids from host plants can disable their green colouration (Nguyen et al., 2019; Valadon et al., 1974; Welch et al., 2017).

Given that chewing insect herbivores feed less on Si-supplemented plants and silicification reduces the digestibility of ingested tissues (Massey & Hartley, 2009), insects may acquire fewer carotenoids when feeding on Si-supplemented plants, which could inhibit their cryptic colouration. This remains to be demonstrated empirically, however. Furthermore, both Si and plant pigments are inducible defences and insect attack can trigger increased Si accumulation and pigment biosynthesis in plants. It remains unknown whether Si supply impacts the levels of carotenoids and chlorophyll pigments upon insect attack. This is of interest, as these pigments function as non-enzymatic antioxidants and there are reports that Si can enhance the activity of enzymatic antioxidants including superoxide dismutase (SOD), catalase, and peroxidase and thus can improve the scavenging of reactive oxygen species (ROS) in plants upon insect attack (Han et al., 2016; Yang et al., 2017).

Using *Brachypodium distachyon*, a model grass (Girin et al., 2014), we investigated the plant-mediated effects of Si on carotenoid sequestration and cryptic colouration of a global chewing insect, the cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae).

Helicoverpa armigera larvae often exhibit colour polyphenism when feeding on different host plants and diets (Chakravarty et al., 2020; Yamasaki et al., 2009). Larvae usually prefer to feed on plant reproductive structures (i.e. flowers and fruits) but can also feed on plant leaves (Rogers & Brier, 2010; Zalucki et al., 1986). Our objectives were (i) to investigate the effects of Si on carotenoid sequestration in the haemolymph of *H. armigera* larvae and the exhibition of larval cryptic colouration; and (ii) to elucidate the effects of Si and insect attack on the levels of carotenoid and chlorophyll pigments in *B. distachyon* leaves. We hypothesise that larvae feed less on Si-supplemented plants and thus sequester decreased levels of carotenoids in the haemolymph, which disables their green cryptic colouration. We grew plants hydroponically with (+Si) or without Si (−Si) and allowed *H. armigera* larvae to feed on plants for 10 days. Subsequently, the contrast between larvae and their green leaf backgrounds was estimated; the levels of carotenoid and chlorophyll pigment were quantified in leaves, insect haemolymph, and frass.

6.3 Materials and Methods

6.3.1 Plant growth conditions and insect herbivore

Brachypodium distachyon seeds (accession Bd21-3, INRAE, Paris, France) were germinated in irrigated perlite as per the procedures described previously (Hall et al., 2020b). Briefly, seeds were soaked in water and dehusked manually using forceps before being sterilised in bleach. Subsequently, seeds were washed in water several times and sown in wet perlite medium. Seeds were then cold stratified at 4°C for seven days and grown for another two weeks to obtain uniform seedlings. Seedlings were transplanted to non-aerated hydroponic containers, each consisting of two nested plastic cups (480 ml) and a fitted foam disc at the top to anchor a seedling, as described in Hall et al. (2020b). Cups

were filled weekly with 370 ml fresh nutrient solution either with or without Si supplementation. The nutrient recipe has been detailed in Hall et al. (2020b).

Plants were provided with Si by adding liquid K_2SiO_3 (containing 21% K_2O and 32% SiO_2, Agsil32, PQ Australia, SA, Australia) to the nutrient solution at 2 mM concentrations (SiO_2 equivalent). To balance the added K^+ ions in +Si solution, KCl (Sigma-Aldrich, MO, USA) was added to −Si solution, and both solutions were adjusted to pH 5.5 using HCl. All plants (N = 60) were grown under natural lighting in a glasshouse at 22/18°C (day/night) temperatures and 50% (± 6%) relative humidity. Plants were rotated weekly inside the glasshouse to curtail position bias. *Helicoverpa armigera* larvae were obtained from CSIRO Agriculture and Food, Narrabri, NSW, Australia. Larvae were kept on an artificial diet adapted from Teakle and Jensen (1985).

6.3.2 Herbivore treatment and sample collection for pigment analysis

Plants were assigned for herbivore treatment (i.e. insect or insect-free) after five weeks of growth in hydroponic containers. Forty plants (20 +Si and 20 −Si) received a single *H. armigera* second instar larva, and another 20 plants remained insect-free. Larvae were starved for 24 hr and weighed before being placed on plants. All plants were caged in transparent acrylic cylinders with mesh apertures, as described previously (Johnson et al., 2020b). After 10 days, larvae were removed from plants, starved singly in Petri dishes for 24 hr to allow frass to be discharged, and weighed. The relative growth rate (RGR) of larvae was calculated as mass gain (mg) relative to initial mass (mg) per day (Massey & Hartley, 2009). Frass expelled by larvae over the last 48 hrs was collected from hydroponic containers and corresponding Petri dishes. Frass of 2-3 larvae was combined for each Si treatment and 25-30 mg of frass per sample (N = 6 of each Si treatment) was collected and

stored at −80°C for pigment analysis.

Twenty larvae (10 fed on −Si plants and 10 fed on +Si plants) were randomly photographed using a stereo microscope (Zeiss AX10) attached camera (Zeiss AxioCam MRc) by placing individually over a leaf from matching plants. Photos were processed in ImageJ to estimate the contrast of larvae against their green leaf backgrounds (Welch et al., 2017). The RGB values of larvae and their leaf backgrounds were quantified, and the colour contrast was calculated as (1 − larval RGB/leaf RGB), whereby high contrast values indicate greater colour contrast between larvae and their green leaf backgrounds. All larvae fed on plants along with 20 larvae (5^{th} instar) fed on the artificial diet were subsequently placed at −20°C for 5 min to restrict movements, and a proleg was removed from each larva for haemolymph collection.

Twenty microliters of haemolymph was collected using a sterile micropipette from 2-3 larvae combined and flushed immediately into a microcentrifuge tube containing 20 µl of cold phosphate-buffered saline (pH 7.4). Haemolymph samples (N = 6-9 of each treatment) were then stored at −80°C until further analysis. To assess the impacts of Si and insect attack on the levels of plant pigments, the youngest fully expanded leaves of eight insect-free (N = 4 of each Si treatment) and eight insect-attacked plants (N = 4 of each Si treatment) were harvested. Leaves were immediately snap-frozen in liquid nitrogen and stored at −80°C until further analyses were performed.

6.3.3 Pigment extraction and quantification

Pigments in leaf and frass samples were extracted as per the procedures described in Dhami et al. (2018) and Alagoz et al. (2020). Briefly, 25-40 mg of leaf tissue or 25-30 mg of frass

per sample was placed in a microcentrifuge tube and ball-milled using a tissue lysing system (Qiagen, TissueLyser II). Five hundred microliters of extraction buffer (6:4 v/v acetone:ethyl acetate with 0.1% butylated hydroxytoluene) was added to ground leaf tissue or larval frass and vortexed for one minute. Subsequently, 500 µl of water (Milli-Q) was added to the mixture for phase separation and centrifuged (15000 rpm, 4°C) for 5 min. The pigment-rich, upper ethyl acetate phase (~250 µl) was collected in a separate microcentrifuge tube and centrifuged again for 5 min before collecting the final ca. 150 µl of the upper ethyl acetate phase. Pigments in haemolymph samples were extracted similarly except the initial ball-milling step was omitted.

Extracts were stored at 4°C until analysis by an HPLC system (Agilent 1260 Infinity, California, USA) equipped with a diode array detector following the protocols described in Alagoz et al. (2020). In short, extracts (20 µl per sample) were injected and run through a C18 HPLC column (GraceSmart, 4.6 mm × 250 mm column, 4 µm size). Pigment separations were performed using three mobile phase solvents (i.e. 9:1:0.01 v/v/v acetonitrile:water:triethylamine, 100% ethyl acetate, and 100% acetonitrile). The retention times of pigments relative to synthetic standards and pigment absorption spectra at 440 nm were considered for identifying chlorophyll and carotenoid pigments. Finally, pigment contents were calculated from the standard curve based upon peak area and expressed as micrograms per gram of sample fresh weight for leaf and frass samples and as micrograms per millilitre of sample volume for haemolymph samples.

6.3.4 Si quantification

Concentrations of total leaf Si (% dry weight) were quantified in 20 +Si plants (10 insect-fed and 10 insect-free plants) as per Reidinger et al. (2012). Plants were harvested and oven-dried at 60°C for seven days, and leaves were ball milled to a fine powder. Approximately 80 mg of ground leaf tissue per sample was analysed with an X-ray fluorescence spectrometer (Epsilon 3^x; PANalytical, EA Almelo, The Netherlands). Spectrometer readings were standardised against a citrus leaf sample of known Si concentration (NCS ZC73018 Citrus leaves, China National Institute for Iron and Steel).

6.3.5 Statistical analysis

Statistical software environment R (version 3.6.1) (R Core Team, 2019) was used to analyse all data. The effects of Si and insect attack and their interactions on carotenoid and chlorophyll contents in leaf tissues were analysed using two-way ANOVA tests with post-hoc Tukey's HSD tests. For ANOVA tests, QQ-plots were used to confirm the assumptions of distributions, and residual versus fits plots were used to confirm the homogeneity of variance. Haemolymph samples of artificial diet-fed larvae did not contain any pigments and hence were omitted from the analysis. All other parameters were compared using Welch's *t*-tests.

6.4 Results

Si significantly reduced larval RGR (−63%) (Fig. 6-1; Table 6-1) while significantly increasing larval contrast (+310%) against green leaf backgrounds (Fig. 6-2; Table 6-1). Larvae fed on −Si plants exhibited green cryptic colouration, whereas larvae fed on +Si plants exhibited brown to blackish colouration (Fig. 6-2). Si in interaction with insect attack significantly impacted the contents of individual carotenoids (i.e. neoxanthin, violaxanthin, lutein, and β-carotene), total carotenoids, and chlorophyll pigments (i.e. chlorophyll a, chlorophyll b, and total chlorophyll) in *B. distachyon* leaves (Fig. 6-3a and 6-3b; Table 6-2). Specifically, insect-free −Si plants had higher contents of violaxanthin, lutein, β-carotene, total carotenoids, and chlorophyll in leaves compared to insect-free +Si plants. Subsequently, insect attack caused a minor non-significant reduction in the contents of carotenoid and chlorophyll pigments in the leaves of −Si plants while significantly increasing the contents of all pigments except neoxanthin in the leaves of +Si plants (Fig. 6-3a and 6-3b; Table 6-2). Precisely, insect attack increased the total carotenoid and total chlorophyll contents by 64% and 68%, respectively, in the leaves of +Si plants (Fig. 6-3a and 6-3b). Likewise, insect attack significantly increased leaf Si concentrations (+87%) in +Si plants (Fig. 6-4; Table 6-1), suggesting induction of Si defences by insect herbivory.

When feeding on +Si plants, larvae sequestered significantly lower contents of lutein (−53%) and total carotenoids (−44%) and a significantly higher content of β-carotene (+58%) in the haemolymph compared to when feeding on −Si plants (Fig. 6-5; Table 6-1). Larvae fed on the artificial diet did not sequester any carotenoids in the haemolymph. Chlorophyll pigments were generally absent in the haemolymph, except for a few larvae (20%) that sequestered a negligible content of chlorophyll a when feeding on −Si plants

(Fig. 6-5; Table 6-1). All larvae that fed on plants partially excreted carotenoid and chlorophyll pigments irrespective of the Si status of their host plants. Larvae fed on +Si plants had lower contents of β-carotene (−63%) and chlorophyll b (−87%) in the frass compared to larvae fed on −Si plants, although the contents of total carotenoids and total chlorophyll in the frass were unaffected by Si (Fig. 6-6a and 6-6b; Table 6-1).

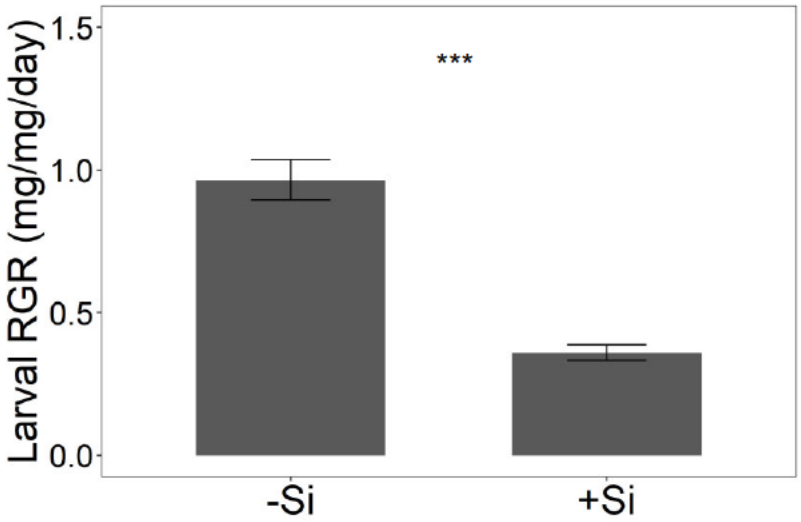

Figure 6-1 Relative growth rate (mg/mg/day) of larvae fed on –Si and +Si plants. Mean ± SE shown (N = 20). Treatment means were compared using a Welch's t-test. Asterisks indicate the level of statistical significance (*** $p < 0.001$) at a 95% confidence interval.

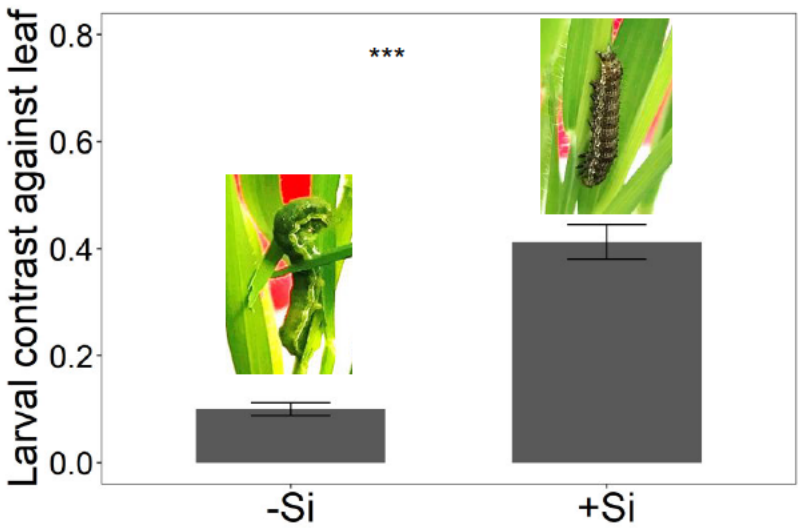

Figure 6-2 Colour contrast of larvae against their green leaf backgrounds. Mean ± SE shown ($N = 10$). Treatment means were compared using a Welch's *t*-test. Asterisks indicate the level of statistical significance (** $p < 0.01$) at a 95% confidence interval. Inset photos showing colour polyphenism of larvae. Larvae exhibited green cryptic colouration when feeding on −Si plants and brown to blackish colouration when feeding on +Si plants.

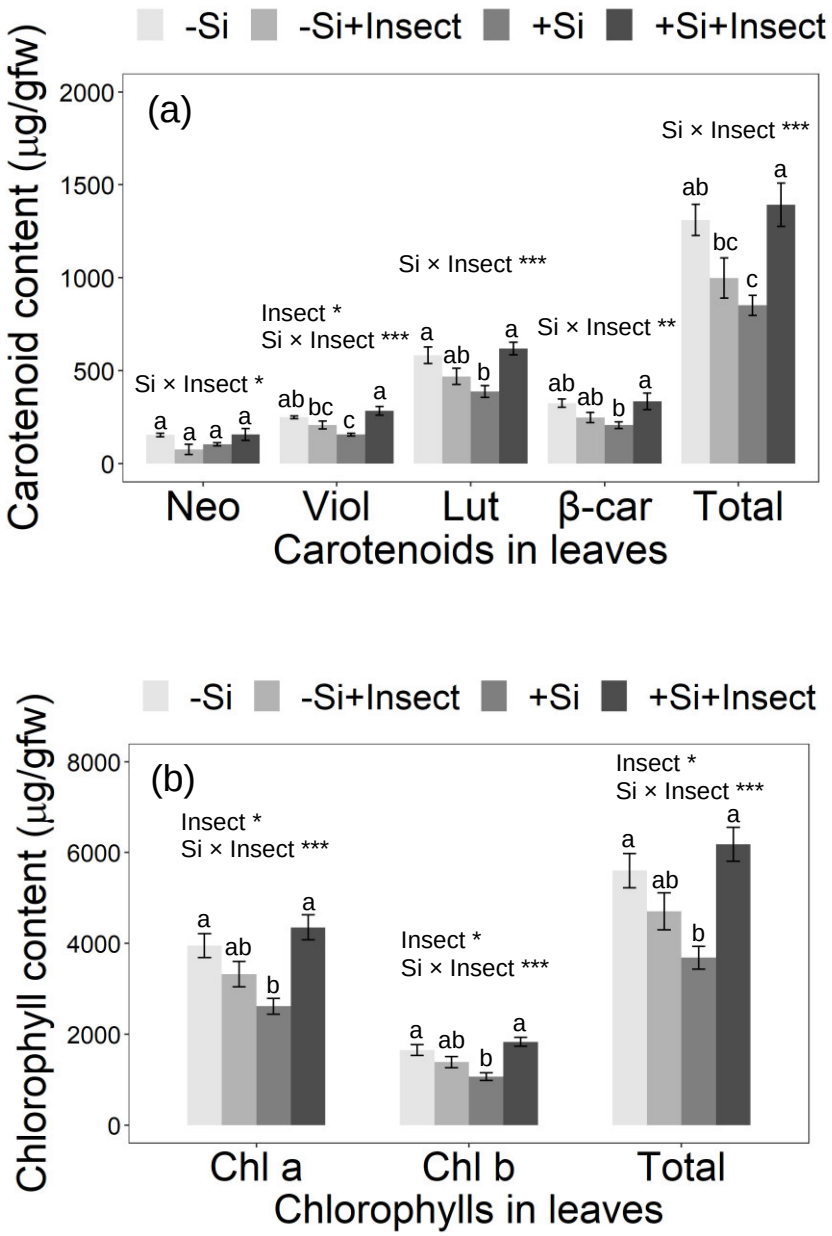

Figure 6-3 (a) Carotenoid and (b) chlorophyll contents in leaves of –Si and +Si plants in the presence and absence of insect attack. Mean ± SE shown (N = 4). Differences between treatments were determined using two-way ANOVAs with post-hoc Tukey's HSD tests. Asterisks indicate the level of statistical significance (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) at 95% confidence intervals. Neoxanthin (Neo), violaxanthin (Viol), lutein (Lut), β-carotene (β-car), chlorophyll a (Chl a), and chlorophyll b (Chl b).

Figure 6-4 Si concentrations (% dry weight) in the leaves of +Si, insect-fed and insect-free plants. Mean ± SE shown ($N = 10$). Differences between treatment means were determined using a Welch's *t*-test. Asterisks indicate the level of statistical significance (** $p < 0.01$) at a 95% confidence interval.

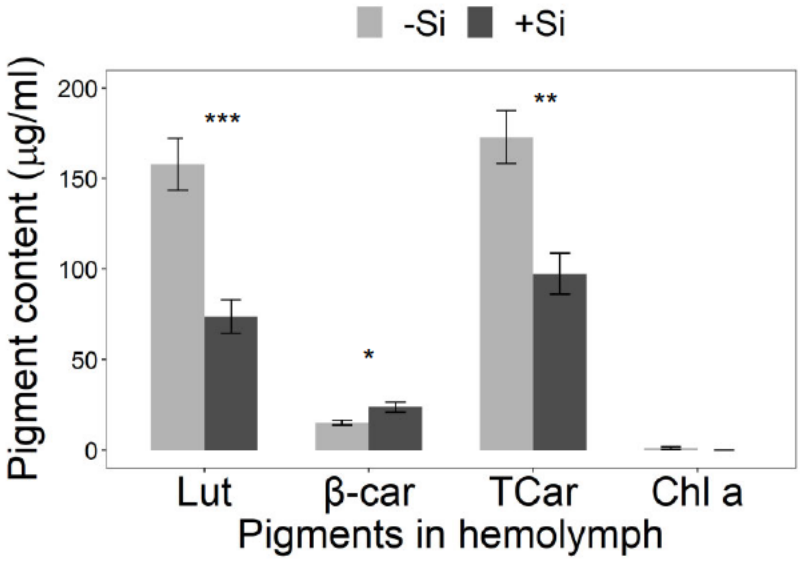

Figure 6-5 Pigment contents in the haemolymph of larvae fed on –Si and +Si plants. Mean ± SE shown ($N = 6$-9). Treatments were compared using Welch's *t*-tests. Asterisks indicate the level of statistical significance (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) at 95% confidence intervals. Lutein (Lut), β-carotene (β-car), total carotenoids (TCar), and chlorophyll a (Chl a).

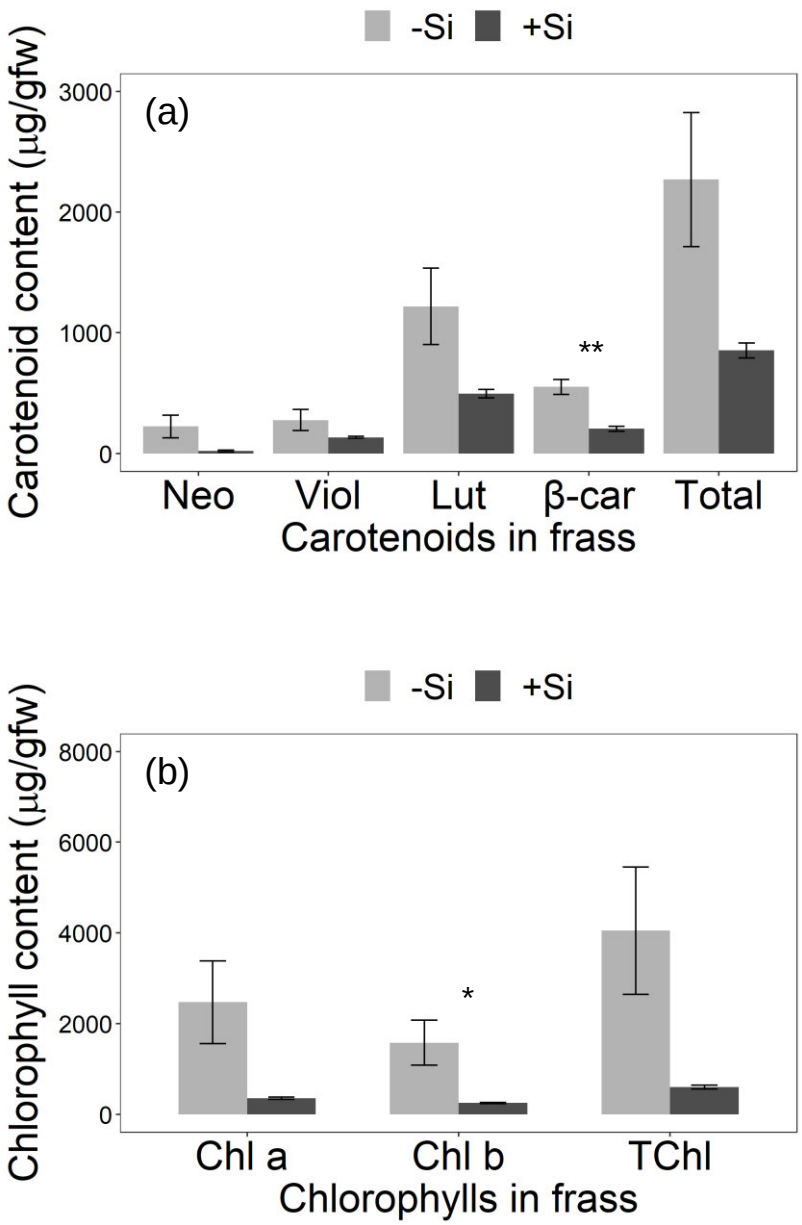

Figure 6-6 (a) Carotenoid and (b) chlorophyll contents in frass of larvae fed on –Si and +Si plants. Mean ± SE shown (N = 6). Differences between treatments were determined using Welch's *t*-tests. Asterisks indicate the level of statistical significance (** $p < 0.01$) at 95% confidence intervals. Neoxanthin (Neo), violaxanthin (Viol), lutein (Lut), β-carotene (β-car), chlorophyll a (Chl a), and chlorophyll b (Chl b).

Table 6-1 Summary output of Welch's *t*-tests for comparing treatment means. Statistically significant effects ($p < 0.05$) are indicated in bold.

Response variable	Fig.	Statistical analysis		
		df	Test statistic (*t*)	*p*
Larval RGR	1	24.73	8.05	**<0.001**
Larval contrast against leaves	2	11.63	−9.01	**<0.001**
Leaf Si	4	11.48	4.11	**0.002**
Pigments in the haemolymph				
Lutein	5	12.53	4.95	**<0.001**
β-carotene		7.71	−2.84	**0.023**
Total carotenoids		13.00	4.07	**0.001**
Chl a		8.00	1.51	0.169
Carotenoids in the frass				
Neoxanthin	6a	5.05	2.19	0.080
Violaxanthin		5.06	1.62	0.166
Lutein		5.13	2.27	0.071
β-carotene		6.12	5.32	**0.002**
Total carotenoids		5.13	2.53	0.051
Chlorophyll in frass				
Chl a		5.01	2.33	0.067
Chl b	6b	5.01	2.68	**0.044**
Total chlorophyll		5.01	2.45	0.058

Table 6-2 Summary output of two-way ANOVA tests for the effects of Si and insect herbivore on carotenoid and chlorophyll contents in leaves. Statistically significant effects ($p < 0.05$) are indicated in bold.

Response variable	Fig.	*df*	Si		Insect attack		Si × Insect attack	
			F	*p*	*F*	*p*	*F*	*p*
Carotenoids in leaves								
Neoxanthin	3a	1,12	0.50	0.491	0.31	0.585	8.92	**0.011**
Violaxanthin		1,12	0.28	0.604	6.73	**0.023**	26.78	<**0.001**
Lutein		1,12	0.37	0.553	2.26	0.159	19.92	<**0.001**
β-carotene		1,12	0.27	0.614	0.74	0.407	11.99	**0.005**
Total carotenoids		1,12	0.12	0.738	1.48	0.247	20.70	<**0.001**
Chlorophyll in leaves								
Chl a	3b	1,12	0.37	0.552	4.83	**0.048**	21.97	<**0.001**
Chl b		1,12	0.39	0.544	5.32	**0.040**	22.95	<**0.001**
Total chlorophyll		1,12	0.38	0.548	5.02	**0.045**	22.45	<**0.001**

6.5 Discussion

We provide the first evidence that Si supplementation of plants can disable cryptic colouration of an insect herbivore (*H. armigera*) via decreased carotenoid sequestration in the haemolymph. The content of total carotenoids was almost half in the haemolymph of larvae fed on +Si plants compared to larvae fed on −Si plants. Consequently, larvae fed on −Si plants exhibited green colouration that resembled their green leaf backgrounds, while larvae feeding on +Si plants exhibited brown to black colouration which contrasted markedly against their green leaf backgrounds. Furthermore, Si reduced the levels of carotenoid and chlorophyll pigments in the leaves of *B. distachyon* in the absence of insect attack. Insect attack further induced pigment synthesis and Si accumulation in Si-supplemented plants.

6.5.1 Si diminished insect performance and disabled its cryptic colouration

Si supplementation of plants has been shown to suppress the growth and damage potential of lepidopteran larvae (Andama et al., 2020; Nikpay et al., 2015; Pereira et al., 2021), including *H. armigera* (Biru et al., 2021; Islam et al., 2020; Waterman et al., 2021). Our results show for the first time that Si supplementation not only suppresses the performance of *H. armigera* larvae but also inhibits one of their primary anti-predator defences, cryptic colouration. This suggests that larvae feeding on +Si plants are more likely to be attacked by arthropod (e.g. spiders, ants) and avian predators, which predominantly depend on their sight for hunting (Greeney et al., 2012; Sugiura, 2020). For example, *Trichoplusia ni* larvae that did not sequester any carotenoids in the haemolymph and thus exhibited high colour contrast against a green leaf background were five times more likely to be attacked by the generalist predator, *Podisus maculiventris*, compared to larvae that sequestered carotenoids

and exhibited green cryptic colouration (Welch et al., 2017).

6.5.2 Si impacted pigment sequestration in the insect haemolymph

Although both −Si and +Si plants synthesised four different carotenoids and chlorophyll pigments in leaves, larvae fed on those plants sequestered only lutein and β-carotene in the haemolymph, with the exception that a few larvae sequestered chlorophyll a when feeding on −Si plants. No pigments were present in the haemolymph of larvae fed on the artificial diet, confirming the dietary source of haemolymph pigments. Our results substantiate the previous findings that carotenoids are the main haemolymph pigments in lepidopteran larvae and are responsible for larval green cryptic colouration (Hackman, 1952; Nguyen et al., 2019; Welch et al., 2017).

Lutein was the dominant carotenoid in the haemolymph of larvae irrespective of their host plants, which is consistent with the previous body of literature (Cromartie, 1959; Hackman, 1952). In particular, Eichenseer et al. (2002) found that *Helicoverpa zea* larvae accumulated high levels of lutein compared to other carotenoids, regardless of their feeding on different host plants. We found that Si supplementation of plants reduced lutein sequestration by more than half in larval haemolymph. Even though larvae fed on +Si plants sequestered a higher content of β-carotene than larvae fed on −Si plants, the total carotenoid content in the haemolymph was still 44% lower compared to their −Si counterparts. Considering that both β-carotene and lutein can combine with larval bile pigments to develop green colouration (Cromartie, 1959; Hackman, 1952), we suggest that there could be a minimum threshold for lutein or total carotenoid contents in the haemolymph for exhibiting green colouration of larvae.

6.5.3 Possible mechanisms underlying the reduced carotenoid sequestration in larvae

Larvae fed on +Si plants could fail to accumulate the minimum threshold of carotenoids due to their reduced consumption of leaf tissues. In accordance with this, Welch et al. (2017) reported that *Trichoplusia ni* larvae with longer feeding bouts on cabbage leaves contrasted minimally against a green leaf background compared to larvae with shorter feeding bouts. Furthermore, carotenoids in insects can function as antioxidants; they scavenge reactive oxygen species (ROS) that can be generated as metabolic by-products of insect immune defences in response to phytotoxins, UV-radiation, or pathogenic infection (Ahmad, 1992; Cornet et al., 2007; Heath et al., 2012; Shao et al., 2011). Recently, feeding on Si-supplemented plants has been shown to enhance the activity of phenoloxidase, a key enzyme that regulates insect immune responses, in the haemolymph of *H. armigera* larvae (Islam et al., 2022b). Hence, we speculate that larvae fed on +Si plants may partly exploit carotenoids to neutralise the oxidative stress caused by heightened immune responses, which may reduce the levels of stored carotenoids in the haemolymph.

6.5.4 Si induced carotenoid and chlorophyll contents in plants following insect attack

We found that +Si plants contained low levels of carotenoids (i.e. violaxanthin, lutein, and β-carotene) and chlorophyll pigments in leaves compared to those of −Si plants in the absence of insect attack. This may have partially contributed to low carotenoid accumulation in the haemolymph and could be the reason behind the low contents of pigments in the frass of larvae fed on +Si plants. Insect attack, however, induced pigment synthesis in +Si plants while slightly depleting pigment contents in −Si plants. Given the antioxidant function of pigments in plants, our results suggest that the induction of pigment

levels in +Si plants following insect herbivory might better scavenge ROS produced in plants in response to insect damage and thus minimise plant oxidative stress.

6.5.5 Conclusions

Our results provide novel evidence for the effects of Si on insect crypsis. Feeding by *H. armigera* larvae on Si-supplemented plants reduced their RGR and ability to sequester carotenoids in the haemolymph, which in turn disabled their cryptic colouration against a green leaf background. Furthermore, Si supplementation reduced the contents of carotenoid and chlorophyll pigments in *B. distachyon* leaves, whereas insect attack induced pigment contents in +Si plants, potentially to scavenge ROS produced in response to insect damage. Assessing how the disabled cryptic colouration of larvae when feeding on +Si plants impacts their biocontrol by natural enemies is a ripe topic for future research. We suggest that high colour contrast of larvae when feeding on +Si plants would enhance their detectability to, and the likelihood of predation by, visually hunting predators. This would also make predators more efficient biocontrol agents by reducing their search time for prey.

Chapter 7

General Discussion

7.1 Key findings and synthesis

Overall, this work examined some novel aspects of plant Si defences across different Si-accumulating plants against herbivorous pests belonging to different feeding guilds. Specifically, this work investigated (i) the magnitude and locality of Si induction in plants following insect herbivory (chapter 2); (ii) how Si impacts induced direct (chapters 2, 4, 5, and 6) and indirect (chapter 3) plant defences against herbivorous pests; (iii) the plant-mediated effects of Si on the anti-predator defences of insect herbivores (chapters 4 and 6); and (iv) the impacts of Si on plant-mediated interspecific interactions between chewing and sap-feeding insect herbivores (chapter 5).

7.1.1 Effects of Si on induced defences in plants

Induced defences refer to any changes in plant morphology, physiology, or chemistry in response to herbivore attack, which can reduce herbivore performance on, or preference for, plants (Gatehouse, 2002; Karban & Myers, 1989). For example, following herbivore attack, plants can increase the density of physical defence structures such as spines or trichomes on leaves, enhance the production of secondary metabolites, or show compensatory growth for herbivory (i.e. tolerance) (Gatehouse, 2002; War et al., 2012). Si accumulation is primarily considered a constitutive physical defence in plants (Epstein, 1999). However, plants can accumulate increased amounts of Si upon damage by vertebrate and invertebrate herbivores, including insects (Hartley et al., 2016; Massey et

al., 2007; McNaughton & Tarrants, 1983). Induction of Si defences can better defend plants against insect herbivory (Massey et al., 2007); however, the locality of Si induction (i.e. whether induction occurs locally in attacked tissues or systemically in all tissues) was uncertain. Chapter 2 provided novel evidence that Si defences in plants can be induced both locally and systemically in response to insect attack and that the magnitude of such induction can be consistent between plant varieties.

Previous research has shown that the feeding guild of herbivores can be a determining factor for Si induction in plants. For example, damage by folivorous chewing insects was found to induce high Si accumulation in plants (Johnson et al., 2020b; Massey et al., 2007), whereas prior feeding by aphids did not make a difference in the magnitude of Si induction (Johnson et al., 2020a). This thesis demonstrated that Si induction can occur in response to chewing (chapters 2, 4, 5, and 6) and cell-content feeding (chapter 3) herbivores but not in response to sap-feeding insects such as aphids (chapter 5). Chapter 3 is the first to demonstrate Si induction in plants following attack by a cell-content feeding herbivore (*T. urticae*). Moreover, this work confirmed the inducibility of Si defences across different Si-accumulating plants including low (chapter 3), moderate (chapter 2), and high (chapters 4, 5, and 6) Si-accumulators.

Si defences are generally deployed as solid amorphous silica (SiO_2) deposited in the leaf epidermis, hairs, trichomes, and spines (Guntzer et al., 2011; Hartley et al., 2015; Mandlik et al., 2020). Furthermore, Si supply can enhance the constitutive production of plant physical defence structures such as trichomes and spines (Biru et al., 2021; Hartley et al., 2015; Johnson et al., 2021b). Chapter 4 showed that the increased density of constitutively

produced trichomes on Si-supplemented plants impeded further induction of trichome defences following herbivory because plants were already defended against herbivores (chapter 5). This may have aided resource allocation for higher compensatory growth in Si-supplemented plants following herbivory (chapter 5), given that a trade-off may exist between plant growth and defences (Herms & Mattson, 1992).

Plant pigments such as carotenoids and chlorophyll can play defensive roles against herbivorous insects in addition to light energy harvesting (Heath et al., 2012; Schaefer & Rolshausen, 2006). As a matter of fact, some hypotheses proposed that non-green plant colouration evolved as a defence against herbivores (Schaefer & Rolshausen, 2006). Chapter 6 found that the levels of carotenoid and chlorophyll pigments in *B. distachyon* leaves were decreased when plants had access to Si supply. Insect attack, however, induced increased pigment contents in the leaves of these plants. This result suggested that plants invested less in pigment defences when they were defended by silica deposition. Following insect attack, siliceous plants increased pigment levels, possibly to limit the oxidative stress imposed by insect herbivory, as carotenoids and chlorophyll can function as antioxidants in plants (Ahmad, 1992; Pérez-Gálvez et al., 2020).

Herbivore attack can induce indirect defences in plants whereby plants release specialised volatiles known as herbivore-induced plant volatiles (HIPVs) (Dicke, 2009). HIPVs can function as reliable cues for carnivorous natural enemies of herbivores and thus promote natural enemy attraction (Aartsma et al., 2017; Mumm & Dicke, 2010). The positive effects of Si on plant indirect defences against chewing (Liu et al., 2017) and sap-feeding herbivores (de Oliveira et al., 2020) have been reported, although it was untested for cell-

content feeders (Leroy et al., 2019). Chapter 2 within this thesis showed that Si can quantitatively shift the ratio of compounds in volatile blends released from plants in response to attack by *T. urticae* mites that feed on the cell-contents of plants. Moreover, such altered volatile emission from Si-supplemented plants promoted the attraction of predatory mites (chapter 2). This result suggested that Si fertilisation could play a role in the biological control of herbivorous pests by attracting a greater number of natural enemies upon herbivore attack. Therefore, Si fertilisation may be used as a supplement or alternative to commercial volatile attractants of natural enemies.

7.1.2 Effects of Si against different feeding guilds of herbivores

The anti-herbivore effects of Si are well documented for chewing herbivores (Frew et al., 2018; Reynolds et al., 2016), and a range of underlying mechanisms have been revealed including the abrasiveness of plant tissues due to silica deposition (Hartley et al., 2015; Johnson et al., 2019b; Massey & Hartley, 2009), wear of insect mandibles (Massey & Hartley, 2009; Waterman et al., 2021) and gut damage (Andama et al., 2020) when feeding on silicified plant tissues and reduced nutrient assimilation in insects from ingested plant materials (Massey & Hartley, 2009). Compared to chewers, sap-feeding herbivores (e.g. aphids) are less affected by Si, as they could avoid silica structures when feeding using their long, flexible, and retractable stylets (Johnson et al., 2021a; Massey et al., 2006). This thesis substantiated the previous findings that Si reduces the performance of chewing herbivores (chapters 2 and 4-6). Si supplementation of plants was found to diminish the performance of the polyphagous chewing insect, *H. armigera*, when feeding on moderate (*Cucumis sativus*) and high (*Brachypodium distachyon*) Si-accumulating plants (chapters

2 and 4-6). In contrast, Si supply was found to enhance aphid colonisation, potentially by increasing plant growth and shoot biomass (chapter 5).

Compared to chewing and phloem-feeding herbivores, the effect of Si against cell-content feeders remains less explored. Chapter 3 showed that Si supply lowered the damage potential and population growth of a cell-content feeding mite (*T. urticae*) and partially ameliorated plant photosynthetic rates and stomatal conductance following *T. urticae* herbivory. *Tetranychus urticae* mites have stylet-like mouthparts that are adapted for piercing tissues and sucking cell-contents (Bensoussan et al., 2016), which in turn produces chlorotic spots on leaves and impairs plant gas exchange by triggering stomatal closure (de Freitas Bueno et al., 2009; Park & Lee, 2002). Although the feeding modes of *T. urticae* somewhat resemble aphids, *T. urticae* mites primarily feed on mesophyll cells that are in direct contact with the leaf epidermis (Bensoussan et al., 2016). Given that the epidermis is the primary site for silica deposition in leaves (Mandlik et al., 2020), *T. urticae* may encounter more silica bodies when feeding on leaf mesophyll cells compared to aphids, which feed on deep phloem tissues. Furthermore, silica deposition in leaf stomata may partially negate abrupt stomatal closure caused by *T. urticae* herbivory, ameliorating *T. urticae*-induced reductions in leaf gas exchange rates (chapter 3).

7.1.3 Effects of Si on the anti-predator defences of herbivorous insects

Herbivorous insects can show a range of anti-predator defences against their natural enemies before, during, and following attack (Greeney et al., 2012; Ruxton et al., 2005). Insect morphological defences such as cryptic colouration to match the environment and integument resistance can form one of the first lines of defences against predators and

parasitoids, respectively (Greeney et al., 2012; Sugiura, 2020). Insects can exploit defensive behaviours (e.g. biting, thrashing, regurgitating) when attacked, which can deter natural enemies or even kill them (Gross, 1993). Furthermore, the immune defences of insects can determine their vulnerability following attack by parasitoids and pathogens (Moreno-García et al., 2013).

Anti-predator defences of herbivorous insects can be influenced by anti-herbivore defences of their host plants (Garvey et al., 2021; Winde & Wittstock, 2011). Chapter 4 is the first to assess the plant-mediated effects of Si on the anti-predator defences of the chewing insect, *H. armigera*, combining morphological, behavioural, and immune responses. Si decreased the relative growth rate and integument resistance of *H. armigera* larvae while enhancing PO activity in the haemolymph (chapter 4). However, high PO activity did not translate into high melanisation responses when larvae were immunologically challenged, indicating that high PO does not necessarily imply high immune responses; rather, it may indicate larval physiological stress due to feeding on silicified tissues and trichomes (chapter 4). Chapter 6 demonstrated that *H. armigera* larvae were unable to produce green cryptic colouration and blend into their green leaf backgrounds when feeding on Si-supplemented plants because of decreased sequestration of carotenoid contents in their haemolymph. Larvae fed less on silicified tissues and hence sequestered lower contents of lutein and total carotenoids in the haemolymph.

The inability to produce cryptic colouration and lower integument resistance of larvae when feeding on Si-supplemented plants can make larvae more susceptible to some natural enemies, including visual predators and parasitoids. Moreover, high PO activity may

impose fitness costs, as PO is costly to produce and maintain (González-Santoyo & Córdoba-Aguilar, 2012). These results signified that Si defences could play a role in the biological control of pest insects by hindering insect defences against their natural enemies. Furthermore, given that carotenoids also function as antioxidants in insects and most insects must obtain carotenoids from host plants (Ahmad, 1992; Catalan et al., 2012), the reduced accumulation of dietary carotenoids can exacerbate insect immune defences and intensify the effects of plant toxins, UV-radiation, and microbial infections (Cornet et al., 2007; Heath et al., 2012).

7.1.4 Effects of Si on interspecific interactions between insect herbivores

Plant-mediated interactions between herbivorous insects, for example, competition for resources, are widespread, particularly when insect herbivores co-occur spatiotemporally on their host plants (Denno et al., 1995; Kaplan & Denno, 2007). Chapter 5 within this thesis is the first to assess how the dissimilar effects of Si against chewing versus sap-feeding insect herbivores influence their interspecific competition on contemporaneously shared host plants. Results demonstrated that Si supplies reduced the performance of the chewing herbivore, *H. armigera*, which benefitted colonisation by aphids, potentially by reducing exploitative competition for resources between the two herbivores. Furthermore, when allowed to choose between Si-free and Si-supplemented plants, the chewing and sap-feeding insects preferred Si-free and Si-supplemented plants, respectively, which was consistent with their superior performance on these host plants. These results suggested that Si fertilisation of plants can provide a competitive advantage to sap-feeding herbivores by suppressing the performance of co-occurring chewing insects on shared host plants (Staley et al., 2011).

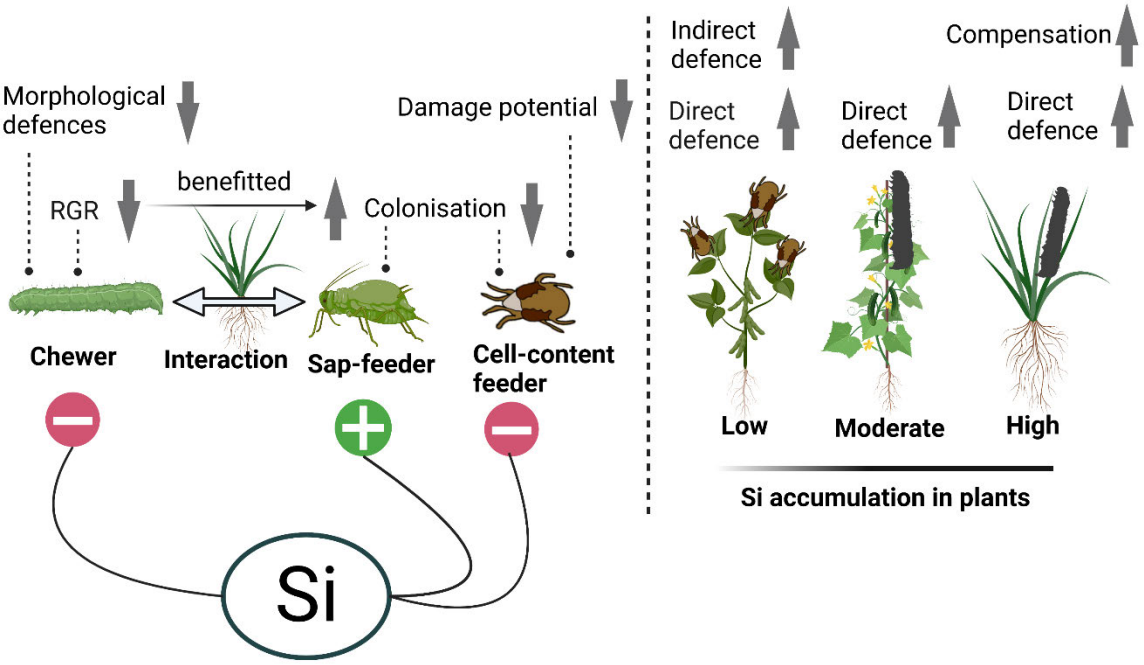

Figure 7-1 Schematic illustrations of the major findings of this work. Si supplementation of plants negatively impacted the performance of a chewing (*Helicoverpa armigera*) and a cell-content feeding (*Tetranychus urticae*) herbivore while enhancing the colonisation success of a sap-feeding aphid (*Rhopalosiphum padi*). Consequently, Si fertilisation impacted plant-mediated interactions between the chewing and sap-feeding insect herbivores on contemporaneously shared host plants. *Helicoverpa armigera* larvae feeding on Si-supplemented plants exhibited diminished morphological defences; larvae were unable to produce green cryptic colouration and showed low integument resistance. Furthermore, Si supplementation enhanced plant direct and indirect defences across different Si-accumulating plants. Upwards and downwards pointing arrows indicate positive and negative effects of Si, respectively, on the measured parameters.

7.2 Constraints, caveats, and future research

All experiments reported in this thesis were performed under controlled temperatures and relative humidity conditions in a glasshouse. Hence, the results of this work may differ in natural settings due to environmental variation or the presence of other biotic and abiotic factors. Plants were grown hydroponically across all experiments to create Si-free and Si-supplemented conditions. Thus, growing plants in the soil may cause variation in results, as plants often develop symbiotic relationships with soil microbiota (e.g. mycorrhizae, rhizobia), which can influence plant defences, including Si accumulation (Oye Anda et al., 2016), against herbivorous insect pests (Gehring & Bennett, 2009; Kempel et al., 2009; Koricheva et al., 2009). For instance, colonisation of plant roots by arbuscular mycorrhizal fungi was found to increase Si accumulation in plants and diminish the performance of an insect herbivore (*Dermolepida albohirtum*) (Frew et al., 2017a). Furthermore, plants were grown in individual hydroponic containers, which provided confined spaces for root development and prevented plant-plant or plant-microbe communication via root exudates or root volatiles (Elhakeem et al., 2018; Rolfe et al., 2019). Therefore, further validation of the results of this work based on field studies would be valuable.

Chapter 2 demonstrated the local and systemic induction of Si defences in plants following insect attack. Si induction was measured following feeding by a single *H. armigera* larva on a confined leaf of each plant. Further studies should consider measuring the level of Si induction in plants when insects roam and feed freely on plants. Furthermore, the underlying molecular mechanism behind Si induction remains to be investigated. Transcriptome analysis of plants following Si and herbivory treatments may provide valuable insight into the molecular basis of Si induction. Chapter 3 assessed the impacts of

Si on plant direct and indirect defences against a herbivorous mite (*T. urticae*). Although Si was found to diminish the population growth of *T. urticae*, it is unclear whether Si acted solely as a physical defence or also impacted plant chemical defences. *Tetranychus urticae* mites can suppress plant defences by secreting effector proteins with their saliva, thereby hindering plant recognition of herbivory (Blaazer et al., 2018). Si deposited in cell apoplasts is hypothesised to impede effecter proteins, as proposed in the apoplastic obstruction hypothesis (Coskun et al., 2019), allowing plants to exploit their optimal defences against herbivores. Future research on the effects of Si on plant chemical defences against *T. urticae* utilising high throughput omics approaches would be useful. Furthermore, the ameliorating effects of Si on plant gas exchange rates following *T. urticae* herbivory also call for further research on how Si ameliorates the physiological injury caused by herbivorous mites.

In chapter 3, the preference of predatory mites was assessed based on Y-tube olfactometer bioassays. Results of such dual-choice behavioural studies do not always reflect the actual feeding preference of natural enemies in realistic settings (Ballhorn & Kautz, 2013). Thus, assessing whether increased attraction of natural enemies under Si supplementation provides enhanced biological control of herbivorous pests is a ripe topic for further research. Moreover, volatile compounds (HIPVs) emitted from plants (i.e. indirect defence) upon *T. urticae* attack, as shown in chapter 3, were tentatively identified by library search based on the probability match. The identity and concentrations of these compounds remain to be confirmed using synthetic standards. Moreover, the physiological and molecular mechanisms underlying Si-mediated shifts in volatile emission are unclear and require further investigation.

The effects of Si on *H. armigera* performance (chapters 2 and 4-6) were assessed by measuring a narrow set of parameters (e.g. relative growth rates, relative consumption). It remains to be seen whether diminished performance of larvae when feeding on Si-supplemented plants impacts their metamorphosis and adult reproduction and whether Si has any transgenerational effects on insect herbivores. Although chapters 4 and 6 established that the anti-predator defences of *H. armigera* larvae are impacted by Si fertilisation of plants, these studies did not directly assess how changes in anti-predator defences impact insect vulnerability to biological control agents. Future studies on the impacts of Si on biocontrol should consider experimenting under both controlled and open field conditions integrating arthropod and avian predators. Particularly, how low integument resistance of, and failure to exhibit crypsis by, insects when feeding on siliceous plants impact their interactions with natural enemies would be a vital area for further research.

Chapter 4 found that Si supplementation enhanced plant compensation for insect herbivory. The underlying physiological mechanisms behind this have yet to be investigated, however. Measuring the effects of Si on plant gas exchange rates, release of apical dominance, and resource allocation and translocation can provide further understanding of compensation mechanisms. Specifically, the use of stable isotopes to trace the translocation of non-structural carbohydrates between source and sink organs is likely to provide insight into the role of Si in plant compensation for herbivory (Dawson et al., 2002).

In chapter 5, the plant-mediated, indirect effects of Si on interspecific competition between a chewing and a sap-feeding insect herbivore were assessed by caging plants with insects

in acrylic cylinders. Such confined habitat may have intensified competition between the two herbivores (Kaplan & Denno, 2007), which could produce variations in results under natural open habitat. Considering the effects of Si on natural enemy attraction and anti-predator defences of insect herbivores, further field studies on the effects of Si on interguild interactions between insect herbivores integrating natural enemies would be valuable. Furthermore, like olfactometer bioassays, the preference of insect herbivores in dual-choice tests in rearing cages, as demonstrated in chapter 5, may not reflect the actual feeding preference of insects under natural conditions. It is also not evident how caterpillars and aphids selected their optimal host plants in dual-choice tests. Further work should consider focusing on the impacts of Si on the perception and recognition of chemical cues (e.g. HIPVs) by herbivorous insects for host plant selection.

7.3 Conclusions

Despite significant increases in global pesticide use and costly management practices for crop protection over the past decades, pest herbivores still ruin crops that can be fed to a billion people every year (Birch et al., 2011). Global population is expected to surpass 11 billion by 2100, and to ensure food security for this burgeoning population, crop production must be increased and crop losses by pest herbivores must be minimised without disrupting the environment. Si fertilisation could be a viable option to simultaneously enhance the productivity and defences of plants, especially given that grasses, which supply a large share of human calories (O'Mara, 2012), use Si for defence (O'Reagain & Mentis, 1989; Vicari & Bazely, 1993).

This work has explored some novel aspects of plant Si defences against pest herbivores and has broadened our understanding of the effects of Si on direct and indirect plant defences against herbivorous pests belonging to different feeding guilds. Specifically, this work provided evidence for systemic induction of Si defences in plants in response to insect attack. Si supplementation of plants was found to diminish the performance and damage potential of a generalist chewing (*H. armigera*) and a cell-content feeding (*T. urticae*) herbivore while enhancing the colonisation success of a sap-feeding aphid (*R. padi*). Such contrasting effects of Si were further found to impact the plant-mediated, indirect interactions between chewing and sap-feeding insects when sharing the host plants. Furthermore, this work has indicated that Si supplementation of plants may play a part in the biological control of pest herbivores in two ways. First, Si supplementation could attract a greater number of natural enemies of herbivores by changing plant volatile (HIPV) emissions upon herbivore attack. Second, feeding on Si-supplemented plants could compromise the anti-predator defences of insect herbivores, such as integument resistance and cryptic colouration, thereby making insect herbivores more vulnerable to their natural enemies. Collectively, this work has suggested that Si defences in plants are multifaceted and affect arthropod herbivores in a variety of ways beyond physical resistance.

Bibliography

Aartsma, Y., Bianchi, F. J. J. A., van der Werf, W., Poelman, E. H., & Dicke, M. (2017). Herbivore-induced plant volatiles and tritrophic interactions across spatial scales. *New Phytologist,* 216, 1054-1063.

Abbasi, A., Sufyan, M., Arif, M. J., & Sahi, S. T. (2022). Effect of silicon on oviposition preference and biology of *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) feeding on *Gossypium hirsutum* (Linnaeus). *International Journal of Pest Management,* 68, 124-134.

Abd-Alkarim, E., Bayoumi, Y., Metwally, E., & Rakha, M. (2017). Silicon supplements affect yield and fruit quality of cucumber (*Cucumis sativus* L.) grown in net houses. *African Journal of Agricultural Research,* 12, 2518-2523.

Acevedo, F. E., Peiffer, M., Ray, S., Tan, C. W., & Felton, G. W. (2021). Silicon-mediated enhancement of herbivore resistance in agricultural crops. *Frontiers in plant science,* 12, 631824.

Adamo, S. A., Davies, G., Easy, R., Kovalko, I., & Turnbull, K. F. (2016). Reconfiguration of the immune system network during food limitation in the caterpillar *Manduca sexta*. *Journal of Experimental Biology,* 219, 706-718.

Agut, B., Pastor, V., Jaques, J. A., & Flors, V. (2018). Can plant defence mechanisms provide new approaches for the sustainable control of the two-spotted spider mite *Tetranychus urticae*? *International Journal of Molecular Sciences,* 19, 614.

Ahmad, M., Arif, M. I., & Attique, M. R. (1997). Pyrethroid resistance of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Pakistan. *Bulletin of Entomological Research,* 87, 343-347.

Ahmad, S. (1992). Biochemical defence of pro-oxidant plant allelochemicals by herbivorous insects. *Biochemical Systematics and Ecology,* 20, 269-296.

Ahmad, S., Cheema, H. M. N., Khan, A. A., Khan, R. S. A., & Ahmad, J. N. (2019). Resistance status of *Helicoverpa armigera* against Bt cotton in Pakistan. *Transgenic Research,* 28, 199-212.

Alagoz, Y., Dhami, N., Mitchell, C., & Cazzonelli, C. I. (2020). *cis/trans* carotenoid extraction, purification, detection, quantification, and profiling in plant tissues. In M. Rodríguez-Concepción & R. Welsch (Eds.), *Plant and Food Carotenoids* (pp. 145-163). New York, NY: Humana.

Alba, J. M., Schimmel, B. C., Glas, J. J., Ataide, L. M., Pappas, M. L., Villarroel, C. A., . . . Kant, M. R. (2015). Spider mites suppress tomato defenses downstream of jasmonate and salicylate independently of hormonal crosstalk. *New Phytologist,* 205, 828-840.

Aldea, M., Hamilton, J. G., Resti, J. P., Zangerl, A. R., Berenbaum, M. R., & DeLucia, E. H. (2005). Indirect effects of insect herbivory on leaf gas exchange in soybean. *Plant, Cell & Environment,* 28, 402-411.

Alhousari, F., & Greger, M. (2018). Silicon and mechanisms of plant resistance to insect pests. *Plants (Basel),* 7, 33.

Aljbory, Z., & Chen, M.-S. (2018). Indirect plant defense against insect herbivores: a review. *Insect Science,* 25, 2-23.

Alyousuf, A., Hamid, D., Desher, M. A., Nikpay, A., & Laane, H. M. (2022). Effect of silicic acid formulation (silicon 0.8%) on two major insect pests of tomato under greenhouse conditions. *Silicon,* 14, 3019-3025.

Andama, J. B., Mujiono, K., Hojo, Y., Shinya, T., & Galis, I. (2020). Nonglandular silicified trichomes are essential for rice defense against chewing herbivores. *Plant, Cell & Environment,* 43, 2019-2032.

Arab, A., & Trigo, J. R. (2011). Host plant invests in growth rather than chemical defense when attacked by a specialist herbivore. *Journal of Chemical Ecology,* 37, 492-495.

Arimura, G.-i., Kost, C., & Boland, W. (2005). Herbivore-induced, indirect plant defences. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids,* 1734, 91-111.

Bakhat, H. F., Bibi, N., Zia, Z., Abbas, S., Hammad, H. M., Fahad, S., . . . Saeed, S. (2018). Silicon mitigates biotic stresses in crop plants: a review. *Crop Protection,* 104, 21-34.

Ballhorn, D. J., & Kautz, S. (2013). How useful are olfactometer experiments in chemical ecology research? *Communicative & Integrative Biology,* 6, e24787.

Barber, A., Campbell, C. A. M., Crane, H., Lilley, R., & Tregidga, E. (2003). Biocontrol of two-spotted spider mite *Tetranychus urticae* on dwarf hops by the phytoseiid mites *Phytoseiulus persimilis* and *Neoseiulus californicus*. *Biocontrol Science and Technology,* 13, 275-284.

Bardner, R., & Fletcher, K. E. (1974). Insect infestations and their effects on the growth and yield of field crops: a review. *Bulletin of Entomological Research,* 64, 141-160.

Beckage, N. E., & Riddiford, L. M. (1978). Developmental interactions between the tobacco hornworm *Manduca sexta* and its braconid parasite *Apanteles congregatus*. *Entomologia Experimentalis et Applicata,* 23, 139-151.

Belsky, A. J. (1986). Does herbivory benefit plants? A review of the evidence. *The American Naturalist,* 127, 870-892.

Bensoussan, N., Santamaria, M. E., Zhurov, V., Diaz, I., Grbić, M., & Grbić, V. (2016). Plant-herbivore interaction: Dissection of the cellular pattern of *Tetranychus urticae* feeding on the host plant. *Frontiers in plant science,* 7, 1105.

Bernays, E. A. (1997). Feeding by lepidopteran larvae is dangerous. *Ecological Entomology,* 22, 121-123.

Birch, A. N. E., Begg, G. S., & Squire, G. R. (2011). How agro-ecological research helps to address food security issues under new IPM and pesticide reduction policies for global crop production systems. *Journal of Experimental Botany,* 62, 3251-3261.

Biru, F. N., Islam, T., Cibils-Stewart, X., Cazzonelli, C. I., Elbaum, R., & Johnson, S. N. (2021). Anti-herbivore silicon defences in a model grass are greatest under Miocene levels of atmospheric CO_2. *Global change biology,* 27, 2959-2969.

Blaazer, C. J. H., Villacis-Perez, E. A., Chafi, R., Van Leeuwen, T., Kant, M. R., & Schimmel, B. C. J. (2018). Why do herbivorous mites suppress plant defenses? *Frontiers in plant science,* 9, 1057.

Boevé, J.-L., & Schaffner, U. (2003). Why does the larval integument of some sawfly species disrupt so easily? The harmful hemolymph hypothesis. *Oecologia,* 134, 104-111.

Burt, J. (2007). *Growing cucumbers in protected cultivation in Western Australia*. Perth: Department of Agriculture and Food, Western Australia.

Callis-Duehl, K. L., McAuslane, H. J., Duehl, A. J., & Levey, D. J. (2017). The effects of silica fertilizer as an anti-herbivore defense in cucumber. *Journal of Horticultural Research,* 25, 89-98.

Cardona, C. (1989). Insects and other invertebrate bean pests in Latin America. In H. F. Schwartz & M. A. Pastor-Corrales (Eds.), *Bean production problems in the tropics* (pp. 505-570). Cali, CO: Centro Internacional de Agricultura Tropical (CIAT).

Carmona, D., Lajeunesse, M. J., & Johnson, M. T. J. (2011). Plant traits that predict resistance to herbivores. *Functional Ecology,* 25, 358-367.

Catalan, T. P., Wozniak, A., Niemeyer, H. M., Kalergis, A. M., & Bozinovic, F. (2012). Interplay between thermal and immune ecology: effect of environmental temperature on insect immune response and energetic costs after an immune challenge. *Journal of Insect Physiology,* 58, 310-317.

Cazaux, M., Navarro, M., Bruinsma, K. A., Zhurov, V., Negrave, T., Van Leeuwen, T., . . . Grbic, M. (2014). Application of two-spotted spider mite *Tetranychus urticae* for plant-pest interaction studies. *Journal of Visualized Experiments*, e51738.

Cazzonelli, C. I. (2011). Carotenoids in nature: insights from plants and beyond. *Functional Plant Biology,* 38, 833-847.

Chakravarty, S., Padwal, K. G., & Srivastava, C. P. (2020). Larval body colour polymorphism in *Helicoverpa armigera* (hubner) populations across India. *Indian Journal of Entomology,* 82, 415-422.

Chérif, M., Asselin, A., & Bélanger, R. R. (1994). Defense responses induced by soluble silicon in cucumber roots infected by *Pythium* spp. *Phytopathology,* 84, 236-242.

Chérif, M., Benhamou, N., Menzies, J. G., & Bélanger, R. R. (1992). Silicon induced resistance in cucumber plants against *Pythium ultimum*. *Physiological and Molecular Plant Pathology,* 41, 411-425.

Christalcatalani, G., Vieira, M. R., De Camargos, L. S., Bomfim, N. C. P., & Agustin, J. A. (2017). Effects of potassim silicate application on papaya plants in the twospotted spider mite population. *Revista Brasileira de Fruticultura*, 39, e-840.

Cipollini, D., Purrington, C. B., & Bergelson, J. (2003). Costs of induced responses in plants. *Basic and Applied Ecology*, 4, 79-89.

Cipollini, D., Walters, D., & Voelckel, C. (2018). Costs of resistance in plants: from theory to evidence. *Annual Plant Reviews online,* 47, 263-307.

Colazza, S., McElfresh, J. S., & Millar, J. G. (2004). Identification of volatile synomones, induced by *Nezara viridula* feeding and oviposition on bean spp., that attract the egg parasitoid *Trissolcus basalis*. *Journal of Chemical Ecology,* 30, 945-964.

Connick, V. J. (2011). *The impact of silicon fertilisation on the chemical ecology of the grapevine, Vitis vinifera; constitutive and induced chemical defenses against arthropod pests and their natural enemies.* (Master of Philosophy Master's thesis), Charles Sturt University, Bathurst NSW, Australia.

Cooke, J., & Leishman, M. R. (2011). Is plant ecology more siliceous than we realise? *Trends in Plant Science,* 16, 61-68.

Cooke, J., & Leishman, M. R. (2016). Consistent alleviation of abiotic stress with silicon addition: a meta-analysis. *Functional Ecology,* 30, 1340-1357.

Cornell, J. C., Stamp, N. E., & Bowers, M. D. (1987). Developmental change in aggregation, defense and escape behavior of buckmoth caterpillars, *Hemileuca lucina* (Saturniidae). *Behavioral Ecology and Sociobiology,* 20, 383-388.

Cornet, S., Biard, C., & Moret, Y. (2007). Is there a role for antioxidant carotenoids in limiting self-harming immune response in invertebrates? *Biology Letters*, 3, 284-288.

Coskun, D., Deshmukh, R., Sonah, H., Menzies, J. G., Reynolds, O., Ma, J. F., . . . Bélanger, R. R. (2019). The controversies of silicon's role in plant biology. *New Phytologist*, 221, 67-85.

Cotter, S. C., Myatt, J. P., Benskin, C. M., & Wilson, K. (2008). Selection for cuticular melanism reveals immune function and life-history trade-offs in *Spodoptera littoralis*. *Journal of Evolutionary Biology*, 21, 1744-1754.

Crawley, M. J. (2012). *The R book*: John Wiley & Sons.

Cromartie, R. I. T. (1959). Insect pigments. *Annual Review of Entomology,* 4, 59-76.

Cunningham, J. P., & Zalucki, M. P. (2014). Understanding heliothine (Lepidoptera: Heliothinae) pests: what is a host plant? *Journal of Economic Entomology,* 107, 881-896.

Czeczuga, B. (1986). The presence of carotenoids in various species of Lepidoptera. *Biochemical Systematics and Ecology*, 14, 345-351.

Dalin, P., Ågren, J., Björkman, C., Huttunen, P., & Kärkkäinen, K. (2008). Leaf trichome formation and plant resistance to herbivory. In A. Schaller (Ed.), *Induced Plant Resistance to Herbivory* (pp. 89-105). Dordrecht: Springer.

Dara, S. K., Peck, D., & Murray, D. (2018). Chemical and non-chemical options for managing twospotted spider mite, western tarnished plant bug and other arthropod pests in strawberries. *Insects,* 9, 156.

Dawson, T. E., Mambelli, S., Plamboeck, A. H., Templer, P. H., & Tu, K. P. (2002). Stable isotopes in plant ecology. *Annual Review of Ecology and Systematics,* 33, 507-559.

De Angelis, J. D., Larson, K., Berry, R. E., & Krantz, G. (1982). Effects of spider mite injury on transpiration and leaf water status in peppermint. *Environmental Entomology,* 11, 975-978.

De Barro, P. J. (1992). The role of temperature, photoperiod, crowding and plant quality on the production of alate viviparous females of the bird cherry-oat aphid, *Rhopalosiphum padi*. *Entomologia Experimentalis et Applicata,* 65, 205-214.

De Boer, J. G., & Dicke, M. (2004). The role of methyl salicylate in prey searching behavior of the predatory mite *Phytoseiulus persimilis*. *Journal of Chemical Ecology,* 30, 255-271.

de Freitas Bueno, A., de Freitas Bueno, R. C. O., Nabity, P. D., Higley, L. G., & Fernandes, O. A. (2009). Photosynthetic response of soybean to twospotted spider mite (Acari: Tetranychydae) injury. *Brazilian Archives of Biology and Technology,* 52, 825-834.

de la Peña, M., González-Moro, M. B., & Marino, D. (2019). Providing carbon skeletons to sustain amide synthesis in roots underlines the suitability of *Brachypodium distachyon* for the study of ammonium stress in cereals. *AoB Plants,* 11.

de Oliveira, R. S., Peñaflor, M., Gonçalves, F. G., Sampaio, M. V., Korndörfer, A. P., Silva, W. D., & Bento, J. M. S. (2020). Silicon-induced changes in plant volatiles

reduce attractiveness of wheat to the bird cherry-oat aphid *Rhopalosiphum padi* and attract the parasitoid *Lysiphlebus testaceipes*. *PloS one,* 15, e0231005.

Denno, R. F., & Kaplan, I. (2007). Plant-mediated interactions in herbivorous insects: mechanisms, symmetry, and challenging the paradigms of competition past. In T. Ohgushi, T. P. Craig, & P. W. Price (Eds.), *Ecological communities : Plant mediation in indirect interaction webs* (pp. 19-50). Cambridge: Cambridge University Press.

Denno, R. F., McClure, M. S., & Ott, J. R. (1995). Interspecific interactions in phytophagous insects: Competition reexamined and resurrected. *Annual Review of Entomology,* 40, 297-331.

Denno, R. F., Peterson, M. A., Gratton, C., Cheng, J., Langellotto, G. A., Huberty, A. F., & Finke, D. L. (2000). Feeding-induced changes in plant quality mediate interspecific competition between sap-feeding herbivores. *Ecology,* 81, 1814-1827.

Deren, C. W. (2001). Plant genotype, silicon concentration, and silicon-related responses. In L. E. Datnoff, G. H. Snyder, & G. H. Korndörfer (Eds.), *Silicon in Agriculture* (Vol. 8, pp. 149-158). Amsterdam: Elsevier.

Deren, C. W., Datnoff, L. E., Snyder, G. H., & Martin, F. G. (1994). Silicon concentration, disease response, and yield components of rice genotypes grown on flooded organic histosols. *Crop Science,* 34, 733-737.

Deshmukh, R. K., Vivancos, J., Guérin, V., Sonah, H., Labbé, C., Belzile, F., & Bélanger, R. R. (2013). Identification and functional characterization of silicon transporters in soybean using comparative genomics of major intrinsic proteins in Arabidopsis and rice. *Plant Molecular Biology,* 83, 303-315.

Detmann, K. C., Araújo, W. L., Martins, S. C., Sanglard, L. M., Reis, J. V., Detmann, E., . . . DaMatta, F. M. (2012). Silicon nutrition increases grain yield, which, in turn, exerts a feed-forward stimulation of photosynthetic rates via enhanced mesophyll conductance and alters primary metabolism in rice. *New Phytologist,* 196, 752-762.

Dhami, N., Tissue, D. T., & Cazzonelli, C. I. (2018). Leaf-age dependent response of carotenoid accumulation to elevated CO_2 in Arabidopsis. *Archives of Biochemistry and Biophysics,* 647, 67-75.

Diamond, S. E., & Kingsolver, J. G. (2011). Host plant quality, selection and trade-offs shape the immunity of *Manduca sexta*. *Proceedings of the Royal Society B,* 278, 289-297.

Dias, P. A., Sampaio, M. V., Rodrigues, M. P., Korndörfer, A. P., Oliveira, R. S., Ferreira, S. E., & Korndörfer, G. H. (2014). Induction of resistance by silicon in wheat plants to alate and apterous morphs of *Sitobion avenae* (Hemiptera: Aphididae). *Environmental Entomology,* 43, 949-956.

Dicke, M. (2009). Behavioural and community ecology of plants that cry for help. *Plant, Cell & Environment,* 32, 654-665.

Dicke, M., & Baldwin, I. T. (2010). The evolutionary context for herbivore-induced plant volatiles: beyond the 'cry for help'. *Trends in Plant Science,* 15, 167-175.

Dicke, M., Beek, T. A. V., Posthumus, M. A., Dom, N. B., Bokhoven, H. V., & Groot, A. D. (1990). Isolation and identification of volatile kairomone that affects acarine predator-prey interactions: involvement of host plant in its production. *Journal of Chemical Ecology,* 16, 381-396.

Döker, İ., & Kazak, C. (2019). Non-target effects of five acaricides on a native population of *Amblyseius swirskii* (Acari: Phytoseiidae). *International Journal of Acarology,* 45, 69-74.

Döring, T. F. (2014). How aphids find their host plants, and how they don't. *Annals of Applied Biology,* 165, 3-26.

Ehrlich, P. R., & Raven, P. H. (1964). Butterflies and plants: a study in coevolution. *Evolution,* 18, 586-608.

Eichenseer, H., Murphy, J. B., & Felton, G. W. (2002). Sequestration of host plant carotenoids in the larval tissues of *Helicoverpa zea*. *Journal of Insect Physiology,* 48, 311-318.

Eleftherianos, I., & Revenis, C. (2011). Role and importance of phenoloxidase in insect hemostasis. *Journal of Innate Immunity,* 3, 28-33.

Elhakeem, A., Markovic, D., Broberg, A., Anten, N. P. R., & Ninkovic, V. (2018). Aboveground mechanical stimuli affect belowground plant-plant communication. *PloS one,* 13, e0195646.

Epstein, E. (1994). The anomaly of silicon in plant biology. *Proceedings of the National Academy of Sciences, USA,* 91, 11-17.

Epstein, E. (1999). Silicon. *Annual Review of Plant Physiology and Plant Molecular Biology,* 50, 641-664.

Epstein, E. (2001). Silicon in plants: facts vs. concepts. In L. E. Datnoff, G. H. Snyder, & G. H. Korndörfer (Eds.), *Studies in Plant Science* (Vol. 8, pp. 1-15). Amsterdam: Elsevier.

Epstein, E. (2009). Silicon: its manifold roles in plants. *Annals of Applied Biology,* 155, 155-160.

Erb, M., Meldau, S., & Howe, G. A. (2012). Role of phytohormones in insect-specific plant reactions. *Trends in Plant Science,* 17, 250-259.

Faisal, S., Callis, K. L., Slot, M., & Kitajima, K. (2012). Transpiration-dependent passive silica accumulation in cucumber (*Cucumis sativus*) under varying soil silicon availability. *Botany,* 90, 1058-1064.

Ferreira, R. S., Moraes, J. C., & Antunes, C. S. (2011). Silicon influence on resistance induction against *Bemisia tabaci* biotype B (Genn.) (Hemiptera: Aleyrodidae) and on vegetative development in two soybean cultivars. *Neotropical Entomology,* 40, 495-500.

Finlay, K. J., & Luck, J. E. (2011). Response of the bird cherry-oat aphid (*Rhopalosiphum padi*) to climate change in relation to its pest status, vectoring potential and function in a crop–vector–virus pathosystem. *Agriculture, Ecosystems & Environment,* 144, 405-421.

Forkner, R. E., & Hunter, M. D. (2000). What goes up must come down? Nutrient addition and predation pressure on oak herbivores. *Ecology,* 81, 1588-1600.

Frew, A., Powell, J. R., Allsopp, P. G., Sallam, N., & Johnson, S. N. (2017a). Arbuscular mycorrhizal fungi promote silicon accumulation in plant roots, reducing the impacts of root herbivory. *Plant and soil,* 419, 423-433.

Frew, A., Powell, J. R., Hiltpold, I., Allsopp, P. G., Sallam, N., & Johnson, S. N. (2017b). Host plant colonisation by arbuscular mycorrhizal fungi stimulates immune

function whereas high root silicon concentrations diminish growth in a soil-dwelling herbivore. *Soil Biology and Biochemistry*, 112, 117-126.

Frew, A., Powell, J. R., Sallam, N., Allsopp, P. G., & Johnson, S. N. (2016). Trade-offs between silicon and phenolic defenses may explain enhanced performance of root herbivores on phenolic-rich plants. *Journal of Chemical Ecology*, 42, 768-771.

Frew, A., Weston, L. A., & Gurr, G. M. (2019). Silicon reduces herbivore performance via different mechanisms, depending on host-plant species. *Austral Ecology*, 44, 1092-1097.

Frew, A., Weston, L. A., Reynolds, O. L., & Gurr, G. M. (2018). The role of silicon in plant biology: a paradigm shift in research approach. *Annals of Botany*, 121, 1265-1273.

Garvey, M., Bredlau, J., Kester, K., Creighton, C., & Kaplan, I. (2021). Toxin or medication? Immunotherapeutic effects of nicotine on a specialist caterpillar. *Functional Ecology*, 35, 614-626.

Gatarayiha, M. C., Laing, M. D., & Miller, R. M. (2010). Combining applications of potassium silicate and *Beauveria bassiana* to four crops to control two spotted spider mite, *Tetranychus urticae* Koch. *International Journal of Pest Management*, 56, 291-297.

Gatehouse, J. A. (2002). Plant resistance towards insect herbivores: a dynamic interaction. *New Phytologist*, 156, 145-169.

Gavloski, J. E., & Lamb, R. J. (2000). Compensation for herbivory in cruciferous plants: Specific responses to three defoliating insects. *Environmental Entomology*, 29, 1258-1267.

Gehring, C., & Bennett, A. (2009). Mycorrhizal fungal–plant–insect interactions: The importance of a community approach. *Environmental Entomology,* 38, 93-102.

Gherlenda, A. N., Haigh, A. M., Moore, B. D., Johnson, S. N., & Riegler, M. (2016). Climate change, nutrition and immunity: Effects of elevated CO_2 and temperature on the immune function of an insect herbivore. *Journal of Insect Physiology*, 85, 57-64.

Ghule, T. M., Uikey, B. L., Barma, P., & Jha, S. (2014). Incidence studies on some important insect pests of cucumber (*Cucumis sativus* L.). *The Ecoscan,* 8, 177-180.

Girin, T., David, L. C., Chardin, C., Sibout, R., Krapp, A., Ferrario-Méry, S., & Daniel-Vedele, F. (2014). *Brachypodium*: a promising hub between model species and cereals. *Journal of Experimental Botany,* 65, 5683-5696.

Gish, M., & Inbar, M. (2006). Host location by apterous aphids after escape dropping from the plant. *Journal of Insect Behavior,* 19, 143-153.

Gomes, E. S., Santos, V., & Ávila, C. J. (2017). Biology and fertility life table of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in different hosts. *Entomological Science,* 20, 419-426.

González-Santoyo, I., & Córdoba-Aguilar, A. (2012). Phenoloxidase: a key component of the insect immune system. *Entomologia Experimentalis et Applicata,* 142, 1-16.

Grbić, M., Van Leeuwen, T., Clark, R. M., Rombauts, S., Rouzé, P., Grbić, V., . . . Ortego, F. (2011). The genome of *Tetranychus urticae* reveals herbivorous pest adaptations. *Nature,* 479, 487-492.

Greeney, H. F., Dyer, L. A., & Smilanich, A. M. (2012). Feeding by lepidopteran larvae is dangerous: A review of caterpillars' chemical, physiological, morphological, and

behavioral defenses against natural enemies. *Invertebrate Survival Journal,* 9, 7-34.

Gross, P. (1993). Insect behavioral and morphological defenses against parasitoids. *Annual Review of Entomology,* 38, 251-273.

Guerrieri, E., & Digilio, M. C. (2008). Aphid-plant interactions: a review. *Journal of Plant Interactions,* 3, 223-232.

Guntzer, F., Keller, C., & Meunier, J.-D. (2011). Benefits of plant silicon for crops: a review. *Agronomy for Sustainable Development,* 32, 201-213.

Hackman, R. H. (1952). Green pigments of the hemolymph of insects. *Archives of Biochemistry and Biophysics,* 41.

Hall, C. R., Dagg, V., Waterman, J. M., & Johnson, S. N. (2020a). Silicon alters leaf surface morphology and suppresses insect herbivory in a model grass species. *Plants (Basel),* 9, 643.

Hall, C. R., Mikhael, M., Hartley, S. E., & Johnson, S. N. (2020b). Elevated atmospheric CO_2 suppresses jasmonate and silicon-based defences without affecting herbivores. *Functional Ecology,* 34, 993-1002.

Hall, C. R., Rowe, R. C., Mikhael, M., Read, E., Hartley, S. E., & Johnson, S. N. (2021). Plant silicon application alters leaf alkaloid concentrations and impacts parasitoids more adversely than their aphid hosts. *Oecologia,* 196, 145-154.

Hall, C. R., Waterman, J. M., Vandegeer, R. K., Hartley, S. E., & Johnson, S. N. (2019). The role of silicon in antiherbivore phytohormonal signalling. *Frontiers in plant science,* 10, 1132.

Han, Y., Lei, W., Wen, L., & Hou, M. (2015). Silicon-mediated resistance in a susceptible rice variety to the rice leaf folder, *Cnaphalocrocis medinalis* Guenée (Lepidoptera: Pyralidae). *PloS one,* 10, e0120557.

Han, Y., Li, P., Gong, S., Yang, L., Wen, L., & Hou, M. (2016). Defense responses in rice induced by silicon amendment against Infestation by the leaf folder *Cnaphalocrocis medinalis*. *PloS one,* 11, e0153918.

Harizanova, A., Koleva-Valkova, L., Stoeva, A., & Sevov, A. (2019). Effect of silicon on the activity of antioxidant enzymes and the photosynthetic rate of cucumber under mite infestation. *Sci Papers Series A Agron,* 62, 519-528.

Harmon, J. P., Losey, J. E., & Ives, A. R. (1998). The role of vision and color in the close proximity foraging behavior of four coccinellid species. *Oecologia,* 115, 287-292.

Hartley, S. E., DeGabriel, J. L., & Cooke, J. (2016). The ecology of herbivore-induced silicon defences in grasses. *Functional Ecology*, 30, 1311-1322.

Hartley, S. E., Fitt, R. N., McLarnon, E. L., & Wade, R. N. (2015). Defending the leaf surface: intra- and inter-specific differences in silicon deposition in grasses in response to damage and silicon supply. *Frontiers in plant science,* 6, 35.

Heath, J. J., Cipollini, D. F., & Stireman III, J. O. (2012). The role of carotenoids and their derivatives in mediating interactions between insects and their environment. *Arthropod-Plant Interactions,* 7, 1-20.

Herms, D. A., & Mattson, W. J. (1992). The dilemma of plants: to grow or defend. *The Quarterly Review of Biology*, 67, 283-335.

Hochmuth, G. (2001). Fertilizer management for greenhouse vegetables, Florida greenhouse vegetable production handbook. *HS787. Gainesville. Horticultural*

Sciences Department, Florida Cooperative Extension Service, University of Florida. Retrieved April, 5, 2007.

Hochmuth, R. (2015). Greenhouse cucumber production-Florida greenhouse vegetable production handbook, vol. 3. *IFAS Extension, University of Florida. Florida, EEUU. HS790*.

Hodson, M. J., White, P. J., Mead, A., & Broadley, M. R. (2005). Phylogenetic variation in the silicon composition of plants. *Annals of Botany,* 96, 1027-1046.

Hoffland, E., Dicke, M., Van Tintelen, W., Dijkman, H., & Van Beusichem, M. L. (2000). Nitrogen availability and defense of tomato against two-spotted spider mite. *Journal of Chemical Ecology,* 26, 2697-2711.

Honěk, A. (1991). Nitrogen fertilization and abundance of the cereal aphids *Metopolophium dirhodum* and *Sitobion avenae* (Homoptera, Aphididae). *Journal of Plant Diseases and Protection,* 98, 655-660.

Honěk, A., Jarošik, V., Lapchin, L., & Rabasse, J.-M. (1998). The effect of parasitism by *Aphelinus abdominalis* and drought on the walking movement of aphids. *Entomologia Experimentalis et Applicata,* 87, 191-200.

Hosseini, M., Ashouri, A., Enkegaard, A., Goldansaz, S. H., Nassiri Mahalati, M., & Hosseininaveh, V. (2010). Performance and population growth rate of the cotton aphid, and associated yield losses in cucumber, under different nitrogen fertilization regimes. *International Journal of Pest Management,* 56, 127-135.

Hosseini, S. Z., Jelodar, N. B., & Bagheri, N. (2012). Study of silicon effects on plant growth and resistance to stem borer in rice. *Communications in Soil Science and Plant Analysis,* 43, 2744-2751.

Hou, M., & Han, Y. (2010). Silicon-mediated rice plant resistance to the asiatic rice borer (Lepidoptera: Crambidae): effects of silicon amendment and rice varietal resistance. *Journal of Economic Entomology,* 103, 1412-1419.

Iltis, C., Martel, G., Thiéry, D., Moreau, J., & Louâpre, P. (2018). When warmer means weaker: high temperatures reduce behavioural and immune defences of the larvae of a major grapevine pest. *Journal of Pest Science,* 91, 1315-1326.

Inbar, M., Doostdar, H., & Mayer, R. T. (1999). Effects of sessile whitefly nymphs (Homoptera: Aleyrodidae) on leaf-chewing larvae (Lepidoptera: Noctuidae). *Environmental Entomology,* 28, 353-357.

Islam, T., Moore, B. D., & Johnson, S. N. (2020). Novel evidence for systemic induction of silicon defences in cucumber following attack by a global insect herbivore. *Ecological Entomology,* 45, 1373-1381.

Islam, T., Moore, B. D., & Johnson, S. N. (2022a). Plant silicon defences reduce the performance of a chewing insect herbivore which benefits a contemporaneous sap-feeding insect. *Ecological Entomology*, https://doi.org/10.1111/een.13183.

Islam, T., Moore, B. D., & Johnson, S. N. (2022b). Silicon fertilisation affects morphological and immune defences of an insect pest and enhances plant compensatory growth. *Journal of Pest Science*, https://doi.org/10.1007/s10340-022-01478-4.

Islam, T., Moore, B. D., & Johnson, S. N. (2022c). Silicon suppresses a ubiquitous mite herbivore and promotes natural enemy attraction by altering plant volatile blends. *Journal of Pest Science,* 95, 423-434.

Jeppson, L. R., Keifer, H. H., & Baker, E. W. (1975). *Mites injurious to economic plants*. Berkeley: University of California Press.

Johnson, M.-L., & Zalucki, M. P. (2007). Feeding and foraging behaviour of a generalist caterpillar: are third instars just bigger versions of firsts? *Bulletin of Entomological Research,* 97, 81-88.

Johnson, M. T. J., Bertrand, J. A., & Turcotte, M. M. (2016). Precision and accuracy in quantifying herbivory. *Ecological Entomology,* 41, 112-121.

Johnson, S. N., & Hartley, S. E. (2018). Elevated carbon dioxide and warming impact silicon and phenolic-based defences differently in native and exotic grasses. *Global change biology,* 24, 3886-3896.

Johnson, S. N., Hartley, S. E., Ryalls, J. M. W., Frew, A., DeGabriel, J. L., Duncan, M., . . . Biere, A. (2017). Silicon-induced root nodulation and synthesis of essential amino acids in a legume is associated with higher herbivore abundance. *Functional Ecology,* 31, 1903-1909.

Johnson, S. N., Hartley, S. E., Ryalls, J. M. W., Frew, A., & Hall, C. R. (2021a). Targeted plant defense: silicon conserves hormonal defense signaling impacting chewing but not fluid-feeding herbivores. *Ecology,* 102, e03250.

Johnson, S. N., Reynolds, O. L., Gurr, G. M., Esveld, J. L., Moore, B. D., Tory, G. J., & Gherlenda, A. N. (2019a). When resistance is futile, tolerate instead: silicon promotes plant compensatory growth when attacked by above- and belowground herbivores. *Biology Letters,* 15, 20190361.

Johnson, S. N., Rowe, R. C., & Hall, C. R. (2020a). Aphid feeding induces phytohormonal cross-talk without affecting silicon defense against subsequent chewing herbivores. *Plants,* 9, 1009.

Johnson, S. N., Rowe, R. C., & Hall, C. R. (2020b). Silicon is an inducible and effective herbivore defence against *Helicoverpa punctigera* (Lepidoptera: Noctuidae) in soybean. *Bulletin of Entomological Research,* 110, 417-422.

Johnson, S. N., Ryalls, J. M. W., Barton, C. V. M., Tjoelker, M. G., Wright, I. J., Moore, B. D., & Rasmann, S. (2019b). Climate warming and plant biomechanical defences: Silicon addition contributes to herbivore suppression in a pasture grass. *Functional Ecology,* 33, 587-596.

Johnson, S. N., Waterman, J. M., Wuhrer, R., Rowe, R. C., Hall, C. R., & Cibils–Stewart, X. (2021b). Siliceous and non-nutritious: nitrogen limitation increases anti-herbivore silicon defences in a model grass. *Journal of Ecology,* 109, 3767-3778.

Jones, C. M., Parry, H., Tay, W. T., Reynolds, D. R., & Chapman, J. W. (2019). Movement ecology of pest *Helicoverpa*: implications for ongoing spread. *Annual Review of Entomology,* 64, 277-295.

Jones, L. H. P., & Handreck, K. A. (1965). Studies of silica in the oat plant. *Plant and soil,* 23, 79-96.

Jung, H.-i., Gayomba, S. R., Yan, J., & Vatamaniuk, O. K. (2014). *Brachypodium distachyon* as a model system for studies of copper transport in cereal crops. *Frontiers in plant science,* 5, 236.

Jung, H. I., Yan, J., Zhai, Z., & Vatamaniuk, O. K. (2015). Gene functional analysis using protoplast transient assays. In J. Alonso & J. Stepanova (Eds.), *Plant Functional*

Genomics. Methods in Molecular Biology (Vol. 1284, pp. 433-452). New York, NY: Humana Press.

Kanost, M. R., & Gorman, M. J. (2008). Phenoloxidases in insect immunity. In N. E. Beckage (Ed.), *Insect Immunology* (pp. 69-96). San Diego: Academic Press.

Kant, M. R., Jonckheere, W., Knegt, B., Lemos, F., Liu, J., Schimmel, B. C., . . . Alba, J. M. (2015). Mechanisms and ecological consequences of plant defence induction and suppression in herbivore communities. *Annals of Botany,* 115, 1015-1051.

Kaplan, I. (2012). Attracting carnivorous arthropods with plant volatiles: The future of biocontrol or playing with fire? *Biological Control,* 60, 77-89.

Kaplan, I., & Denno, R. F. (2007). Interspecific interactions in phytophagous insects revisited: a quantitative assessment of competition theory. *Ecology Letters,* 10, 977-994.

Karban, R. (2011). The ecology and evolution of induced resistance against herbivores. *Functional Ecology,* 25, 339-347.

Karban, R. (2020). The ecology and evolution of induced responses to herbivory and how plants perceive risk. *Ecological Entomology,* 45, 1-9.

Karban, R., Agrawal, A. A., Thaler, J. S., & Adler, L. S. (1999). Induced plant responses and information content about risk of herbivory. *Trends in Ecology & Evolution,* 14, 443-447.

Karban, R., & Myers, J. H. (1989). Induced plant responses to herbivory. *Annual Review of Ecology and Systematics,* 20, 331-348.

Kaur, S., Kaur, S., Srinivasan, R., Cheema, D. S., Lal, T., Ghai, T. R., & Chadha, M. L. (2010). Monitoring of major pests on cucumber, sweet pepper and tomato under

net-house conditions in Punjab, India. *Pest Management in Horticultural Ecosystems,* 16, 148-155.

Kempel, A., Brandl, R., & Schädler, M. (2009). Symbiotic soil microorganisms as players in aboveground plant–herbivore interactions – the role of rhizobia. *OIKOS,* 118, 634-640.

Kessler, A. (2015). The information landscape of plant constitutive and induced secondary metabolite production. *Current Opinion in Insect Science,* 8, 47-53.

Kim, Y. H., Khan, A. L., Waqas, M., Jeong, H. J., Kim, D. H., Shin, J. S., . . . Lee, I. J. (2014). Regulation of jasmonic acid biosynthesis by silicon application during physical injury to *Oryza sativa* L. *Journal of Plant Research,* 127, 525-532.

Koricheva, J. (2002). Meta-analysis of sources of variation in fitness costs of plant antiherbivore defenses. *Ecology,* 83, 176-190.

Koricheva, J., Gange, A. C., & Jones, T. (2009). Effects of mycorrhizal fungi on insect herbivores: a meta-analysis. *Ecology,* 90, 2088-2097.

Krugner, R., Wallis, C. M., & Walse, S. S. (2014). Attraction of the egg parasitoid, *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae) to synthetic formulation of a (*E*)-β-ocimene and (*E*,*E*)-α-farnesene mixture. *Biological Control,* 77, 23-28.

Kulkarni, J., Kapse, N., & Kulkarni, D. K. (2009). Plant-based pesticide for control of *Helicoverpa armigera* on *Cucumis sativus*. *Asian Agri-History,* 13, 327-332.

Kvedaras, O. L., An, M., Choi, Y. S., & Gurr, G. M. (2010). Silicon enhances natural enemy attraction and biological control through induced plant defences. *Bulletin of Entomological Research,* 100, 367-371.

Kvedaras, O. L., Byrne, M. J., Coombes, N. E., & Keeping, M. G. (2009). Influence of plant silicon and sugarcane cultivar on mandibular wear in the stalk borer *Eldana saccharina*. *Agricultural and Forest Entomology,* 11, 301-306.

Kvedaras, O. L., Keeping, M. G., Goebel, F. R., & Byrne, M. J. (2007). Larval performance of the pyralid borer *Eldana saccharina* Walker and stalk damage in sugarcane: Influence of plant silicon, cultivar and feeding site. *International Journal of Pest Management,* 53, 183-194.

Laing, J. E. (1968). Life history and life table of *Phytoseiulus persimilis* Aathias-Henriot. *Acarologia,* 10, 578-588.

Lampert, E. (2012). Influences of plant traits on immune responses of specialist and generalist herbivores. *Insects,* 3, 573-592.

Lavine, M. D., & Strand, M. R. (2002). Insect hemocytes and their role in immunity. *Insect Biochemistry and Molecular Biology,* 32, 1295-1309.

Leroy, N., Hanciaux, N., Cornelis, J. T., & Verheggen, F. J. (2022). Silicon accumulation in maize negatively impacts the feeding and life history traits of *Spodoptera exigua* Hubner. *Entomologia Generalis*, 42, 413-420.

Leroy, N., Tombeur, F., Walgraffe, Y., Cornélis, J. T., & Verheggen, F. J. (2019). Silicon and plant natural defenses against insect pests: impact on plant volatile organic compounds and cascade effects on multitrophic interactions. *Plants (Basel),* 8, 444.

Liang, Y., Nikolic, M., Bélanger, R., Gong, H., & Song, A. (2015). Silicon and insect pest resistance. In *Silicon in Agriculture* (pp. 197-207). Dordrecht: Springer.

Liang, Y., Si, J., & Römheld, V. (2005). Silicon uptake and transport is an active process in *Cucumis sativus*. *New Phytologist,* 167, 797-804.

Lin, Y. B., Lin, X. H., Ding, C. H., Xia, M., Xue, R. R., Sun, Z. X., . . . Song, Y. Y. (2022). Priming of rice defense against a sap-sucking insect pest brown planthopper by silicon. *Journal of Pest Science,* 95, 1371-1385.

Liu, J., Zhu, J., Zhang, P., Han, L., Reynolds, O. L., Zeng, R., . . . Gurr, G. M. (2017). Silicon supplementation alters the composition of herbivore induced plant volatiles and enhances attraction of parasitoids to infested rice plants. *Frontiers in plant science,* 8, 1265.

Lynch, M. E., Kaplan, I., Dively, G. P., & Denno, R. F. (2006). Host-plant-mediated competition via induced resistance: Interactions between pest herbivores on potatoes. *Ecological Applications,* 16, 855-864.

Lyytinen, A. (2001). *Insect coloration as a defence mechanism against visually hunting predators.* (PhD thesis), University of Jyväskylä, Finland.

Ma, J. F., Higashitani, A., Sato, K., & Takeda, K. (2003). Genotypic variation in silicon concentration of barley grain. *Plant and soil,* 249, 383-387.

Ma, J. F., Tamai, K., Yamaji, N., Mitani, N., Konishi, S., Katsuhara, M., . . . Yano, M. (2006). A silicon transporter in rice. *Nature,* 440, 688-691.

Ma, J. F., & Yamaji, N. (2006). Silicon uptake and accumulation in higher plants. *Trends in Plant Science,* 11, 392-397.

Ma, J. F., & Yamaji, N. (2008). Functions and transport of silicon in plants. *Celllular and Molecular Life Sciences,* 65, 3049-3057.

Ma, J. F., & Yamaji, N. (2015). A cooperative system of silicon transport in plants. *Trends in Plant Science,* 20, 435-442.

Ma, J. F., Yamaji, N., Mitani, N., Tamai, K., Konishi, S., Fujiwara, T., . . . Yano, M. (2007a). An efflux transporter of silicon in rice. *Nature,* 448, 209-212.

Ma, J. F., Yamaji, N., Tamai, K., & Mitani, N. (2007b). Genotypic difference in silicon uptake and expression of silicon transporter genes in rice. *Plant Physiology,* 145, 919-924.

Mandlik, R., Thakral, V., Raturi, G., Shinde, S., Nikolić, M., Tripathi, D. K., . . . Deshmukh, R. (2020). Significance of silicon uptake, transport, and deposition in plants. *Journal of Experimental Botany,* 71, 6703–6718.

Massey, F. P., Ennos, A. R., & Hartley, S. E. (2006). Silica in grasses as a defence against insect herbivores: contrasting effects on folivores and a phloem feeder. *Journal of Animal Ecology,* 75, 595-603.

Massey, F. P., Ennos, A. R., & Hartley, S. E. (2007). Herbivore specific induction of silica-based plant defences. *Oecologia,* 152, 677-683.

Massey, F. P., & Hartley, S. E. (2009). Physical defences wear you down: progressive and irreversible impacts of silica on insect herbivores. *Journal of Animal Ecology,* 78, 281-291.

McLarnon, E., McQueen-Mason, S., Lenk, I., & Hartley, S. E. (2017). Evidence for active uptake and deposition of Si-based defenses in tall fescue. *Frontiers in plant science,* 8, 1199.

McNaughton, S., & Tarrants, J. (1983). Grass leaf silicification: natural selection for an inducible defense against herbivores. *Proceedings of the National Academy of Sciences, USA,* 80, 790-791.

Miresmailli, S., & Isman, M. B. (2006). Efficacy and persistence of rosemary oil as an acaricide against twospotted spider mite (Acari: Tetranychidae) on greenhouse tomato. *Journal of Economic Entomology*, 99, 2015-2023.

Mithöfer, A., & Boland, W. (2012). Plant defense against herbivores: chemical aspects. *Annual Review of Plant Biology.*, 63, 431-450.

Mohaghegh, P., Shirvani, M., & Ghasemi, S. (2010). Silicon application effects on yield and growth of two cucumber genotypes in hydroponics system. *Journal of Science and Technology of Greenhouse Culture,* 1, 35-40.

Mohammadi, D., Abad, R. F. P., Rashidi, M. R., & Mohammadi, S. A. (2010). Study of cotton bollworm, *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) using Dyar's rule. *Munis Entomology & Zoology*, 5, 216-224.

Moise, E. R., McNeil, J. N., Hartley, S. E., & Henry, H. A. (2019a). Plant silicon effects on insect feeding dynamics are influenced by plant nitrogen availability. *Entomologia Experimentalis et Applicata,* 167, 91-97.

Moise, E. R. D., McNeil, J. N., Hartley, S. E., & Henry, H. A. L. (2019b). Plant silicon effects on insect feeding dynamics are influenced by plant nitrogen availability. *Entomologia Experimentalis et Applicata,* 167, 91-97.

Moore, B. D., & Johnson, S. N. (2016). Get tough, get toxic, or get a bodyguard: Identifying candidate traits conferring belowground resistance to herbivores in grasses. *Frontiers in plant science*, 7, 1925.

Moraes, J. C., Goussain, M. M., Basagli, M. A. B., Carvalho, G. A., Ecole, C. C., & Sampaio, M. V. (2004). Silicon influence on the tritrophic interaction: wheat plants, the greenbug *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae), and its

natural enemies, *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) and *Aphidius colemani* Viereck (Hymenoptera: Aphidiidae). *Neotropical Entomology,* 33, 619-624.

Moran, N. A., & Jarvik, T. (2010). Lateral transfer of genes from fungi underlies carotenoid production in aphids. *Science,* 328, 624-627.

Moreno-García, M., Córdoba-Aguilar, A., Condé, R., & Lanz-Mendoza, H. (2013). Current immunity markers in insect ecological immunology: assumed trade-offs and methodological issues. *Bulletin of Entomological Research,* 103, 127-139.

Mumm, R., & Dicke, M. (2010). Variation in natural plant products and the attraction of bodyguards involved in indirect plant defense. *Canadian Journal of Zoology,* 88, 628-667.

Murray, T. J., Tissue, D. T., Ellsworth, D. S., & Riegler, M. (2013). Interactive effects of pre-industrial, current and future [CO_2] and temperature on an insect herbivore of *Eucalyptus*. *Oecologia,* 171, 1025-1035.

Nachappa, P., Margolies, D. C., Nechols, J. R., & Loughin, T. (2006). *Phytoseiulus persimilis* response to herbivore-induced plant volatiles as a function of mite-days. *Experimental and Applied Acarology,* 40, 231-239.

Nagaratna, W., Kalleshwaraswamy, C. M., Dhananjaya, B. C., Sharanabasappa, & Prakash, N. B. (2022). Effect of silicon and plant growth regulators on the biology and fitness of fall armyworm, *Spodoptera frugiperda*, a recently invaded pest of maize in India. *Silicon,* 14, 783-793.

Nascimento, A. M., Assis, F. A., Moraes, J. C., & Souza, B. H. S. (2018). Silicon application promotes rice growth and negatively affects development of

Spodoptera frugiperda (J. E. Smith). *Journal of Applied Entomology*, 142, 241-249.

Neu, S., Schaller, J., & Dudel, E. G. (2017). Silicon availability modifies nutrient use efficiency and content, C: N: P stoichiometry, and productivity of winter wheat (*Triticum aestivum* L.). *Scientific Reports*, 7, 40829.

Nguyen, K. O., Al-Rashid, S., Clarke Miller, M., Tom Diggs, J., & Lampert, E. C. (2019). *Trichoplusia ni* (Lepidoptera: Noctuidae) qualitative and quantitative sequestration of host plant carotenoids. *Environmental Entomology*, 48, 540-545.

Nikpay, A., & Soleyman-Nejadian, E. (2014). Field applications of silicon-based fertilizers against sugarcane yellow mite *Oligonychus sacchari*. *Sugar Tech*, 16, 319-324.

Nikpay, A., Soleyman-Nejadian, E., Goldasteh, S., & Farazmand, H. (2015). Response of sugarcane and sugarcane stalk borers *Sesamia* spp. (Lepidoptera: Noctuidae) to calcium silicate fertilization. *Neotropical Entomology*, 44, 498-503.

Núñez-Farfán, J., Fornoni, J., & Valverde, P. L. (2007). The evolution of resistance and tolerance to herbivores. *Annual Review of Ecology, Evolution, and Systematics*, 38, 541-566.

Nuñez-Palenius, H. G., Hopkins, D., & Cantliffe, D. J. (2006). Powdery mildew of cucurbits in Florida. In *Publication HS1067. Horticultural Sciences Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL*.

O'Mara, F. P. (2012). The role of grasslands in food security and climate change. *Annals of Botany*, 110, 1263-1270.

O'Reagain, P. J., & Mentis, M. T. (1989). Leaf silicification in grasses — A review. *Journal of the Grassland Society of Southern Africa,* 6, 37-43.

Opit, G., Nechols, J., & Margolies, D. (2004). Biological control of twospotted spider mites, *Tetranychus urticae* Koch (Acari: Tetranychidae), using *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseidae) on ivy geranium: assessment of predator release ratios. *Biological Control,* 29, 445-452.

Ostlie, K., & Pedigo, L. (1984). Water loss from soybeans after simulated and actual insect defoliation. *Environmental Entomology,* 13, 1675-1680.

Oye Anda, C. C., Opfergelt, S., & Declerck, S. (2016). Silicon acquisition by bananas (c.V. Grande Naine) is increased in presence of the arbuscular mycorrhizal fungus *Rhizophagus irregularis* MUCL 41833. *Plant and soil,* 409, 77-85.

Park, Y.-L., & Lee, J.-H. (2002). Leaf cell and tissue damage of cucumber caused by twospotted spider mite (Acari: Tetranychidae). *Journal of Economic Entomology,* 95, 952-957.

Pascua, L. T., & Pascua, M. E. (2002). The distribution and movement of cotton bollworm, *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) larvae on cotton. *Philippine Journal of Science,* 131, 91-98.

Patil, C., Udikeri, S., & Karabhantanal, S. (2018). A note on pesticide induced resurgence of two spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae) on grape. *Persian Journal of Acarology,* 7, 75-84.

Peixoto, M. L., Moraes, J. C., Silva, A. A., & Assis, F. A. (2011). Effect of silicon on the oviposition preference of *Bemisia tabaci* Biotype B (GENN.) (Hemiptera:

Aleyrodidae) on bean (*Phaseolus vulgaris* L.) plants. *Ciencia E Agrotecnologia*, 35, 478-481.

Pekas, A., & Wäckers, F. L. (2020). Bottom-up effects on tri-trophic interactions: Plant fertilization enhances the fitness of a primary parasitoid mediated by its herbivore host. *Journal of Economic Entomology*, 113, 2619-2626.

Pereira, P., Nascimento, A. M., de Souza, B. H. S., & Peñaflor, M. F. G. V. (2021). Silicon supplementation of maize impacts fall armyworm colonization and increases predator attraction. *Neotropical Entomology*, 50, 654-661.

Pérez-Gálvez, A., Viera, I., & Roca, M. (2020). Carotenoids and chlorophylls as antioxidants. *Antioxidants*, 9, 505.

Peterson, R. K. D., Varella, A. C., & Higley, L. G. (2017). Tolerance: the forgotten child of plant resistance. *PeerJ*, 5, e3934.

Piñera, A. V., Charles, H. M., Dinh, T. A., & Killian, K. A. (2013). Maturation of the immune system of the male house cricket, *Acheta domesticus*. *Journal of Insect Physiology*, 59, 752-760.

Ponzio, C., Gols, R., Pieterse, C. M., & Dicke, M. (2013). Ecological and phytohormonal aspects of plant volatile emission in response to single and dual infestations with herbivores and phytopathogens. *Functional Ecology*, 27, 587-598.

Preisser, E. L., Gibson, S. E., Adler, L. S., & Lewis, E. E. (2007). Underground herbivory and the costs of constitutive defense in tobacco. *Acta Oecologica*, 31, 210-215.

Quigley, K. M., Griffith, D. M., Donati, G. L., & Anderson, T. M. (2020). Soil nutrients and precipitation are major drivers of global patterns of grass leaf silicification. *Ecology*, 101, e03006.

R Core Team. (2019). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.R-project.org/

Rasband, W. S. (1997). ImageJ. U. S. National Institutes of Health, Bethesda, MD.

Rasmann, S., Köllner, T. G., Degenhardt, J., Hiltpold, I., Toepfer, S., Kuhlmann, U., . . . Turlings, T. C. J. (2005). Recruitment of entomopathogenic nematodes by insect-damaged maize roots. *Nature,* 434, 732-737.

Reddall, A., Sadras, V., Wilson, L., & Gregg, P. (2004). Physiological responses of cotton to two-spotted spider mite damage. *Crop Science,* 44, 835-846.

Redman, A. M., & Scriber, J. M. (2000). Competition between the gypsy moth, *Lymantria dispar*, and the northern tiger swallowtail, *Papilio canadensis*: interactions mediated by host plant chemistry, pathogens, and parasitoids. *Oecologia,* 125, 218-228.

Reidinger, S., Ramsey, M. H., & Hartley, S. E. (2012). Rapid and accurate analyses of silicon and phosphorus in plants using a portable X-ray fluorescence spectrometer. *New Phytologist,* 195, 699-706.

Remmel, T., Davison, J., & Tammaru, T. (2011). Quantifying predation on folivorous insect larvae: the perspective of life-history evolution. *Biological Journal of the Linnean Society,* 104, 1-18.

Reynolds, O. L., Keeping, M. G., & Meyer, J. H. (2009). Silicon-augmented resistance of plants to herbivorous insects: a review. *Annals of Applied Biology,* 155, 171-186.

Reynolds, O. L., Padula, M. P., Zeng, R., & Gurr, G. M. (2016). Silicon: potential to promote direct and indirect effects on plant defense against arthropod pests in agriculture. *Frontiers in plant science,* 7, 744.

Ribeiro, C., & Brehélin, M. (2006). Insect haemocytes: What type of cell is that? *Journal of Insect Physiology,* 52, 417-429.

Ribeiro, E. B., Castellani, M. A., de Andrade, D., Prado, R. D., Souza, L., Freire, D. S., & Savi, P. J. (2021). Biological aspects of the two-spotted spider mite on strawberry plants under silicon application. *Horticultura Brasileira,* 39, 5-10.

Rodrigues, J. H. V., Angelini, M. R., de Oliveira, R. S., & Queiroz, A. A. (2018). Silicon doses, through soil, in the induction of resistance to *Spodoptera frugiperda* in the bean plant. *Revista De Agricultura Neotropical,* 5, 13-19.

Rogers, D. J., & Brier, H. B. (2010). Pest-damage relationships for *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) on vegetative soybean. *Crop Protection,* 29, 39-46.

Rolfe, S. A., Griffiths, J., & Ton, J. (2019). Crying out for help with root exudates: adaptive mechanisms by which stressed plants assemble health-promoting soil microbiomes. *Current Opinion in Microbiology,* 49, 73-82.

Rousselin, A., Bevacqua, D., Vercambre, G., Sauge, M.-H., Lescourret, F., & Jordan, M.-O. (2018). Rosy apple aphid abundance on apple is shaped by vegetative growth and water status. *Crop Protection,* 105, 1-9.

Rowe, R. C., Trębicki, P., Gherlenda, A. N., & Johnson, S. N. (2020). Cereal aphid performance and feeding behaviour largely unaffected by silicon enrichment of host plants. *Journal of Pest Science,* 93, 41-48.

Ruxton, G. D., Sherratt, T. N., & Speed, M. P. (2005). *Avoiding attack: the evolutionary ecology of crypsis, warning signals and mimicry*. Oxford: Oxford University Press.

Ryalls, J. M. W., Hartley, S. E., & Johnson, S. N. (2017). Impacts of silicon-based grass defences across trophic levels under both current and future atmospheric CO_2 scenarios. *Biology Letters,* 13, 20160912.

Sabelis, M. W., & Van de Baan, H. E. (1983). Location of distant spider mite colonies by phytoseiid predators: demonstration of specific kairomones emitted by *Tetranychus urticae* and *Panonychus ulmi*. *Entomologia Experimentalis et Applicata*, 33, 303-314.

Savaris, M., Lampert, S., Salvadori, J. R., Lau, D., Pereira, P. R. V. S., & Smaniotto, M. A. (2013). Population growth and damage caused by *Rhopalosiphum padi* (L.) (Hemiptera, Aphididae) on different cultivars and phenological stages of wheat. *Neotropical Entomology,* 42, 539-543.

Schaefer, H. M., & Rolshausen, G. (2006). Plants on red alert: do insects pay attention? *Bioessays,* 28, 65-71.

Schausberger, P., Peneder, S., Jürschik, S., & Hoffmann, D. (2012). Mycorrhiza changes plant volatiles to attract spider mite enemies. *Functional Ecology,* 26, 441-449.

Scholthof, K.-B. G., Irigoyen, S., Catalan, P., & Mandadi, K. K. (2018). *Brachypodium*: A monocot grass model genus for plant biology. *The Plant Cell*, 30, 1673-1694.

Schoonhoven, L. M. (1990). Insect-plant interactions. *ESF Communications,* 23, 40-41.

Schoonhoven, L. M., van Loon, J. J. A., & Dicke, M. (2005). *Insect–Plant Biology* (Second ed.). Oxford: Oxford University Press.

Schuman, M. C., & Baldwin, I. T. (2016). The layers of plant responses to insect herbivores. *Annual Review of Entomology,* 61, 373-394.

Shao, Y., Spiteller, D., Tang, X., Ping, L., Colesie, C., Münchberg, U., . . . Boland, W. (2011). Crystallization of α- and β-carotene in the foregut of *Spodoptera* larvae feeding on a toxic food plant. *Insect Biochemistry and Molecular Biology,* 41, 273-281.

Shaw, N. L., Cantliffe, D. J., Funes, J., & Shine, C. I. (2004). Successful Beit Alpha cucumber production in the greenhouse using pine bark as an alternative soilless media. *HortTechnology,* 14, 289-294.

Shiao, S. H., Higgs, S., Adelman, Z., Christensen, B. M., Liu, S. H., & Chen, C. C. (2001). Effect of prophenoloxidase expression knockout on the melanization of microfilariae in the mosquito *Armigeres subalbatus*. *Insect Molecular Biology*, 10, 315-321.

Shih, C.-i. T., Poe, S. L., & Cromroy, H. L. (1976). Biology, life table, and intrinsic rate of increase of *Tetranychus urticae*. *Annals of the Entomological Society of America,* 69, 362-364.

Sidhu, J. K., Stout, M. J., Blouin, D. C., & Datnoff, L. E. (2013). Effect of silicon soil amendment on performance of sugarcane borer, *Diatraea saccharalis* (Lepidoptera: Crambidae) on rice. *Bulletin of Entomological Research,* 103, 656-664.

Simelane, D. O. (2006). Effect of herbivory by *Teleonemia scrupulosa* on the performance of *Longitarsus bethae* on their shared host, *Lantana camara*. *Biological Control,* 39, 385-391.

Singer, M. S., & Stireman, J. O. I. (2005). The tri-trophic niche concept and adaptive radiation of phytophagous insects. *Ecology Letters*, 8, 1247-1255.

Siva–Jothy, M. T. (2000). A mechanistic link between parasite resistance and expression of a sexually selected trait in a damselfly. *Proceedings of the Royal Society B*, 267, 2523-2527.

Staley, J. T., Stafford, D. B., Green, E. R., Leather, S. R., Rossiter, J. T., Poppy, G. M., & Wright, D. J. (2011). Plant nutrient supply determines competition between phytophagous insects. *Proceedings of the Royal Society B*, 278, 718-724.

Stiling, P., & Cornelissen, T. (2005). What makes a successful biocontrol agent? A meta-analysis of biological control agent performance. *Biological Control*, 34, 236-246.

Sugiura, S. (2020). Predators as drivers of insect defenses. *Entomological Science*, 23, 316-337.

Sun, H., Duan, Y., Qi, X., Zhang, L., Huo, H., & Gong, H. (2018). Isolation and functional characterization of *CsLsi2*, a cucumber silicon efflux transporter gene. *Annals of Botany*, 122, 641-648.

Sun, H., Guo, J., Duan, Y., Zhang, T., Huo, H., & Gong, H. (2017). Isolation and functional characterization of *CsLsi1*, a silicon transporter gene in *Cucumis sativus*. *Physiologia plantarum*, 159, 201-214.

Takahashi, E., Ma, J., & Miyake, Y. (1990). The possibility of silicon as an essential element for higher plants. *Comments on Agricultural and Food Chemistry*, 2, 99-102.

Tang, T., Zhao, C., Xu, L., & Qiu, L. (2016). Factors affecting larval cannibalism in the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Oriental Insects,* 50, 23-33.

Teakle, R. E., & Jensen, J. M. (1985). *Heliothis punctigera*. In P. Singh & R. F. Moore (Eds.), *Handbook of insect rearing* (Vol. 2, pp. 313-322). Amsterdam: Elsevier.

Tholl, D., Boland, W., Hansel, A., Loreto, F., Röse, U. S. R., & Schnitzler, J.-P. (2006). Practical approaches to plant volatile analysis. *The Plant Journal,* 45, 540-560.

Tiffin, P. (2001). Mechanisms of tolerance to herbivore damage: what do we know? *Evolutionary Ecology,* 14, 523-536.

Tomlin, E. S., & Sears, M. K. (1992). Indirect competition between the Colorado potato beetle (Coleoptera: Chrysomelidae) and the potato leafhopper (Homoptera: Cicadellidae) on potato: Laboratory study. *Environmental Entomology,* 21, 787-792.

Traw, M. B., & Dawson, T. E. (2002). Reduced performance of two specialist herbivores (Lepidoptera: Pieridae, Coleoptera: Chrysomelidae) on new leaves of damaged black mustard plants. *Environmental Entomology,* 31, 714-722.

Ueno, O., & Agarie, S. (2015). Silica deposition in cell walls of the stomatal apparatus of rice leaves. *Plant Production Science,* 8, 71-73.

Valadon, L. R. G., Mummery, R. S., Rothschild, M., & Feltwell, J. S. E. (1974). The relationship between carotenoids of certain Lepidoptera and Coleoptera and of their food plants. *Biochemical Society Transactions,* 2, 1061-1062.

Van Leeuwen, T., Vontas, J., Tsagkarakou, A., Dermauw, W., & Tirry, L. (2010). Acaricide resistance mechanisms in the two-spotted spider mite *Tetranychus*

urticae and other important Acari: a review. *Insect Biochemistry and Molecular Biology,* 40, 563-572.

Van Poecke, R. M., Posthumus, M. A., & Dicke, M. (2001). Herbivore-induced volatile production by *Arabidopsis thaliana* leads to attraction of the parasitoid *Cotesia rubecula*: chemical, behavioral, and gene-expression analysis. *Journal of Chemical Ecology,* 27, 1911-1928.

van Wijk, M., De Bruijn, P. J., & Sabelis, M. W. (2008). Predatory mite attraction to herbivore-induced plant odors is not a consequence of attraction to individual herbivore-induced plant volatiles. *Journal of Chemical Ecology,* 34, 791-803.

van Wijk, M., de Bruijn, P. J., & Sabelis, M. W. (2010). The predatory mite *Phytoseiulus persimilis* does not perceive odor mixtures as strictly elemental objects. *Journal of Chemical Ecology,* 36, 1211-1225.

van Wijk, M., Wadman, W. J., & Sabelis, M. W. (2006). Morphology of the olfactory system in the predatory mite *Phytoseiulus persimilis*. *Experimental & applied acarology,* 40, 217-229.

Vet, L. E. M., & Dicke, M. (1992). Ecology of infochemical use by natural enemies in a tritrophic context. *Annual Review of Entomology,* 37, 141-172.

Vicari, M., & Bazely, D. R. (1993). Do grasses fight back? The case for antiherbivore defences. *Trends in Ecology & Evolution,* 8, 137-141.

Vidal, M. C., & Murphy, S. M. (2018). Bottom-up vs. top-down effects on terrestrial insect herbivores: a meta-analysis. *Ecology Letters,* 21, 138-150.

Villarroel, C. A., Jonckheere, W., Alba, J. M., Glas, J. J., Dermauw, W., Haring, M. A., . . . Kant, M. R. (2016). Salivary proteins of spider mites suppress defenses in

Nicotiana benthamiana and promote mite reproduction. *The Plant Journal,* 86, 119-131.

Vogelweith, F., Moret, Y., Monceau, K., Thiéry, D., & Moreau, J. (2016). The relative abundance of hemocyte types in a polyphagous moth larva depends on diet. *Journal of Insect Physiology,* 88, 33-39.

Vogelweith, F., Thiéry, D., Moret, Y., Colin, E., Motreuil, S., & Moreau, J. (2014). Defense strategies used by two sympatric vineyard moth pests. *Journal of Insect Physiology,* 64, 54-61.

Vogelweith, F., Thiéry, D., Quaglietti, B., Moret, Y., & Moreau, J. (2011). Host plant variation plastically impacts different traits of the immune system of a phytophagous insect. *Functional Ecology,* 25, 1241-1247.

Vosteen, I., Weisser, W. W., & Kunert, G. (2016). Is there any evidence that aphid alarm pheromones work as prey and host finding kairomones for natural enemies? *Ecological Entomology,* 41, 1-12.

Wang, H.-S., Yu, C., Fan, P.-P., Bao, B.-F., Li, T., & Zhu, Z.-J. (2015). Identification of two cucumber putative silicon transporter genes in *Cucumis sativus*. *Journal of Plant Growth Regulation,* 34, 332-338.

Wang, J., Hüner, N., & Tian, L. (2019). Identification and molecular characterization of the *Brachypodium distachyon NRT2* family, with a major role of *BdNRT2.1*. *Physiologia plantarum,* 165, 498-510.

War, A. R., Paulraj, M. G., Ahmad, T., Buhroo, A. A., Hussain, B., Ignacimuthu, S., & Sharma, H. C. (2012). Mechanisms of plant defense against insect herbivores. *Plant Signalling & Behaviour,* 7, 1306-1320.

Waterman, J. M., Cibils-Stewart, X., Cazzonelli, C. I., Hartley, S. E., & Johnson, S. N. (2021). Short-term exposure to silicon rapidly enhances plant resistance to herbivory. *Ecology,* 102, e03438.

Waterman, J. M., Hall, C. R., Mikhael, M., Cazzonelli, C. I., Hartley, S. E., Johnson, S. N., & Cooke, J. (2020). Short-term resistance that persists: Rapidly induced silicon anti-herbivore defence affects carbon-based plant defences. *Functional Ecology,* 35, 82-92.

Wei, J.-N., Zhu, J., & Kang, L. (2006). Volatiles released from bean plants in response to agromyzid flies. *Planta,* 224, 279-287.

Welch, B. J., Obadi, O. M., & Lampert, E. C. (2017). Effects of carotenoid sequestration on a caterpillar's cryptic coloration and susceptibility to predation. *Entomologia Experimentalis et Applicata,* 163, 177-183.

Wilson-Rich, N., Dres, S. T., & Starks, P. T. (2008). The ontogeny of immunity: development of innate immune strength in the honey bee (*Apis mellifera*). *Journal of Insect Physiology,* 54, 1392-1399.

Winde, I., & Wittstock, U. (2011). Insect herbivore counteradaptations to the plant glucosinolate-myrosinase system. *Phytochemistry,* 72, 1566-1575.

Wu, J., & Baldwin, I. T. (2010). New insights into plant responses to the attack from insect herbivores. *Annual Review of Genetics,* 44, 1-24.

Wu, X., Yu, Y., Baerson, S. R., Song, Y., Liang, G., Ding, C., . . . Zeng, R. (2017). Interactions between nitrogen and silicon in rice and their effects on resistance toward the brown planthopper *Nilaparvata lugens*. *Frontiers in plant science,* 8, 28.

Ximénez-Embún, M. G., Glas, J. J., Ortego, F., Alba, J. M., Castañera, P., & Kant, M. R. (2017). Drought stress promotes the colonization success of a herbivorous mite that manipulates plant defenses. *Experimental and Applied Acarology,* 73, 297-315.

Yamasaki, A., Shimizu, K., & Eujisaki, K. (2009). Effect of host plant part on larval body-color polymorphism in *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America,* 102, 76-84.

Yang, L., Han, Y. Q., Li, P., Wen, L. Z., & Hou, M. L. (2017). Silicon amendment to rice plants impairs sucking behaviors and population growth in the phloem feeder *Nilaparvata lugens* (Hemiptera: Delphacidae). *Scientific Reports,* 7.

Yang, L., Li, P., Li, F., Ali, S., Sun, X., & Hou, M. (2018). Silicon amendment to rice plants contributes to reduced feeding in a phloem-sucking insect through modulation of callose deposition. *Ecology and Evolution,* 8, 631-637.

Yang, S., Ruuhola, T., & Rantala, M. J. (2007). Impact of starvation on immune defense and other lifehistory traits of an outbreaking geometrid, *Epirrita autumnata*: a possible causal trigger for the crash phase of population cycle. *Annales Zoologici Fennici,* 44, 89-96.

Yano, S., Wakabayashi, M., Takabayashi, J., & Takafuji, A. (1998). Factors determining the host plant range of the phytophagous mite, *Tetranychus urticae* (Acari: Tetranychidae): a method for quantifying host plant acceptance. *Experimental and Applied Acarology,* 22, 595-601.

Ye, M., Song, Y., Long, J., Wang, R., Baerson, S. R., Pan, Z., . . . Zeng, R. (2013). Priming of jasmonate-mediated antiherbivore defense responses in rice by silicon. *Proceedings of the National Academy of Sciences, USA,* 110, E3631-E3639.

Yoshida, S., Ohnishi, Y., & Kitagishi, K. (1962). Histochemistry of silicon in rice plant: III. The presence of cuticle-silica double layer in the epidermal tissue. *Soil Science and Plant Nutrition,* 8, 1-5.

Zalucki, M. P., Daglish, G., Firempong, S., & Twine, P. (1986). The biology and ecology of *Heliothis armigera* (Hübner) and *Heliothis Punctigera* Wallengren (Lepidoptera, Noctuidae) in Australia - what do we know? *Australian Journal of Zoology*, 34, 779-814.

Zhou, J., Meng, L., & Li, B. (2017). Defensive behaviors of the Oriental armyworm *Mythimna separata* in response to different parasitoid species (Hymenoptera: Braconidae). *PeerJ,* 5, e3690.

Appendices

Appendix I - Chapter 2 supplementary material

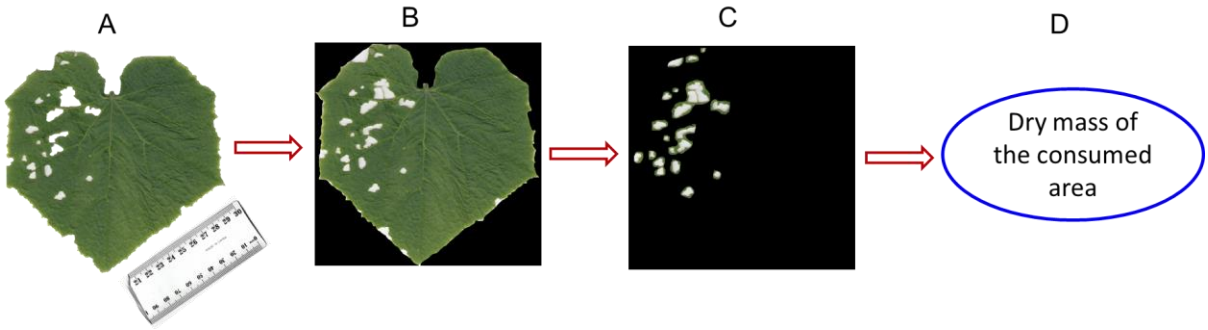

Figure S2-1 Procedures of estimating leaf dry mass consumed by *Helicoverpa armigera* larvae in the *in situ* feeding assays: A) each leaf was scanned in a flat-bed scanner with a ruler, B) total (intact) leaf area was estimated using threshold contrast with the background, C) consumed leaf portions were marked and the area was estimated and D) dry mass of the consumed leaf area was estimated as $\frac{\text{Dry mass of the remaining leaf material}}{(\text{Total leaf area} - \text{Consumed leaf area})} \times$ Consumed leaf area.

Insect bioassay data are posted on the figshare repository https://doi.org/10.6084/m9.figshare.20393979.v1

Appendix II - Chapter 3 supplementary material

Table S3-1 The amounts of volatile compounds (ng/hr/plant) released from different treatment plants. Mean ± 95% confidence interval (lower limit – upper limit) shown. Different letters in the same row between +Si +M and –Si +M and +Si –M and –Si –M plants indicate significant differences (Student's *t*-test, $p < 0.05$).

Compound	+Si +M	–Si +M	+Si –M	–Si –M
Green leaf volatiles				
3-Hexanol	2.42 [b] (2.08 – 2.79)	4.02 [a] (3.37 – 4.77)	12.80 [a] (6.83– 23.31)	4.45 [b] (2.87 – 6.66)
2-Hexanol	2.6 [b] (1.96 – 3.38)	3.84 [a] (3.34 – 4.39)	10.45 (4.91 – 21.19)	3.66 (1.98 – 6.29)
E-2-Hexenyl benzoate	3.86 (2.77 – 5.26)	2.44 (1.65 – 3.47)	2.99 (2.18 – 4.02)	3.08 (1.81 – 4.93)
Hexanal	3.89 [a] (2.55 – 5.73)	2.08 [b] (1.56 – 2.72)		
Terpenoids				
(*E*)-4,8-dimethyl-1,3,7-nonatriene	1.73 (1.22 – 2.35)	1.8 (1.36 – 2.32)		
E-*trans*-β-Ocimene	2.62 [a] (2.13 – 3.19)	1.42 [b] (0.96 - 2)		
D-Limonene	5.8 [a] (4.33 – 7.68)	3.1 [b] (2.31 – 4.08)		
Eucalyptol	4.64 (2.56 – 7.93)	4.45 (2.66 – 7.13)	3.89 (2.43 – 5.97)	8.81 (4.96 – 15.15)
Aromandendrene	1.27 (0.93 – 1.66)	1.5 (0.84 – 2.41)		

trans-Calamenene	1.13 [b]	2.69 [a]		
	(0.74 – 1.59)	(2.21 – 3.23)		
β-Caryophyllene	2.03	1.37		
	(1.42 – 2.78)	(1.04 – 1.77)		
Other compounds				
Indole	3.03	3.45		
	(2.2 – 4.08)	(2.52 – 4.63)		
o-Xylene	3.09 [b]	4.79 [a]	2.8	3.34
	(2.32 – 4.03)	(4.17 – 5.47)	(2.12 – 3.61)	(2.8 – 3.95)
o-Cymene	10.86 [b]	20.41 [a]	8.83 [a]	3.35 [b]
	(6.38 – 18.06)	(16.89 – 24.62)	(6.73 – 11.50)	(2.17 – 4.96)
Methyl salicylate	1.91 [a]	1.0 [b]		
	(1.34 – 2.63)	(0.6 – 1.49)		
1-	3.29	4.03	4.27	5.77
Methylcyclopentanol	(2.6 – 4.11)	(3.38 – 4.78)	(3.01 – 5.93)	(4.34 – 7.57)
Dodecane	3.79	4.64	3.05 [b]	5.84 [a]
	(2.4 – 5.74)	(3.85 – 5.57)	(2.19 – 4.14)	(3.99 – 8.38)

Table S3-2 Results of Student's *t*-test examining the differences in % relative emissions of volatile compounds between +Si +M and –Si +M plants and +Si –M and –Si –M plants. Statistically significant effects are highlighted in bold ($p < 0.05$).

Compound	+Si +M versus –Si +M			+Si –M versus –Si –M		
	df	*t*	*p*	*df*	*t*	*p*
Green leaf volatiles						
3-Hexanol	18	−2.56	**0.020**	10	2.99	**0.014**
2-Hexanol	18	−1.96	0.066	10	1.97	0.077
E-2-Hexenyl benzoate	18	3.13	**0.004**	10	−0.76	0.466
Hexanal	18	3.33	**0.004**			
Terpenoids						
(*E*)-4,8-Dimethyl-1,3,7-nonatriene	18	0.44	0.663			
E-*trans*-β-Ocimene	18	3.04	**0.007**			
D-Limonene	18	3.03	**0.007**			
Eucalyptol	18	0.38	0.707	10	−3.26	**0.009**
Aromandendrene	18	−0.14	0.892			
trans-Calamenene	18	−3.70	**0.002**			
β-Caryophyllene	18	2.31	**0.033**			
Other compounds						
Indole	18	−0.27	0.792			
o-Xylene	18	−2.63	**0.017**	10	−2.98	**0.014**
o-Cymene	18	−3.08	**0.007**	10	1.96	0.078
Methyl salicylate	18	3.52	**0.002**			
1-Methylcyclopentanol	18	−0.49	0.627	10	−3.27	**0.008**
Dodecane	18	−0.42	0.677	10	−3.94	**0.003**

Table S3-3 Results of Student's *t*-test examining the differences in the amounts of volatile compounds (ng/hr/plant) between +Si +M and –Si +M plants and +Si –M and –Si –M plants. Statistically significant effects are highlighted in bold ($p < 0.05$).

Compound	+Si +M versus –Si +M			+Si –M versus –Si –M		
	df	*t*	*p*	*df*	*t*	*p*
Green leaf volatiles						
3-Hexanol	18	−4.37	**<0.001**	10	2.76	**0.020**
2-Hexanol	18	−2.59	**0.018**	10	2.21	0.052
E-2-Hexenyl benzoate	18	1.86	0.079	10	−0.094	0.927
Hexanal	18	2.44	**0.025**			
Terpenoids						
(*E*)-4,8-Dimethyl-1,3,7-nonatriene	18	−0.19	0.851			
E-trans-β-Ocimene	18	3.06	**0.007**			
D-Limonene	18	3.06	**0.007**			
Eucalyptol	18	0.11	0.917	10	−2.23	0.050
Aromandendrene	18	−0.56	0.583			
trans-Calamenene	18	−4.48	**<0.001**			
β-Caryophyllene	18	1.76	0.095			
Other compounds						
Indole	18	−0.59	0.564			
o-Xylene	18	−2.89	**0.010**	10	−1.11	0.291
o-Cymene	18	−2.28	**0.035**	10	4.03	**0.002**
Methyl salicylate	18	2.37	**0.029**			
1-Methylcyclopentanol	18	−1.40	0.178	10	−1.36	0.205
Dodecane	18	−0.86	0.398	10	−2.59	**0.027**
Total volatile emission	18	−0.89	0.385	10	1.78	0.106

Table S**3-4** Shoot fresh weight (g) of different treatment plants used for volatile collection. Mean ± SE shown.

Parameter	+Si +M	–Si +M	+Si –M	–Si –M
Shoot fresh weight (g)	15.09 ± 0.48	14.79 ± 0.69	16.06 ± 0.71	15.82 ± 0.48

Table S3-5 Results of Student's *t*-test examining the differences in shoot fresh weight (g) between +Si +M and –Si +M plants and +Si –M and –Si –M plants.

Parameter	+Si +M versus –Si +M			+Si –M versus –Si –M		
	df	*t*	*p*	*df*	*t*	*p*
Shoot fresh weight	18	0.34	0.741	10	0.25	0.806

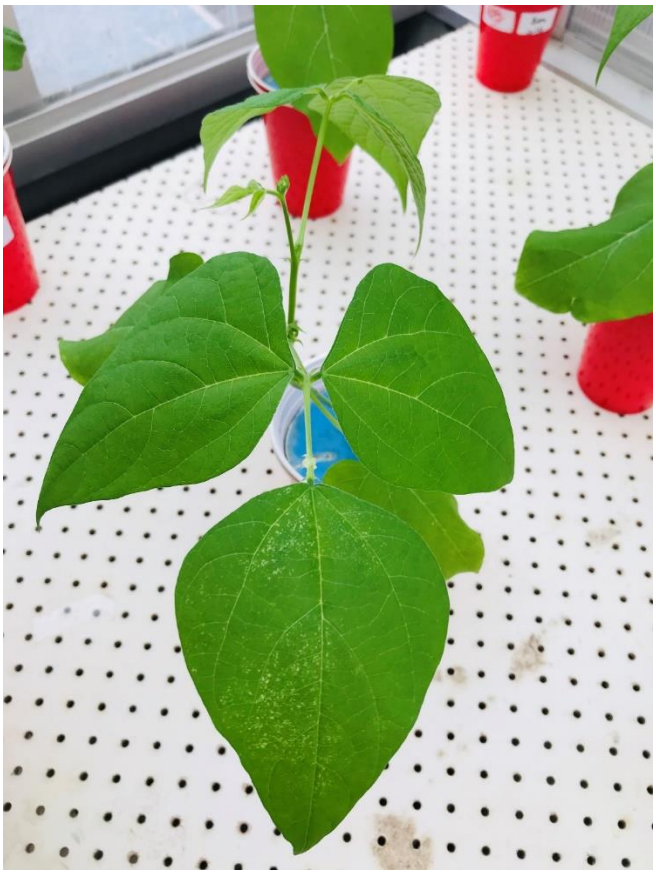

Figure S3-1 Picture showing the central leaflet of a French bean plant with *T. urticae* damage symptoms. *Tetranychus urticae* females were inoculated on the leaflet and confined by creating a lanolin barrier around the petiole.

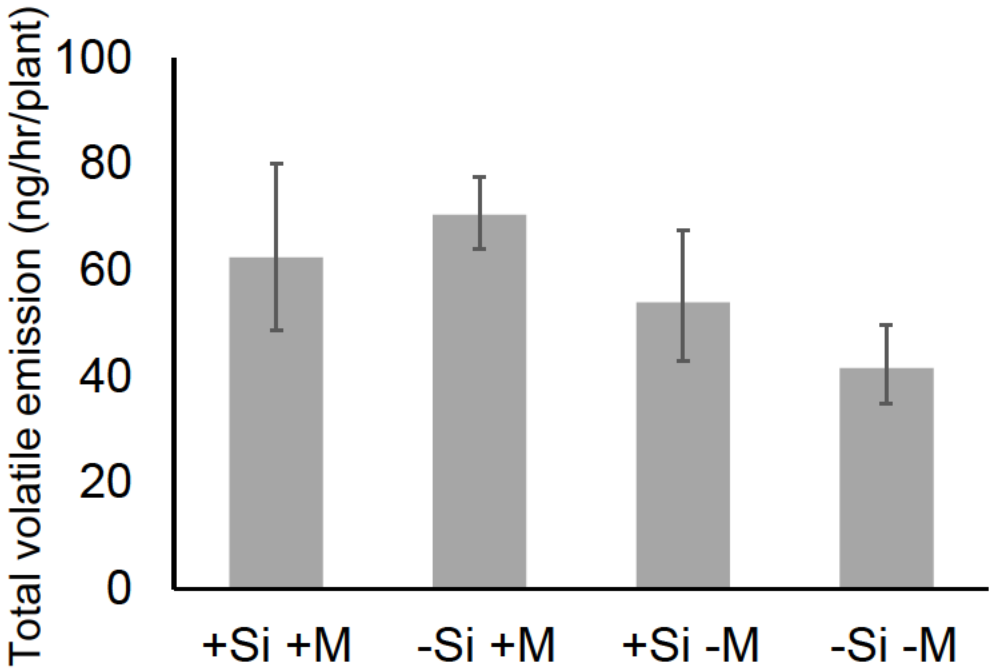

Figure S3-2 Total volatile emissions (ng/hr/plant) from different treatment plants. Mean ± 95% confidence interval shown.

Appendix III - Chapter 4 supplementary material

Table S4-1 Defensive behaviours of larvae fed on –Si and +Si plants. Mean ± SE shown (N = 20). Each defensive behaviour was compared using a Wilcoxon's rank-sum test at a 95% confidence interval.

Behavioural defence	Larvae fed on −Si plants	Larvae fed on +Si plants	Statistical analysis	
			Test statistic (W)	p value
Number of headrears	3.00 ± 0.00	2.85 ± 0.08	230	0.080
Number of thrashes	0.95 ± 0.35	1.00 ± 0.32	192.5	0.818
Number of regurgitations	0.50 ± 0.20	0.50 ± 0.17	193	0.832
Flee (no. of squares crossed)	34.38 ± 3.53	31.08 ± 2.11	111.5	0.758

Table S4-2 Summary output of Student's *t*-tests for comparing PO activity with total-PO for larvae fed on −Si and +Si plants under similar immunological conditions.

Response variable	Statistical analysis		
	df	Test statistic (*t*)	*p* value
No challenge			
Larvae fed on −Si plants	17.82	0.10	0.921
Larvae fed on +Si plants	17.24	−0.22	0.832
Challenge			
Larvae fed on −Si plants	17.71	−0.09	0.927
Larvae fed on +Si plants	17.24	−0.14	0.887

Table S4-3 Summary output of Student's *t*-tests for comparing the differences in haemocyte density and lysozyme-like activity in the haemolymph of larvae fed on –Si and +Si plants.

Response variable	Fig.	Statistical analysis		
		df	Test statistic (*t*)	*p* value
Individual haemocytes				
Prohaemocytes	S3a	18	0.32	0.752
Granulocytes		18	0.22	0.830
Plasmatocytes		18	0.66	0.520
Spherulocytes		18	0.62	0.544
Oenocytoids		18	0.29	0.777
Total haemocyte density	S3b	18	0.48	0.638
Lysozyme-like activity (No challenge)	S4	18	0.27	0.791
Lysozyme-like activity (Challenge)		18	0.11	0.914

Table S4-4 Summary output of a two-way ANOVA for the effects of Si and insect herbivory on the shoot biomass of plants harvested immediately after herbivory. Statistically significant effects ($p < 0.05$) are indicated in bold.

Response variable	Fig.	*df*	Si		Herbivory		Si × Herbivory	
			F	*p* value	*F*	*p* value	*F*	*p* value
Shoot biomass (g)	S5	1,56	263.85	**<0.001**	50.34	**<0.001**	0.38	0.539

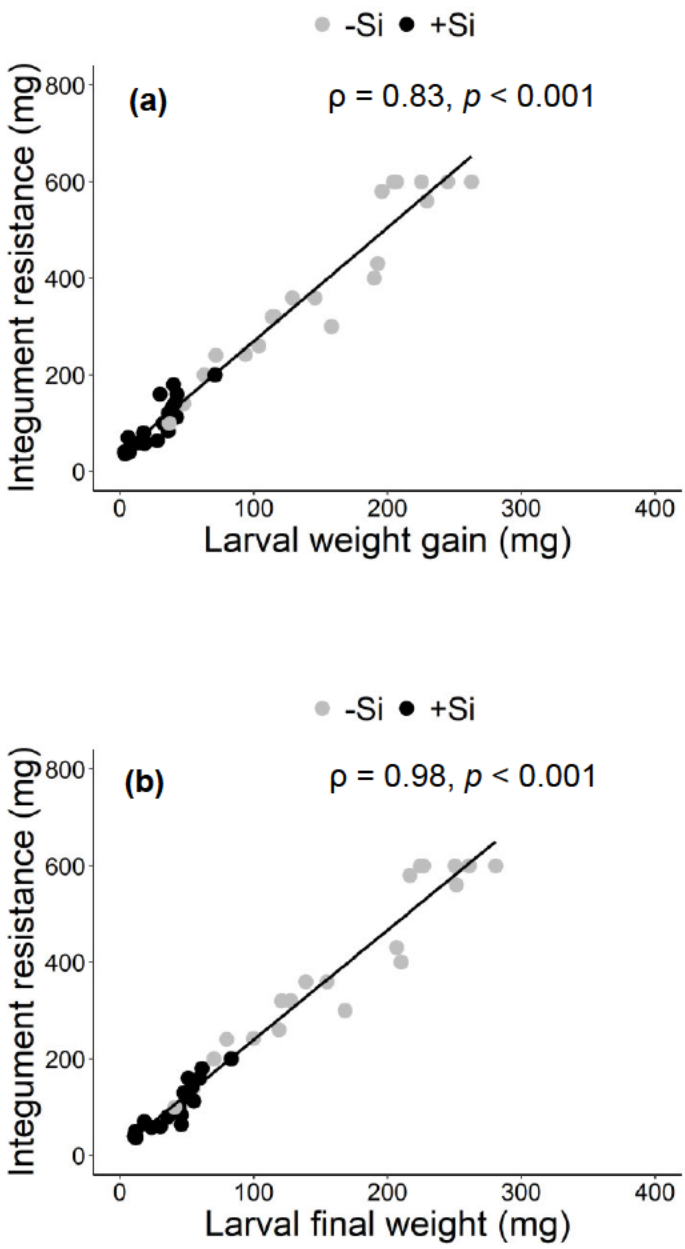

Figure S4-1 Positive correlations between (a) larval weight gain and integument resistance and (b) larval final weight and integument resistance. Larvae were allowed to feed on –Si or +Si plants for seven days before the measurements were undertaken.

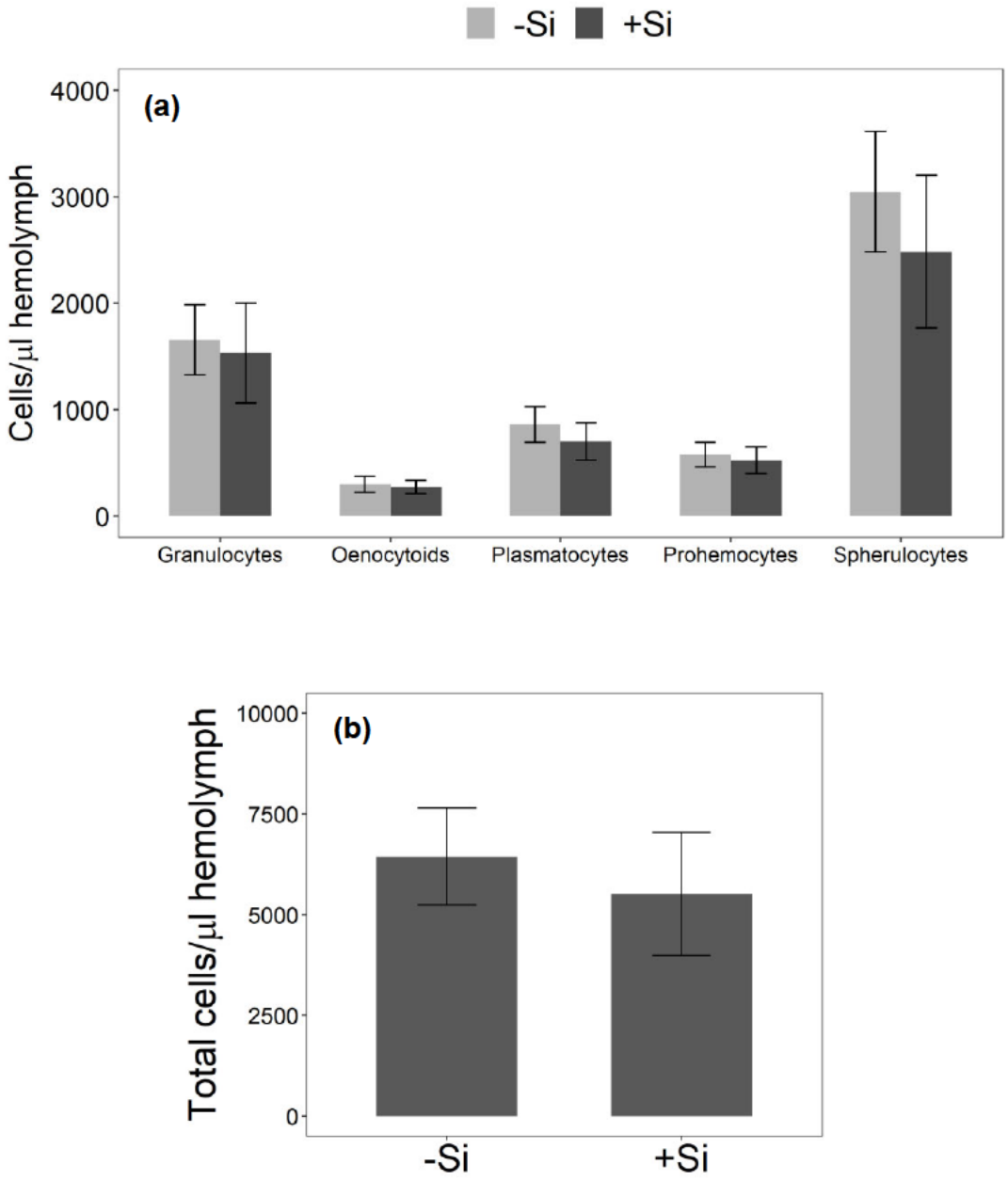

Figure S4-2 The density of haemocytes per microliter of haemolymph in larvae fed on –Si or +Si plants: (a) individual haemocyte density and (b) total haemocyte density. Mean ± SE shown ($N = 10$). Group means were compared using Student's *t*-tests.

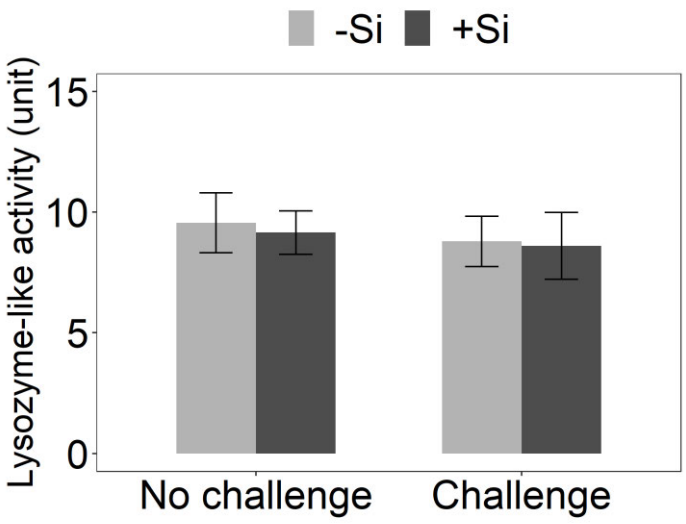

Figure S4-3 Lysozyme-like activity in the haemolymph of immunologically naïve (no challenge) and challenged larvae following feeding on –Si or +Si plants. Mean ± SE shown (*N* = 10). Group means were compared using Student's *t*-tests.

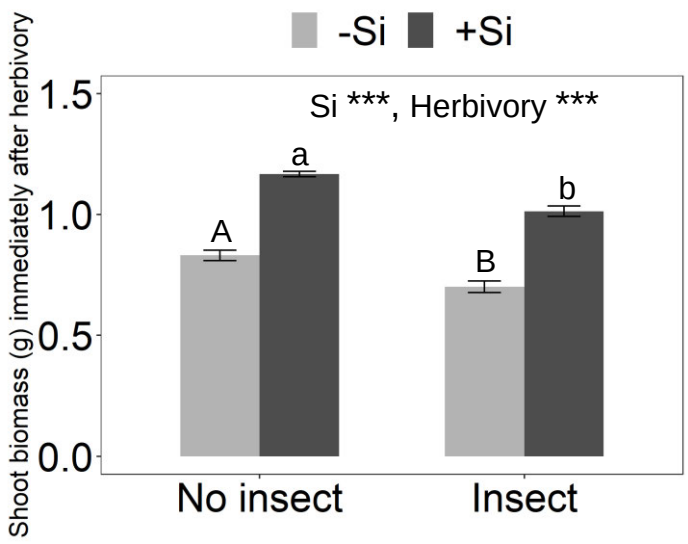

Figure S4-4 Shoot biomass of plants harvested immediately after herbivory along with insect-free control plants. Mean ± SE shown (N = 15). Data were primarily analysed using a two-way ANOVA test, and the levels of statistical significance at 95% confidence intervals are presented (*** $p < 0.001$). A Tukey's HSD test was further used to compare the group means of insect-fed, –Si and +Si plants with their insect-free counterparts. Different uppercase letters indicate a significant difference between insect-free –Si plants and insect-fed –Si plants, whereas different lowercase letters indicate a significant difference between insect-free +Si plants and insect-fed +Si plants.

Appendix IV - Chapter 5 supplementary material

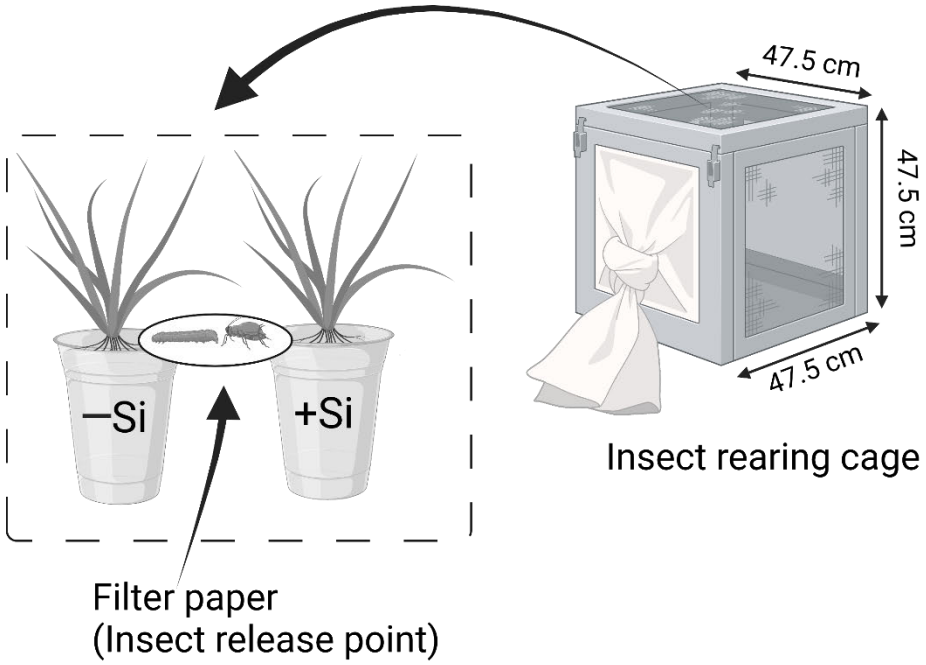

Figure S5-1 Dual-choice tests in rearing cages. Two plants (−Si and +Si) were placed side by side in each cage and filter paper was placed as a connection on top of hydroponic containers. Caterpillars or aphids were released on filter papers.