HOST-PLANT, INDUCED MUTAGENESIS AND SILICON DERIVED RESISTANCE TO APHIDS IN COMMON BEAN, (*PHASEOLUS VULGARIS* L.) AND COWPEA, (*VIGNA UNGUICULATA* (L.) WALP GENOTYPES

by

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A thesis submitted to the University of Zambia in fulfilment of the requirements for the award of the degree of Doctor of Philosophy in Plant Science (Entomology)

THE UNIVERSITY OF ZAMBIA

LUSAKA

CERTIFICATE OF APPROVAL

This thesis of **Kennedy J. Zimba** was approved as fulfilling the requirements for the award of the degree of Doctor of Philosophy in Plant Science (Entomology) by the University of Zambia.

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DECLARATION

I, **Kennedy J. Zimba** do hereby declare that the work presented in this thesis is my own and has not previously been submitted at any university for a degree award.

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ABSTRACT

Common bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata* (L.) Walp) are important food crops across Sub-Saharan Africa. In Zambia, common bean and cowpea yields are typically lower than potential yields due to the impact of plant diseases and invertebrate pests. Black bean aphid (*Aphis fabae* Scopoli) and cowpea aphid (*Aphis craccivora* Koch.) negatively impacts common bean and cowpea productivity directly by ingesting plant assimilates and indirectly by vectoring viral diseases. Aphid management has, for a long time, relied on the use of synthetic pesticides but with variable success due to the development of resistance in many target organisms. Synthetic pesticides may also be harmful to human and environmental health as well as negatively impacting non-target organisms. Current breeding programs aim to develop common bean and cowpea cultivars with improved yield and tolerance to pests. The major objective of this study was to identify and characterise resistance to aphids in common bean and cowpea genotypes while also assessing the potential of silicon to complement mutation derived aphid resistance. Specific objectives of this study were to; (i) identify and characterise resistance to black bean aphid in selected breeding lines and mutation derived common bean genotypes, (ii) identify and characterise resistance to cowpea aphid in mutation derived cowpea genotypes under laboratory and field conditions and, (iii) evaluate the performance of cowpea aphid on silicon treated mutation derived cowpea genotypes under laboratory and field conditions.

Five common bean varieties (Rozi Koko, Mwezi Moja, Majesty, KK25 and AO-1012-29-3A) and four mutation-derived genotypes (CA 3, CA 15, CA 24 and CA 38) were evaluated for resistance to black bean aphid. Bean cultivars, Kabulangeti and Carioca (variety from which all mutants were derived) were used as controls for bean varieties and mutant genotypes, respectively. Several parameters of aphid resistance traits were assessed. Aphid deterrence was assessed by settling preference while physical barriers to aphid feeding were evaluated by nymph survival. Reduction in palatability of phloem sap was evaluated by nymph development and mean relative growth rate. Electrical penetration graph recordings of feeding behaviour were performed in order to localise aphid resistance factors. A complete randomised design was adopted for all laboratory bioassays. Nymph development was significantly longer (13.6 days) on AO-1012-29-3A compared to Kabulangeti (11.2 days) despite the fact that there were significantly fewer glandular trichomes on this line, suggesting the presence of aphid resistance factors.

Cowpea genotypes derived from three susceptible varieties (Bubebe; Lutembwe and Msandile) after mutagenesis by gamma irradiation were assessed. Eleven genotypes were evaluated: six (BB 3-9-7-5, BB 7-9-7-5, BB 8-1-7-5, BB 10-4-2-3, BB 14-16-2-2 and BBV) from Bubebe, three (LT 3-8-4-1, LT 4-2-4-1 and LT 11-3-3-12) from Lutembwe and two (MS 1-8-1-4 and MS 10-7-2-1) from Msandile. Aphid resistance was evaluated by recording aphid colony growth, mean relative growth rate, intrinsic rate of natural increase, doubling time and feeding behaviour when reared on each genotype. A complete randomised design and randomised complete block design were used for laboratory bioassays and field experiments respectively. Where colony growth was recorded, aphid population was significantly reduced by; 48.5 % on BB 7-9-7-5, 69.3 % on LT 3-8-4-1, 67.5 % on LT 4-2-4-1 and 78.7 % on LT 11-3-3-12 compared to their respective parents. LT 3-8-4-1, LT 4-2-4-1 and LT 11-3-3-12 also resulted in lower aphid mean relative growth rate, intrinsic rate of natural increase and doubling time compared to the parent. Slower colony growth, mean relative growth rate, intrinsic rate of natural increase and doubling time on LT 3-8-4-1, LT 4-2-4-1 and LT 11-3-3-12 and slower colony growth only on BB 7-9-7-5 suggests the presence of mutation derived resistance to cowpea aphid. Characterisation of feeding behaviour on LT 3-8-4-1, LT 4-2-4-1 and LT 11-3-3-12 showed that resistance is mediated by epidermal and mesophyll-based factors.

Silicon accumulation capacity of these genotypes as well as aphid performance parameters, as described above, were assessed. A complete randomised design and split-plot design were used for the laboratory bioassays and field experiments respectively. Where silicon was applied, significantly higher concentrations were recorded for Lutembwe and LT 11-3-3-12 plants compared to untreated plants. Silicon application to Lutembwe resulted in slower aphid colony growth and lower mean relative growth rates compared to untreated plants, suggesting the occurrence of silicon-induced resistance. Electrical penetration graph recordings of aphid feeding on silicon-treated Lutembwe plants showed that aphid resistance is mediated by phloem-based factors. Silicon application to LT 3-8-4-1, LT 4-2-4-1 and LT 11-3-3-12 plants did not enhance aphid resistance. This may be due to mutagenesis disrupting their ability to effectively accumulate silicon or because the partial resistance of these mutant genotypes masked any benefits from silicon application to these plants.

Overall, AO-1012-29-3A is a promising common bean parent line with useful genetic attributes that may be used to develop aphid resistant common bean varieties. BB 7-9-7-5, LT 3-8-4-1, LT 4-2-4-1 and LT 11-3-3-12 are promising genotypes that should be evaluated further for genetic improvement of cowpea against the cowpea aphid. Silicon application or use of mutation-derived genotypes may be effective tools with which to manage cowpea aphid on cowpea, but there appears to be little benefit of combining these approaches. These findings have important implications for developing an integrated pest management framework for aphid pests on common bean and cowpea.

DEDICATION

To my wife Chiluba Zimba, children: Walusungu, Chimwemwe, Tawonga and Amos. Thank you all for your love and support.

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CHAPTER ONE

1 INTRODUCTION

1.1 Background

Common bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata* (L.) Walp.), are important leguminous food crops cultivated across sub-Saharan Africa (Ronner *et al.*, 2018; Boukar *et al.*, 2019). The grain and leaves of these staple crops have a high protein content, approximately 23 and 44 % respectively, providing an excellent source of dietary protein and livestock fodder (Horn *et al*. 2016; Samireddypalle *et al*. 2017). Alongside high protein content, common bean and cowpea are also a rich source of vitamins, micronutrients and amino acids (Jayathilake *et al.*, 2018), which are extremely low in sub-Saharan Africa diets typically dominated by cereals (Okoth *et al.*, 2017). Common bean and cowpea consumption is further encouraged due to their high polyphenolic, flavonoids and bioactive peptide levels, which can reduce risk of certain health conditions like obesity or heart disease (Jayathilake *et al.*, 2018). Being drought tolerant, cowpea can be grown in areas with marginal rainfall and limited irrigation potential (Agbicodo *et al.*, 2009). Like most legumes, common bean and cowpea are able to fix atmospheric nitrogen through rhizobium symbiosis (Ehlers & Hall, 1996) and are adapted to grow in nutrient deficient soils (Elowad & Hall, 1987). Despite the economic and agronomic importance, yields produced by these crops in sub-Saharan Africa are low, ranging from 300-500kg in common bean and 250-350 kg/ha in cowpea, compared to potential yields of 2000 kg/ha in both crops (Boukar *et al.*, 2019). Insect pests, particularly aphids and the viral diseases they transmit, are among major constraints to achieving optimal yields (Saranya *et al.*, 2010).

Black bean aphid, *Aphis fabae* Scopoli (Homoptera: Aphididae) and cowpea aphid (*Aphis craccivora* Koch.) (Homoptera: Aphididae), are economically important pests of common bean and cowpea respectively, in sub-Saharan Africa (Abate & Ampofo, 1996; Pettersson *et al.*, 1998). Aphids damage crops directly by feeding on phloem sap or indirectly through transmission of diseases (Ofuya 1997; Obopile & Ositile 2010). While estimates in Zambia are not available, common bean yield losses of up to 37 % in Uganda have been attributed to black bean aphid (Mwangi *et al.*, 2009). Cowpea aphid can cause yield losses of up to 50 % in the absence of control measures. As aphids preferentially feed on seedlings, when plants are at their most vulnerable stage, large populations can reduce plant health (*e.g.*, stunting) through direct feeding (Huynh *et* *al.*, 2015). Ingestion of large volumes of phloem sap by aphids results in excretion of excess sugars as honeydew (Wilkinson & Douglas, 2003), which provides a growth substrate for sooty moulds that reduce photosynthesis (Ouédraogo *et al.*, 2018). The cumulative effect of feeding damage and sooty mould growth is stunting, delayed flowering, abortion of flower buds and plant death (Jackai & Daoust, 1986). Cowpea aphid also transmits Cowpea Mosaic Virus (CMV), a major disease that causes between 10% and 100% yield losses (Atiri *et al*., 1986; Taiwo *et al*., 2007).

Several strategies are used in Zambia to control aphids on common bean crops, but each has limited efficacy. Early planting is one cultural measure used to prevent susceptible seedlings coinciding with high aphid populations soon after the cropping season begins (Musenga *et al.*, 2016). Another widely used measure is intercropping common bean with cereal crops like maize, which has been found to reduce aphid colonisation of common bean plants (Ogenga-Latigo *et al.*, 1993). Foliar application of synthetic insecticides such as deltamethrin, cypermethrin, lambda-cyhalothrin and thiamethoxam early in the season can also help to reduce aphid colonisation of seedlings (Musenga *et al.*, 2016). The use of synthetic insecticides, however, may be detrimental to pollinators and natural enemies (Desneux *et al.*, 2006) as well as human health (Carvalho, 2017; Kim *et al.*, 2017). Black bean aphid is also known to have developed resistance to certain synthetic insecticides (e.g., carbamates and organophosphates), further reducing the reliability of chemical control (van Emden & Harrington, 2017). In addition to environmental and health concerns, high costs and limited availability further prevents widespread use of synthetic insecticides by resource poor farmers (Souleymane *et al.*, 2013). Therefore, there is an urgent need for cost-effective and sustainable alternatives to manage black bean aphid on common bean.

Developing common bean and cowpea cultivars that are resistant to aphid pests could provide a sustainable, environmentally friendly and cost effective option for their management (Miklas *et al.*, 2006; Mwangi *et al.*, 2009). Aphid resistance traits in plants may be classified in to three categories: (i) chemical deterrence to settling, (ii) physical barriers to feeding, and (iii) reduction in palatability (Züst & Agrawal, 2016; Nalam *et al.*, 2019). Plant cells on leaf surfaces often harbour lipids and secondary metabolites that may release aphid deterrent volatiles (Nalam *et al.*, 2019). Trichomes on plant surfaces provide a physical barrier to aphid movement and feeding (Jaouannet *et al.*, 2014). Plants may contain compounds such as protease inhibitors and lectins which reduce palatability of phloem sap to aphids. Lectins bind to carbohydrates in the midgut of insects, interfering with their digestion processes and consequently reducing the performance of aphids (Chougule & Bonning, 2012). Protease inhibitors interfere with protease function in herbivorous insects and inhibit protein metabolism (Zhu-Salzman & Zeng, 2015). These anti-aphid plant traits may be expressed either constitutively or induced by feeding (Westwood & Stevens, 2010; Smith & Chuang, 2014).

To successfully breed resistant cultivars, sources of resistance are needed. Such resistance sources could include existing cultivars, wild relatives of crops, germplasm collections or induced mutations (Omoigui *et al.*, 2017; Olasupo *et al.*, 2018). Mutations can be induced by exposing plant propagules to physical or chemical mutagens that cause DNA changes, resulting in altered traits of treated plants (Novak & Brunner, 1992; Mba *et al.*, 2010). Such induced mutations also often produce genes or alleles not present in the natural population, increasing the chances of generating novel resistance traits (Novak & Brunner, 1992). Mutants showing desired traits could be used as parental genotypes for future breeding programs or further processed into varieties using systematic breeding procedures (Mba *et al.*, 2010). Selected examples of legume cultivars developed through induced mutations include high protein cowpea (Adekola & Oluleye, 2007), drought tolerant cowpea, high yielding cowpea (Horn *et al.*, 2015) and early maturing common bean (Tulmann Neto *et al.*, 2011).

1.2 Statement of the problem

In Zambia, common bean and cowpea yields are typically lower compared to potential yields. Black bean aphid and cowpea aphid negatively impacts common bean and cowpea productivity directly by ingesting plant assimilates and indirectly by vectoring viral diseases. Aphid management has, like for many crop pest species, predominantly relied on the application of synthetic pesticides (Roubos *et al.,* 2014; van Emden & Harrington, 2017). While use of synthetic pesticides has greatly enhanced crop productivity during the last century (Naik *et al.*, 2019), issues of resistance due to their overuse as well as their negative effects on human and environmental health are now widely recognized (Kim *et al.*, 2017). Public concerns regarding pesticide use, particularly chemical residues on harvested crops and their products, have also increased significantly during the past few decades (Dunlap & Beus, 1992; Schaub *et al.,* 2020). Globally, the United Nation's Sustainable Development Goals advocate sustainable agri-food production (FAO, 2018), further limiting the use of synthetic pesticides. Therefore, aphid control options that optimise crop yields while also enhancing biodiversity and food safety are urgently needed (Crowder & Jabbour, 2014; Smith & Chuang, 2014). Additionally, aphids often overcome varietal aphid resistance by the emergence of biotypes adapted to survive on resistant plants (Yates & Michel, 2018). Aphid species constitute over 50 % of all virulent arthropod biotypes (Smith & Chuang 2014). For example, single dominant genes *Rac-1* and *Rac-2* found in most African cowpea cultivars that historically conferred resistance to cowpea aphid have succumbed to resistance-breaking aphid biotypes (Boukar *et al.*, 2019).

1.3 Justification of study

Resistant crop varieties contribute to reduced reliance on synthetic pesticides and as such promotes biodiversity and natural pest suppression (Pertot *et al.*, 2017). Timely supply of resistant varieties through conventional breeding approaches is, however, difficult due to limited genetic variability for aphid resistance in most crop species (Mensah *et al.*, 2005; Dogimont *et al.*, 2010; Macaulay *et al.*, 2020). Induced mutagenesis may offer a quicker and effective method to introduce genetic variation into plants for traits like aphid resistance (Penna & Jain, 2017). Although much focus has been given to addressing pathogen resistance in crops using induced mutagenesis (Gottschalk & Wolff, 2012), this approach could also improve breeding efficiency for aphid-resistant varieties.

In order to address disease and pest constraints to common bean and cowpea productivity in Zambia, a technical cooperation project was initiated between the IAEA and the government of Zambian-research institutions including University of Zambia (UNZA), Zambia Research Institute (ZARI) and National Institute for Scientific Research (NISIR), to improve the performance of local varieties through induced mutation using gamma irradiation. Additionally, a separate bean breeding programme, using molecular techniques, has been established at UNZA (Department of Plant Science) to augment existing efforts. Through these breeding programmes, a range of common bean and cowpea genotypes with promising levels of resistance to diseases and insect pests have been developed. Identification and characterisation of aphid resistance in these genotypes is needed to facilitate deployment of aphid resistance into existing varieties or for use as parent lines in breeding programmes. One approach to address the problem of virulent aphid biotypes is to combine resistant cultivars with other aphid control options in an IPM framework. Silicon application to plants confers a mechanical barrier to insect herbivory and may alter plant chemistry to increase biochemical defense in treated plants. Enhancement of host-plant aphid resistance through silicon application could further enhance durability of aphid resistance in mutation derived cowpea genotypes.

1.4 Study objectives

1.4.1 Major objective of study

The major objective of this study was to identify and characterise resistance to aphids in common bean and cowpea genotypes while also assessing the potential of silicon to complement mutation derived cowpea resistance to aphids.

1.4.2 Specific objective of study

The specific objectives of the study were to;

(i) Characterise black bean aphid resistance in selected breeding lines and mutation derived common bean genotypes
(ii) Characterise cowpea aphid resistance in mutation derived cowpea genotypes under laboratory and field conditions
(iii) Evaluate the performance of cowpea aphid on silicon treated cowpea mutants under laboratory and field conditions

1.5 Hypotheses tested in the study

This study tested the following hypotheses that:

(i) There is genetic variation for aphid resistance in selected breeding lines and mutation derived common bean genotypes
(ii) There is genetic variation for aphid resistance in mutation derived cowpea genotypes
(iii) Silicon application complements aphid resistance in mutation derived cowpea genotypes

CHAPTER TWO

2 LITERATURE REVIEW

2.1 Economic importance of common bean and cowpea

Common bean and cowpea are among the most important food legume crops grown in sub-Saharan Africa (Ronner *et al.*, 2018; Snapp *et al.*, 2018). In Zambia, common bean is the second most widely cultivated legume crop with a total production area of 84,500 hectares (ha) and an average annual production of 52,300 tonnes (t) (Chapoto *et al.*, 2019). Common bean and cowpea provide affordable sources of protein, particularly for women and children in low income communities who cannot afford animal protein sources (Mweetwa *et al.*, 2016; Tembo *et al.*, 2019). Protein content of common bean and cowpea are high, averaging 25% and 26%, respectively (Singh & Rachie, 1985; Mweetwa *et al.*, 2016; Tembo *et al.*, 2017). Both crops are rich in amino acids such as lysine and tryptophan, which are extremely low in the cereal dominated diets in Africa (Mweetwa *et al.*, 2016). These crops are also rich in dietary fibre, starch, vitamins and micronutrients (Mweetwa *et al.*, 2016; Wainaina *et al.*, 2019). Consumption of common bean and cowpea is further encouraged due to the high content of polyphenolic compounds, flavonoids and bioactive peptides found in these crops, which have potential health benefits such as reducing the risk of diabetes, colon cancer, obesity and heart disease (Ramírez-Jiménez *et al.*, 2015; Lin *et al.*, 2016; Awika & Duodu, 2017).

In addition to nutritional benefits, common bean and cowpea are increasingly important for generating income, helping to sustain livelihoods in many farming communities (Sichilima *et al.*, 2016; Tembo *et al.*, 2019). Cowpea is a low input crop able to grow in nutrient deficient soils and tolerates abiotic stresses such as heat and drought, making it a climate-change tolerant crop (Horn *et al.*, 2015; Awika & Duodu, 2017). As legumes, common bean and cowpea form symbiotic relationships with soil rhizobia (bacteria of the genera *Rhizobium*) enabling them to fix atmospheric nitrogen, substantially increasing its availability in the soil by up to 40-80 kg/ha annually (Sikombe *et al.*, 2003; Ngalamu *et al.*, 2015). High biomass production and soil cover reduces soil erosion while increasing soil structure and organic matter content (Sikombe *et al.*, 2003; Tembo *et al.*, 2017). Therefore, common bean and cowpea are important crops for soil fertility improvement and inclusion in mixed or rotation cropping systems with nitrogen demanding crops like maize (*Zea mays* L.) (Chekanai *et al.*, 2018; Simunji *et al.*, 2019). However, productivity of most common bean and cowpea varieties is low with average yields of 0.3-0.5 t/ha, compared to yield potentials of ~2 t/ha for both crops (Simunji *et al.*, 2019; Tembo *et al.*, 2019). Pests and diseases are the major factors limiting productivity, causing up to 50 % reduction in grain yield (Mweetwa *et al.*, 2016).

2.2 Insect pests of common bean and cowpea

Several insect species associated with common bean and cowpea are considered pests, attacking the crops both pre- and post-harvest (Table 2.1). Aphids are among the most important insect pests in Zambia, particularly the black bean aphid and cowpea aphid (Siisii & Moonga, 2015; Musenga *et al.*, 2016). Nymphs and adults feed on plant phloem sap, a highly nutrient rich resource required for plant growth and development (Singh & Rachie, 1985; Abate & Ampofo, 1996). In Africa, black bean aphid and cowpea aphid reproduce entirely by parthenogenesis, enabling large populations to rapidly build up making the control of these pests difficult (Singh & Rachie, 1985). Feeding damage causes leaf distortion, stunting and, in extreme cases, seedling death (Laamari *et al.*, 2008). Both aphid species transmit important viral diseases such as the Bean Common Mosaic Virus (BCMV) and Cowpea Mosaic Virus (CMV) (Spencer & Walkey, 1994; Ramanujam *et al.*, 2017; Wainaina *et al.*, 2019). It is well established that virus transmission has a greater impact on plant productivity than the direct feeding damage caused by aphids on these crops (Singh & Rachie, 1985; Worrall *et al.*, 2015).

Table 2.1 Major insect pests of common bean and cowpea in Africa

Common name	Scientific name	Crop	Damaged parts	Reference
Bean bruchids	*Acanthoscelides obtectus* *Zabrotes subfasciatus*	Bean	Dry seed	Schoonhoven *et al*., 1983
Bean stem maggot	*Ophiomyia* spp.	Bean	Seedling stems	Rodríguez De Luque & Creamer, 2014
Coreid bugs	*Anoplocnemis curvipes* *Clavigralla* spp.	Cowpea	Green pods	Singh & Rachie, 1985
Aphids	*Aphis craccivora* *Aphis fabae*	Bean, cowpea	Leaf, flower, flower bud, green pods	Cardona & Karel, 1990
Cowpea bruchids	*Callosobruchus maculatus* *Callosobruchus chinensis*	Cowpea	Dry seed	Silva *et al.*, 2004
Foliage beetle	*Ootheca* spp.	Bean, cowpea	leaves	Cardona & Karel, 1990
Legume bud thrips	*Megalurothrips sjostedti*	Cowpea	Flower, flower bud	Singh & Rachie, 1985
Pod borer	*Helicoverpa armigera* *Maruca testulalis*	Bean, cowpea	Flower, flower bud, green pod	Sharma, 1998
Whitefly	*Bemisa tabaci*	Bean	Leaves	Abate & Ampofo, 1996

2.2.1 Black bean aphid

2.2.1.1 Distribution

Aphis fabae has a broad distribution, having been reported in the tropical, subtropical and temperate regions of the world (Blackman, 1974; Hort & Birch, 1984; Abate & Ampofo, 1996; Sileshi *et al.*, 2000; Kataria & Kumar, 2012; Mwanauta *et al.*, 2015; van Emden & Harrington, 2017). Despite this, distribution of this aphid species is primarily concentrated in temperate regions of the northern hemisphere (van Emden & Harrington, 2017).

2.2.1.2 Biology

At a temperature of 26.5 °C, black bean aphid takes about 8 days to complete four nymphal instar stages and reach the adult stage (Ogenga-Latigo & Khaemba, 1985), which may live for ~ 6-15 days (Cardona & Karel, 1990). Nymphs are pale green but darken with age (Abate & Ampofo, 1996) while adults are shiny or matt black with white wax or powdery patches on the dorsal segments of the abdomen, including pale or white patches on legs (van Emden & Harrington, 2017) (Figure 2.1). Apterous adults are ~ 1.5-3.1 mm long whereas alate adults are markedly smaller at ~ 1.3-2.6 mm (Abate & Ampofo, 1996) (Figure 2.1).

Figure 2.1: Adult apterous morphs of black bean aphid (source: https://www.flickr.com/photos/148351600@N05/37676889454/)

In temperate climates, black bean aphid has a heteroecious life cycle, during which it feeds on two unrelated species of host plants to complete its life cycle (Cammell *et al.*, 1978) (Figure

2.2). *Aphis fabae* feeds on over 200 secondary host plants, including crops such as common bean in summer but overwinters through an egg stage on single host plant, the spindle tree (*Euonymus europaeus* L.) (van Emden & Harrington, 2017) (Figure 2.2). In tropical regions of Africa, such as Zambia, black bean aphid is known to reproduce entirely by parthenogenesis, surviving on leguminous plants throughout the year (Abate & Ampofo, 1996). Asexual reproduction on crops throughout the summer leads to exponential population build-up of aphids (Blackman, 1974). As populations become denser, aphids begin to compete for feeding sites and the nutritional quality of the plants also deteriorates, stimulating alate morph production for dispersal to new plants (Buruchara *et al*., 2010).

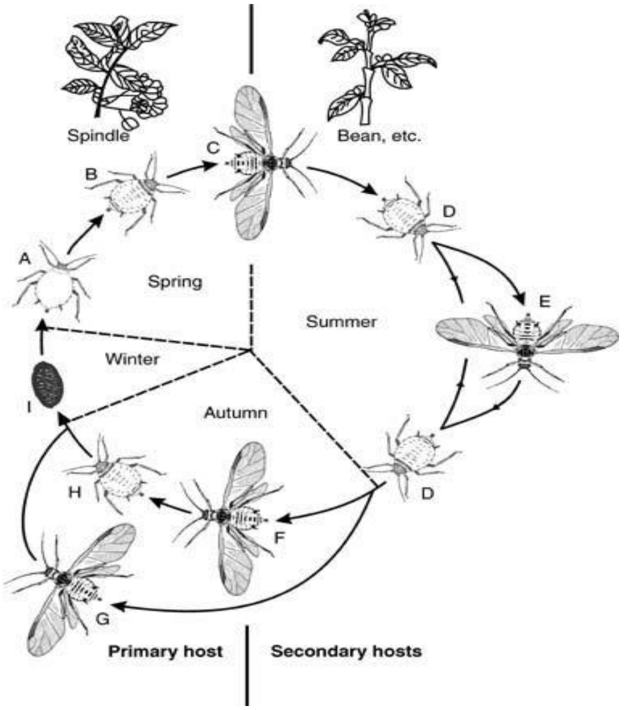

Figure 2.2: Illustration of a tropical-holocyclic (C-D-E) and temperate-heterocyclic (A-B-C-D-E-F-G-H-I) life cycles of black bean aphid on primary and secondary hostplants. A-C=fundatrix or stem mother, D=virginoparae, E=alate virginoparae, F=gynoparae, G=sexual male, H=oviparae, I=Egg (Source: Sullivan 2004)

2.2.2 Cowpea aphid

2.2.2.1 Distribution

Aphis craccivora has been reported in most parts of the world including Africa (Huynh *et al.*, 2015), Asia (Omkar *et al.*, 2005), Australia (Kamphuis *et al.*, 2012), the Middle East (Soffan & Aldawood, 2014), Europe (Borowiak-Sobkowiak *et al.*, 2017), and Americas (Nuessly *et al.*, 2004). However, its centre of origin is thought to be Southern Europe or the Middle East (Borowiak-Sobkowiak *et al.*, 2017; van Emden & Harrington, 2017).

2.2.2.2 Biology

The mean development period of cowpea aphid on cowpea from birth to adult is 5 days at 25 °C, progressing through four nymphal instar stages (Srikanth & Lakkundi, 1988; Borowiak-Sobkowiak *et al.*, 2017). Nymphs are light brown at birth but darken as they develop (Singh & Singh, 2017) while adults are dark brown in colour with a harder, shiny black dorsal surface (van Emden & Harrington, 2017). Body length for adult aphid ranges between 2.0–2.4 mm in apterae and 1.75–2.15 mm for alate individuals (Borowiak-Sobkowiak *et al.*, 2017) (Figure 2.3). In addition, the femur of adults is covered by fine hairs while the cauda bears 10-19 hairs (Abate & Ampofo, 1996). Antennal length for apterae and alate individuals is 1.22–1.45 mm and 1.17–1.40 mm respectively (Borowiak-Sobkowiak *et al.*, 2017).

Figure 2.3: Adult apterous morph of cowpea aphid (source: https://www.flickr.com/photos/116710445@N06/12398559693/)

Cowpea aphid is autoecious, mainly surviving on leguminous plants throughout the year (van Emden & Harrington, 2017). In tropical regions, cowpea aphid is anholocyclic, reproducing exclusively through parthenogenesis (Abate & Ampofo, 1996). In other regions such as parts of, Asia, South America and Europe, cowpea aphid is holocyclic, spending its sexual phase on various

Fabaceae and Menispermaceae (i.e., *Tinospora cordifolia* Miers) plants (Basu *et al.*, 1968; van Emden & Harrington, 2017). During cropping seasons in tropical Africa, populations of cowpea aphid primarily survive on legume crops (i.e., cowpea, groundnuts, common bean). Common weed species such as *Euphorbia hirta* Linn., *E. prostrata* Ait., *Gliricidia sepium* Jacq., *Cassia tora* Linn., *Centrosema pubescens* Ben. or *Macroptilium atropurpureum* Urb. serve as "green bridges" between cropping seasons (Davies, 1972). Apterous morphs produce dense populations on susceptible host plants (Abate & Ampofo, 1996). As the dietary status of plants deteriorates, alate morphs are produced to facilitate migration to new plants (Abate & Ampofo, 1996).

2.3 Economic importance of black bean aphid and cowpea aphid

2.3.1 Host range

Aphis fabae has been recorded on more than 200 plant species worldwide (Esmaeili-Vardanjani *et al.*, 2013), including field bean (*Vicia faba* L.) (Hort & Birch, 1984), common bean (*Phaseolus vulgaris* L.) (Mwanauta *et al.*, 2015), sugar beet (*Beta vulgaris* L.) (Kennedy & Booth, 1951), cowpea (*Vigna unguiculata* (L.) Walp.) (Singh & Rachie, 1985), pigeon pea (*Cajanus cajan* L. Millsp.) (Cardona & Karel, 1990), sesban (*Sesbania sesban* L.) (Sileshi *et al.*, 2000), sunflower (*Helianthus annuus* L.) (Kataria & Kumar, 2012), tomato (*Lycopersicon esculentum* L.) (Kataria & Kumar, 2012) and potato (*Solanum tuberosum* L.) (Kataria & Kumar, 2012). Several weed and ornamental plants are also suitable hosts - i.e., pigweed (*Chenopodium album* L.), marigold (*Tagetes erecta* L.), *Crysanthemum* spp. and Canada thistle (*Cirsium arvense* L. Scop.) (Tomanović *et al.*, 2003; Kataria & Kumar, 2012).

Aphis craccivora is highly polyphagous, attacking more than 400 plants species in 19 different families, although it has a preference for legumes (Singh & Singh, 2017; van Emden & Harrington, 2017). In Africa, cowpea aphid has been reported on many crops including, cowpea (Obopile & Ositile, 2010), common bean (Laamari *et al.*, 2008), soybean (*Glycine max L.*) (Zhaozhi *et al.*, 2017), groundnut (*Arachis hypogaea* L.) (Farrell, 1976), chickpea (*Cicer arietinum* L.) (Abraham *et al.*, 2006), cotton (*Gossypium* spp.) (Zhaozhi *et al.*, 2017), field bean (*Vicia faba* L.) (Laamari *et al.*, 2008), including some pasture plants (Singh & Singh, 2017).

2.3.2 Pest status on common bean and cowpea

Black bean aphid (Figure 2.4a) and cowpea aphid (Figure 2.4b) are among the important pests responsible for limiting yields of common bean and cowpea in Africa (Benchasri *et al.*, 2011;

Horn & Shimelis, 2013). Feeding damage by nymph and adult aphids is caused by their stylets being inserted into a plant in order to feed on photosynthates from the phloem, causing hormonal imbalances and distorted growth (Morkunas & Mai, 2011). As aphids preferentially feed on seedlings, large aphid populations can lead to considerable feeding damage and plant death (Abate & Ampofo, 1996). Due to the low protein content of the sugar rich photosynthates (Blackman, 1974; Züst & Agrawal, 2016), aphids consume large volumes of phloem sap from the plant in order to meet their demand for amino acids. This process deprives the plant of an essential nutrient resource required for growth and leads to stunting (Blackman & Eastop, 2000). Ingestion of large volumes of phloem sap by aphids results in excretion of copious volumes of excess sugars in form of honeydew (Wilkinson & Douglas, 2003). Honeydew provides a good substrate for the growth of black sooty mould, which blocks photosynthesis in the leaves and pods of common bean and cowpea plants (Laamari *et al*., 2008). The cumulative effect of feeding damage and sooty mould growth results in stunting, delayed flowering, flower bud abortion and occasionally death of the plant (Mwangi *et al*., 2009).

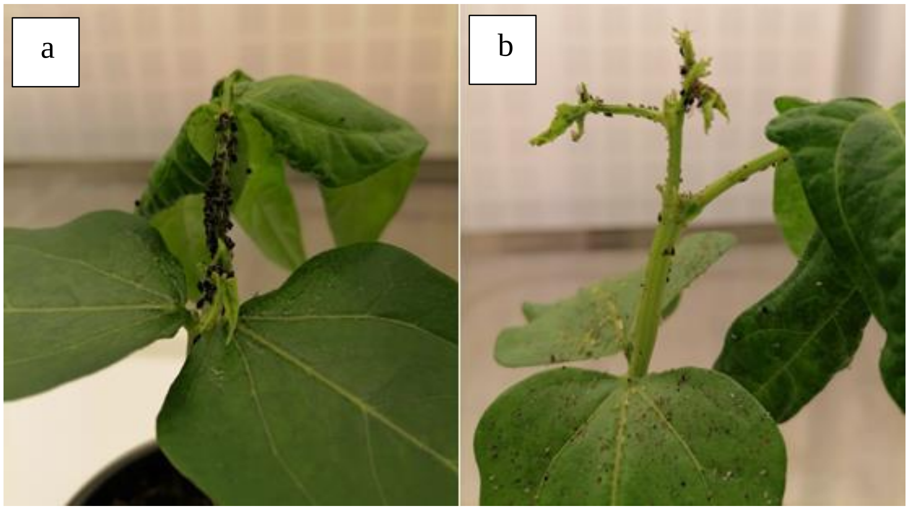

Figure 2.4: Infestation of; (a) common bean and (b) cowpea by black bean aphid and cowpea aphid, respectively

In addition to direct feeding damage caused by black bean aphid and cowpea aphid to common bean and cowpea, these aphid species also cause indirect damage through vectoring of economically important viral diseases such as Bean Common Mosaic Virus (BCMV) (Figure 2.5) and Cowpea Mosaic Virus (CMV) (Spencer & Walkey, 1994; Larocca *et al.*, 2011; Ramanujam *et al.*, 2017). The impact of viral diseases vectored by aphids is often economically more important than direct feeding damage (Mwangi *et al.*, 2009). Yield losses attributed to these viral diseases range between 80-100 % in cowpea and up to 100 % in common bean (Wainaina *et al.*, 2019). Both BCMV and CMV are "non-persistent", meaning that the virus particles are carried on the mouthparts (stylets) of aphids and are easily transmitted from one plant to another during feeding (Blackman, 1974; Abate & Ampofo, 1996; Miklas *et al.*, 2006). These diseases produce similar symptoms, such as: mosaic, stunting, leaf curling and necrosis (Spencer & Walkey, 1994; Worrall *et al.*, 2015). The spread of aphid vectored viral diseases on common bean and cowpea can be rapid, particularly when large numbers of alate aphids are produced (Blackman, 1974).

Figure 2.5: Symptoms of bean common mosaic virus (BCMV) on common bean plant

2.4 Management of black bean aphid and cowpea aphid

2.4.1 Monitoring

Prasanna *et al.*, (2018) defined pest monitoring as a process of tracking the presence, population and distribution of pests in a given area. It is an important action on which integrated pest management (IPM) programmes may be based (Cullen *et al.*, 2000; Abrol & Shankar, 2012).

Monitoring ensures early detection of pest invasions, selection of appropriate management options and timing of control operations (van Emden & Harrington, 2017; Yuan *et al.*, 2017). Aphids usually migrate from overwintering areas to crop fields soon after onset of the cropping season. Therefore, timely detection and control of the initial population of aphids is necessary to prevent population build up later in the season (Knight & Camell, 1994; van Emden & Harrington, 2017). Pest monitoring enhances the cost effectiveness of pesticides and environmental stewardship by ensuring that these products are only applied when necessary (Tatchell, 1985).

In Zambia, growers of common bean begin scouting for black bean aphid on plants twice a week from the 2-3 leaf stage (GS 11-12) (Lancashire *et al.*, 1991) until podding (GS 79) (Siisii & Moonga, 2015). In particular, they look for the presence of aphids under the leaves as well as honeydew and black sooty mould on the upper surface of leaves (Cardona & Karel, 1990). Control efforts are considered when more than 30 aphids are found on individual plants (Siisii & Moonga, 2015). Similarly, cowpea growers begin monitoring for cowpea aphid about 2 weeks after plant emergence, when plants are typically at GS 11, and scouting continues twice a week until podding (Musenga *et al.*, 2016). Growers scout for the presence of aphids under the leaves and on stems of cowpea plants, including honeydew, sooty mould and curled leaves. Generally, control of cowpea aphid is considered when 5-8 % of seedlings or 10-20 % of flowering plants are infested (Musenga *et al.*, 2016). However, such action thresholds are not based on a thorough understanding of ecological factors but rather accumulated years of experience dealing with the aphids. Furthermore, use of monitoring data to make accurate control decisions by small-scale growers in Zambia is limited largely due to lack of research in this area.

2.4.2 Chemical control

2.4.2.1 Synthetic pesticides

Use of synthetic pesticides for management of aphids on crops is common among growers of common bean and cowpea in Africa (Mkenda *et al.*, 2014; Mwanauta *et al.*, 2015). Synthetic pesticides are often used due to their quick knockdown effect and perceived reliability (Carvalho, 2017). In Zambia, pesticides such as acetamiprid, imidacloprid and malathion are used to control black bean aphid (Siisii & Moonga, 2015), while deltamethrin, cypermethrin, lambda-cyhalothrin and thiamethoxam are used to control cowpea aphid (Musenga *et al.*, 2016). In East Africa, seed dressing with endosulfan and lindane have also been used to control black bean aphid on common bean (Mwanauta *et al.*, 2015). In addition, the aphicide pirimicarb has been used against black bean aphid with reduced impact on beneficial insects (Singh, 1990; Mkenda *et al.*, 2014). However, routine use of synthetic pesticides is increasingly being discouraged globally due to negative effects on natural enemies, pollinators, wildlife, human health and food safety (Desneux *et al.*, 2006; Dubey *et al.*, 2010; Biondi *et al.*, 2012; Roubos *et al.*, 2014; Wang *et al.*, 2017). In Europe, for example, withdrawal of several neonicotinoid based pesticides has already taken place due to their negative effects on pollinators (Dewar & Foster, 2017; Holland *et al.*, 2019).

2.4.2.2 Biopesticides

Biopesticides are defined as products derived from microbes or natural products with naturally occurring defence mechanisms (Chandler *et al.*, 2011) and often categorised into three groups based on the active agent: (i) micro-organisms, (ii) semiochemicals, and (iii) biochemicals (Chandler *et al.*, 2011). Biochemicals, also referred to as botanical biopesticides, are derived from plants and suppress pests by several mechanisms: direct toxicity, antifeedant properties, repellency and growth regulation (Prakash *et al.*, 2008). Use of biopesticides in agriculture is increasingly being encouraged due to their reduced environmental impact, affordability, consumer safety, rapid biodegradation of the products and compatibility with IPM programmes (Dubey *et al.*, 2010). In Zambia, water mixtures of, wood ash with neem leaf powder (*Azadirachta indica* L.) or garlic (*Allium sativum* L.) with pepper (*Capsicum annuum* L.) powders have been used as foliar sprays against black bean aphid on common bean (Siisii & Moonga, 2015). Similarly, mixtures of, water and leaf powders from pepper, neem and the leguminous plant *Tephrosia vogelii* Hook. F. are used for control of cowpea aphid on cowpea (Musenga *et al.*, 2016).

Other plants recorded as being used to control aphids on field crops include, tobacco (*Nicotiana tabacum* L.) and eucalyptus (*Eucalyptus camaldulemsis* Dehn.) (Sharma *et al.*, 2014; Mwanauta *et al.*, 2015). Despite the perceived benefits of using botanical biopesticides to the environment and human health, there is limited understanding regarding the effects of these products to natural enemies (Ndakidemi *et al.*, 2016).

2.4.3 Cultural control

Widely used cultural pest control methods include mixed cropping and early planting. In most African countries, including Zambia, common bean and cowpea growers are typically small scale farmers owning small pieces of land (~ 5 ha) on which they plant several crops, making mixed cropping a common practice (Grisley & Shamambo, 1993; Sitko & Jayne, 2014; Mweetwa *et al.*, 2016; Cardona *et al.*, 2019). Mixed cropping offers several benefits, such as soil nutrient recycling, soil moisture conservation, weed suppression and pest management (Perrin & Phillips, 1978; Ratnadass *et al.*, 2012; Sitko & Jayne, 2014). In East Africa, intercropping common bean with maize significantly reduced black bean aphid populations compared to common bean monocrop (Ogenga-Latigo *et al.*, 1993). The low population of black bean aphid in maize-common bean intercrop was attributed to the reduced ability of aphids to locate bean plants and a reduction in sunlight reaching aphids on bean plants due to shading by the taller maize plants. A similar study in Iran on a field bean (*Vicia faba* L.) and dragonhead (*Dracocephalum moldavica* L.) intercrop indicated significant suppression of the black bean aphid population compared to a field bean monocrop (Azimi & Amini, 2015).

Early planting of common bean and cowpea is widely practiced by farmers in Africa to manage aphids (Singh & Rachie, 1985; Siisii & Moonga, 2015; Musenga *et al.*, 2016) as it helps to avoid the susceptible stage of crop coinciding with high pest populations (Singh & Rachie, 1985; Mwanauta *et al.*, 2015). Resistance of plants to aphids generally increases as the plant grows (Sarwar & Sattar, 2013). A study of cowpea aphid in East Africa indicated consistently higher population densities of aphids on late planted cowpea than early planted crops (Karungi *et al.*, 2000). A similar effect of late planting has been reported for mustard aphid, *Lipaphis pseudobrassicae*, on oil seed rape (*Brassica napus*) (Brown *et al.*, 1999; Saeed & Razaq, 2014).

2.4.4 Biological control

Biological control is an ecologically based pest management option that involves the use of one organism (a natural enemy) to control another (a pest) (DeBach & Hagen, 1964). Owing to the ecological and human health risks associated with the use of synthetic pesticides (Dewar & Foster, 2017; Kim *et al.*, 2017), biological control has become an increasingly important component of IPM systems for managing pests in agriculture (Zehnder *et al.*, 2007; Baker *et al.*, 2020). Aphid natural enemies such as parasitoids, predators and pathogens have been recorded on aphids in many parts of the world (Desneux *et al.*, 2009; Diehl *et al.*, 2013; van Emden & Harrington, 2017). Generally, biological control of aphids on common bean and cowpea in Zambia and the rest of Africa has not been explored in much detail. Populations of black bean aphid and cowpea aphid are naturally regulated by many parasitoids, predators and pathogens (Ofuya, 1997;

van Emden & Harrington, 2017). Parasitoids from the *Aphidius, Trioxys* and *Psyllaephagus* genera are the most common species attacking black bean aphid and cowpea aphid in Africa while predators include adults and larvae of coccinellid beetles and syrphid larvae (Ofuya, 1997). Studies in Burundi and Nigeria have demonstrated good establishment of parasitoids (*Aphidius colemani* Vier.) and coccinellid beetles (*Cheilomenes* spp.) with reasonable suppression of black bean aphid and cowpea aphid (Autrique *et al.*, 1989; Ofuya, 1997). Among the pathogens, entomopathogenic fungi are the most effective (Ofuya, 1997). Therefore, conserving and retaining populations of natural enemies is likely to improve the management of aphids on common bean and cowpea in Zambia.

2.4.5 Resistant cultivars

Resistant crop varieties provide a practical, cost effective and environmentally friendly means of pest management (Arora & Sandhu, 2017). Given the increasing occurrence of insecticide resistant species of aphids (Dewar & Foster, 2017; van Emden & Harrington, 2017), incorporating host-plant resistance in IPM programmes is an important consideration. Resistant cultivars of common bean and cowpea have a long history for being among the most important strategies for supressing pests and diseases including aphids (Singh & Rachie, 1985; Miklas *et al.*, 2006). Singh and Rachie (1985) demonstrated that resistance to cowpea aphid in resistant lines of cowpea were controlled by a single gene. In addition, Macfoy & Dabrowski (1984) showed that resistance in cowpea lines was conferred by the high levels of phenols and flavonoids. However, the ability of aphids to develop biotypes that adapt to resistant cultivars highlights the need for continuous breeding efforts and harmonious integration of pest control methods (van Emden & Harrington, 2017). Most of the common bean and cowpea cultivars currently used in Zambia are, however, considered to be susceptible to aphids.

2.5 Host-plant resistance

There are several definitions of host-plant resistance reported in the literature but they all centre essentially on the characteristics of a plant that negatively affect the activities of pests (van Emden & Harrington, 2017). For example; Snelling (1941) defined host-plant resistance as "*those characteristics which enable a plant to avoid, tolerate or recover from attack of insects under conditions that would cause greater injury to other plants of the same species*". Painter (1951) referred to host-plant resistance as "*the relative amount of heritable qualities possessed by a plant* *which influence the ultimate degree of damage done by the insect*". Three mechanisms of host-plant resistance to insects were initially proposed by Painter (1951) as: non-preference (antixenosis), antibiosis and tolerance. However, this classification of resistance has recently been revised into a simplistic framework consisting of resistance and tolerance (Stout, 2013). In his review, Stout (2013) demonstrated that overlaps exist between the three mechanisms proposed by Painter, making it difficult to separate them. Overlaps between the mechanisms of resistance often result from the coexistence of several resistance factors having deterrent, repellent, toxic or antinutritive effects (Stout, 2013). For example, plant latex reflects a major overlap between antibiosis and antixenosis due to its multiple antiherbivore effects such as insect entrapment, feeding interference and toxicity (Stout, 2013). While recognising the limitations of Painter's classification, his conceptual framework is used in this review as there is lots of literature using it.

2.5.1 Antixenosis

Antixenosis (non-preference) involves plant characteristics that affect the behaviour of insects, resulting in reduced colonisation or acceptance of a plant as a host (Züst & Agrawal, 2016). In aphids, antixenosis is reflected by increased numbers of alate aphids avoiding or leaving the plant soon after landing and superficial probing with their stylets (van Emden & Harrington, 2017). Several factors such as colour, waxiness, palatability and hairiness (trichomes) contribute to non-preference characteristics of plants to aphids (van Emden & Harrington, 2017).

As an example of antixenosis based on colour, Radcliffe & Chapman (1966) demonstrated that red cultivars of cabbage (*Brassica oleracea* L.) were avoided for landing by alate cabbage aphids (*Brevicoryne brassicae* L.) (Homoptera: Aphididae), suggesting the influence of colour in host selection. A similar study in India showed that mustard aphids (*Lipaphis erysimi* Kalt) (Homoptera: Aphididae) had a higher preference for green coloured cultivars compared to the pink-coloured ones (Rana *et al.*, 2001).

The chemical constituents of wax in the epicuticle layers of plant leaves have been linked to antixenosis against aphids (van Emden & Harrington, 2017). Plant leaf wax-mediated antixenosis against aphids has been reported in crops such as wheat (Lowe *et al.*, 1985), pea (White & Eigenbrode, 2000), cucumber (Ren *et al.*, 2014) and brassicas (Ellis *et al.*, 1996). Plant palatability to aphids is predominantly influenced by plant chemistry, where deterrent compounds are detected through superficial probing. For example, defence compounds such as glucosinolates in brassicas (Cole, 1997), phenols in oil seed rape (Kumar & Sangha, 2013), phenols in lucerne (Golawska *et al.*, 2005) and sugar esters in tobacco (Johnson *et al.*, 2002) have been reported to deter aphids.

The mechanical aspect of antixenosis to aphids acts by reducing their mobility on the plant and ease of stylet penetration into plant tissue (van Emden & Harrington, 2017). For example, high levels of cellular pectin in sorghum have been found to hinder stylet penetration by *S. graminum* (Dreyer & Campbell, 1987). Non-glandular trichomes are also important deterrents to aphid colonisation in many crops (van Emden & Harrington, 2017), from wheat (Webster *et al.*, 1994) to common bean (Larocca *et al.*, 2011).

2.5.2 Antibiosis

Antibiosis refers to plant characteristics that affect life table parameters (i.e. growth rate, development time, survival and fecundity) of herbivorous insects after plant colonisation (Machuka *et al.*, 1999). As noted by Stout (2013), this form of resistance is usually a consequence of plant traits (some involved in antixenosis effects) such as glandular trichomes, toxins and nutrition (van Emden & Harrington, 2017). Glandular trichomes contain substrates and enzymes in their apical storage structures that rupture when disturbed by insects. The rupturing process can lead to formation of phenolic compounds that are not only distasteful, but also immobilise mouthparts and legs as they harden. The effect of glandular trichomes against aphids has been reported in several crops, including potato (Simmons *et al.*, 2005) and tomato (Dreyer & Campbell, 1987). Toxins produced by induced plant defence during aphid feeding can affect their performance, e.g. the polyphenol gossypol in some cotton cultivars is reported to reduce fecundity of the melon and cotton aphid (*A. gossypii*) (Du *et al.*, 2009). Similarly, high levels of hydroxamic acid in some wheat and oat varieties reduces cereal aphid growth rates (Fuentes-Contreras & Niemeyer, 1998). Given that the diet of aphids is already protein limited, further reductions in nitrogen containing compounds within the plant sap can negatively affect aphid performance. For example, low levels of essential amino acids in some pea, wheat and barley cultivars reduce aphid fecundity (Weibull, 1994; van Emden & Harrington, 2017). Treating plants with nitrogenous fertilisers usually reduces the impact of protein imbalance on aphid performance (van Emden & Harrington, 2017).

2.5.3 Tolerance

Tolerance to pest damage results when a crop cultivar produces higher yields compared to other varieties when placed under the same level of pest pressure (van Emden & Harrington, 2017). Pest tolerance is usually achieved by the plant's ability to compensate for the damage caused by the pest. The mechanisms involved in tolerance are not fully understood but there is evidence for antibiosis being an important component of this (van Emden & Harrington, 2017).

2.6 Plant-aphid interactions

More than 4000 species of aphid have been described of which approximately 250 are considered to be economically important crop pests (Dixon, 1998; Blackman & Eastop, 2000; Jaouannet *et al.*, 2014). Plant injury and viral transmission is exacerbated by the characteristic rapid population growth of aphids, facilitated through parthenogenetic reproduction and short generation times (Singh & Rachie, 1985; Abate & Ampofo, 1996; van Emden & Harrington, 2017). Furthermore, their feeding behaviour and mobility (alate morphs) make aphids efficient vectors of plant viral diseases (Blackman, 1974; Dixon, 1998). Aphid mouthparts are modified into a sharp needle like "piercing" tube called a stylet, used for puncturing plant cells to draw sap (photosynthates) from the phloem tissue (Blackman, 1974; Züst & Agrawal, 2016).

2.6.1 Host-location

Host-plant location is predominantly undertaken by winged, alate aphids (Dixon, 1998). As weak fliers, aphids usually depend on air currents for long-distance dispersal, often leading them to land on unsuitable host-plants (Blackman, 1974; van Emden & Harrington, 2017). It is estimated that only about 1% of a dispersing population of aphids succeed in locating suitable host-plants (Blackman, 1974; Ward *et al.*, 1998). However, host-location is aided by a steady air flow and the ability of the aphids to fly at a low altitude (Ward *et al.*, 1998). During flight, aphids use visual stimuli (i.e. host-plant colour wavelength) for host-location (Dixon, 1998; van Emden & Harrington, 2017). Using a flight tunnel, Hardie (1989) showed that black bean aphid preferred to land when presented with a wavelength associated with green colour. As the aphid gets closer to the plants, olfactory cues (i.e., volatile organic compounds) play a key role in guiding the insect to a specific host-plant (Webster *et al.*, 2010; van Emden & Harrington, 2017).

2.6.2 Host-acceptance

Once a potential host-plant has been located, a series of evaluations are undertaken by the aphid to assess feeding suitability (Blackman & Eastop, 2000; van Emden & Harrington, 2017). Aphids use chemoreceptors on their antennae and mouthparts to sample and evaluate chemical compounds on the surface of the plant (Blackman, 1974; Dixon, 1998; van Emden & Harrington, 2017). Furthermore, superficial probing and salivation into mesophyll cells is undertaken in order to assess leaf cell content (Blackman, 1974; Dixon, 1998). In particular, plant defence compounds (i.e. secondary metabolites) and other factors such as, pH, sugars and amino acids are assessed at this stage (Hewer *et al.*, 2011; Will *et al.*, 2013). After a positive evaluation, the aphid moves its stylet further into the plant tissue, passing through the intercellular space until the phloem tissue is reached (Züst & Agrawal, 2016; Nalam *et al.*, 2019). The intercellular space is preferred for passage of the stylet to minimise the risk of cell damage and induction of plant defence responses (Tjallingii, 2006; van Bel & Will, 2016).

2.6.3 Plant defence

Plant sap is produced at a high energy cost and its role in plant growth and development is crucial (Dixon, 1998). Consumption of sap by aphids creates a “sink-source” imbalance resulting in distorted growth and stunting of plants (Mwanauta *et al.*, 2015; Naessens *et al.*, 2015; Nalam *et al.*, 2019). Therefore, plants have evolved several defence mechanisms in order to protect their sap from exploitation by aphids and other phloem feeding invertebrates (Dixon, 1998; van Emden & Harrington, 2017). However, as the feeding strategy of aphids causes little cellular damage, plant defence mechanisms must be highly sensitive to detect proteins in aphid saliva (i.e., cellulases, peroxidases, lipase) (Westwood & Stevens, 2010; Nalam *et al.*, 2019). Conversely, aphids have also evolved strategies to counteract or circumvent plant defences (Naessens *et al.*, 2015; van Bel & Will, 2016). These interactions are thought to have shaped the co-evolution of both aphids and their host plants (Westwood & Stevens, 2010; Züst & Agrawal, 2016). Failure to adequately suppress plant defences benefits the plant, leading to reduced crop damage (Mewis *et al.*, 2005).

2.6.4 Phytohormone signalling

Timely perception of aphid attack by plants is crucial for defending energy rich photosynthates (Züst & Agrawal, 2016). Several studies have demonstrated the complex mechanisms involved in the perception of herbivore attack by plants (Jones & Dangl, 2006;

Hogenhout & Bos, 2011; Kaloshian & Walling, 2016). Herbivorous insects, including aphids, contain effectors in their saliva that are used to manipulate or suppress plant defence systems (Mile, 1999) (Figure 2.6). It is established, however, that plants use effectors in aphid saliva as primary signals for aphid infestation, resulting in defence responses being triggered (Nalam *et al.*, 2019). Several proteins in aphid saliva have been found to elicit the activities of defence related enzymes (i.e., cellulase, pectinase, peroxidase, polyphenoloxidase and lipase) in plants (Mile, 1999). Howe & Jander (2008) demonstrated that treating the model plant *Arabidopsis thaliana* with saliva of the peach potato aphid (*M. persicae*) elicited defence responses, indicating the potential role of saliva in plant defence signalling. Recognition of herbivore attack in plants upregulates production of defence hormones such as jasmonic acid (JA), salicylic acid (SA) and ethylene (ET) (Smith *et al.*, 2009). Due to the susceptibility of aphids to products of JA signalling, most aphid species contain effectors that suppress this defence response while inducing the SA pathway, which results in less detrimental effects (Ajlan & Potter, 1992; Stout *et al.*, 1994; Ali & Agrawal, 2012). In another study using *A. thaliana* it was shown that mutation derived plants, which were deficient in SA signalling, were more resistant to aphids compared to normal control plants (Mewis *et al.*, 2005).

2.6.5 Physical defence

Trichomes and the wax cuticle provides the first level of defence against aphids (Nalam *et al.*, 2019). There are two types of trichome, glandular and non-glandular (van Lenteren & De Ponti, 1991), which affect aphids by interfering with their feeding and mobility on plant surfaces (Hanley *et al.*, 2007; Fürstenberg-Hägg *et al.*, 2013) while hooked trichomes can directly injure or kill aphids (Westwood & Stevens, 2010). Glandular trichomes also contain toxic chemical compounds such as polyphenols that are detrimental to insects (van Emden & Harrington, 2017).

2.6.6 Biochemical defence

2.6.6.1 Phloem sealing

Leguminous plants contain proteinaceous particles such as forisomes and callose, which facilitate sealing of sieve tubes damaged by aphid feeding (Will *et al.*, 2013; Nalam *et al.*, 2019) (Figure 2.6). As a short term measure to aphid attack, plants respond by increasing the concentration of calcium ions (Ca^{2+}) in the phloem, resulting in the mobilisation of forisome particles to seal the sieve poles (Peters *et al.*, 2006). A study by Medina-Ortega & Walker (2015) demonstrated that feeding of *M. persicae* on field bean resulted in forisome occlusion and consequently led to a cessation of feeding. Similarly, the influx of Ca^{2+} ions in the phloem induces callose deposition leading to plugging of sieve poles. Botha & Matsiliza (2004) demonstrated that sustained feeding by the Russian wheat aphid (*Diuraphis noxia*) on wheat increased the occlusion of callose, leading to blockage of the sieve poles.

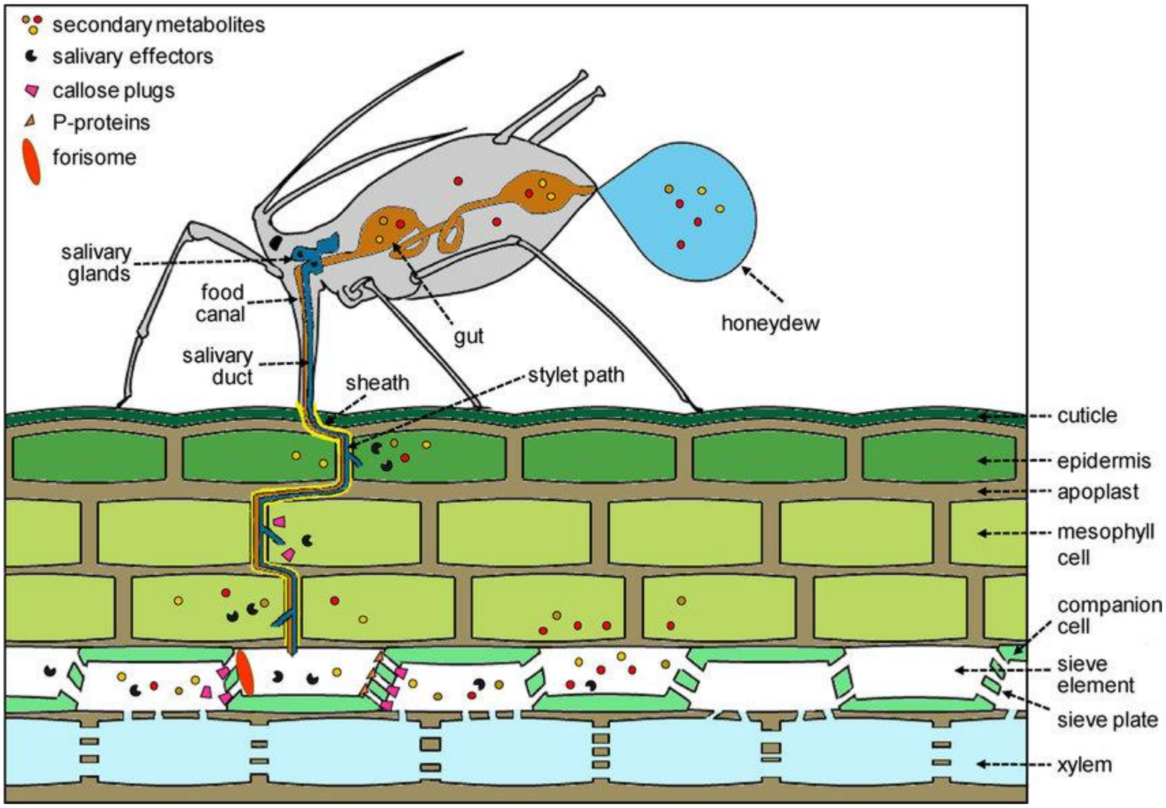

Figure 2.6: Illustration of aphid feeding and plant defence responses (Nalam *et al.*, 2019)

2.6.6.2 Secondary metabolites

Elicitation of antiherbivore phytohormones by aphid feeding triggers a cascade of biochemical reactions that lead to the expression of defence traits and production of secondary metabolites (Pare & Tumlinson, 1999; Smith *et al.*, 2009; Ali & Agrawal, 2012; War *et al.*, 2012). Secondary metabolites such as cardiac glycosides (cardenolides), alkaloids, glucosinolates and benzoxazionid have been found to be toxic against aphids (van Emden & Harrington, 2017; Nalam *et al.*, 2019) (Figure 2.6). Cardenolides are phloem mobile steroidal compounds found in a range of plants (Botha *et al.*, 1977; Ali & Agrawal, 2012) that affect the performance of aphids by inhibiting sodium/potassium-ATPase activity (Züst & Agrawal, 2016). Alkaloids are herbivore deterrent nitrogen containing compounds produced by over 20 % of higher plant species (Dreyer & Campbell, 1987), which interfere with cellular processes such as DNA replication, protein synthesis and neurotransmission (Dreyer & Campbell, 1987). Glucosinolates are defensive compounds found in plants belonging to the Brassicaceae family (Züst & Agrawal, 2016). Activation of glucosinolates by the enzyme myrosinase, which occurs through cell damage results in the formation of isothiocyanates and nitriles that are toxic to aphids (Halkier & Gershenzon, 2006). However, aphids largely avoid the effects of glucosinolates by minimizing cell damage (Kim & Jander, 2007). Aphid induced defence also includes the release of (*E*)-β farnesene, an aphid alarm pheromone, which is repellent to aphids (Petrescu *et al.*, 2001). Parasitoids use (*E*)-β farnesene as a chemical cue to locate aphids (Du *et al.*, 1998).

2.6.6.3 Lectins and protease inhibitors

Lectins are carbohydrate binding proteins that are phloem mobile in a range of plant species (Vandenborre *et al.*, 2011). These proteins bind to carbohydrates in the midgut of insects, interfering with their digestion processes and consequently impacting on the performance of herbivores (Chougule & Bonning, 2012). Beneteau *et al.* (2010) demonstrated the effect of these proteins by adding a phloem lectin (protein2-A1) to an artificial diet, which significantly reduced the growth rate of the soybean aphid (*A. glycines*). Protease inhibitors are plant defence compounds that interfere with protease function in herbivorous insects preventing protein metabolism (Zhu-Salzman & Zeng, 2015). The mechanism of protease inhibition is not fully understood in aphids (Nalam *et al.*, 2019), but a study has demonstrated that adding protease inhibitors (i.e., cystatin) to artificial diet reduced the fecundity of *A. gossypii*, pea aphid (*Acyrthosiphon pisum*) and *M. persicae* (Carrillo *et al.*, 2011).

2.7 Silicon induced defence against pests

2.7.1 Soil silicon

Among the major elements in the Earth's crust, silicon accounts for approximately 28 % by weight and is second only to oxygen (Epstein, 1994). In the soil, silicon mainly exists as insoluble crystalline aluminosilicate, partially soluble polysilicic acid and water soluble monosilicic acid (Richmond & Sussman, 2003). The concentration range of silicon in the soil is similar to that of other major elements (i.e. potassium, calcium, sulphate) and typically ranges between 0.1 to 0.6 mM (Epstein, 1994). Furthermore, the quantity of soluble silicon in the soil is indicative of the soil's capacity to supply essential elements to plants (Liang *et al.*, 2015). Plant available silicon is influenced by several factors: soil texture, organic matter content, land use, parental material, temperature, soil pH and moisture (Miles *et al.*, 2014; Anda *et al.*, 2015).

2.7.2 Silicon uptake by plants

Silicon is taken up by plants in form of monosilicic acid ($H_4 SiO_4$), the only form capable of crossing the root plasma membranes (Epstein, 1994). Different plant species show varied capacities in taking-up and accumulating silicon in their tissue, reflecting differences in the uptake mechanisms employed by roots (Yan *et al.*, 2018). Absorption and movement of silicon through the plant is related to the rate of water uptake. Three modes of silicon uptake have been proposed: (1) active, (2) passive and (3) rejective or exclusive (Takahashi *et al.*, 1990). Active transport is an energy dependent mechanism in which silicon is taken up at a faster rate than water and is only associated with high and intermediate silicon accumulators (Raven, 2003). Passive transport is a general mechanism associated with all plants, which is facilitated by diffusion or facilitated diffusion through proteinaceous channels (Raven, 2003; Yan *et al.*, 2018). In addition, passive uptake is a concentration dependent mechanism in which the rate of silicon uptake is similar to that of water (Yan *et al.*, 2018). By contrast, plants with a rejective mechanism absorb silicon at a much slower rate than water and is characteristic for non-accumulators (Raven, 2003).

Generally, monocotyledonous plants have been found to respond to silicon fertilisation by absorbing and accumulating higher levels of the element in their tissue. For example, crops such as rice (*Oryza sativa* L.) (Takahashi *et al.*, 1990), barley (*Hordeum vulgare* L.) (Nikolic *et al.*, 2007), wheat (*Triticum aestivum* L.) (Rains *et al.*, 2006), maize (Liang *et al.*, 2015), ryegrass (*Lolium perenne* L.) (Nanayakkara *et al.*, 2008), sugarcane (*Saccharum* spp.) (Keeping & Meyer, 2006) and banana (*Musa* spp.) (Henriet *et al.*, 2006) have been reported as active silicon accumulators. While most dicotyledonous plants take up silicon passively (Takahashi *et al.*, 1990), a small number, such as cucumber (*Cucumis sativus* L.) (Nikolic *et al.*, 2007), soybean (*Glycine max* (L.) Merr.) (Reynolds *et al.*, 2016), and cotton (*Gossypium hirsutum* L.) (Reynolds *et al.*, 2016) have been found to be intermediate accumulators, absorbing silicon using active transport. Conversely, some dicotyledonous plants such as tomato (*Lycopersicon esculentum* L.) and field bean (*Vicia faba* L.) are reported to be non-accumulators that exclude silicon from their roots (Takahashi *et al.*, 1990; Nikolic *et al.*, 2007).

From the roots, silicon moves up the plant through the transpiration stream to shoots, leaves and other parts of the plant where it is deposited as opaline phytoliths (Jones & Handreck, 1967; Reynolds *et al.*, 2009). In particular, silicon is deposited as solid, amorphous hydrated silica ($SiO_2.nH_2O$) in the epidermal cells, which adds to the rigidity and abrasiveness of the plant tissue. The mechanisms that stop silicon from polymerising before deposition are poorly understood (Yan *et al.*, 2018). In addition, silicon is never immobilised once deposited and thus permanently adds to the bulk of plant tissue. In general, terrestrial plants accumulate about 1 to 15% by weight of silicon in their dry matter (Epstein, 1994; Reynolds *et al.*, 2016).

2.7.3 Silicon induced defence

A study by McColloch & Salmon (1923) was among the earliest to suggest the contribution of silicon to insect pest resistance in plants. The discovery of other benefits such as disease resistance and tolerance to water stress associated with silicon application has led to its increasing use in agriculture (Reynolds *et al.*, 2016). To date, many studies have reported the positive contribution of silicon in plant defence against pests (Ma *et al.*, 2006; Nakata *et al.*, 2008; Kvedaras *et al.*, 2010; Liang *et al.*, 2015). However, there has been more focus on monocotyledonous plants (Poaceae) due to their increased efficiency of silicon uptake (Hodson *et al.*, 2005). Leguminous plants have for several decades been considered as non-accumulators of silicon (Shone, 1964; Barber & Shone, 1966). Recent studies, however, indicated high silicon accumulation in some legume species, such as pigeon pea (*Cajanus cajan* L.), soya bean, common bean and cowpea, suggesting important functional roles in these plants (Hodson *et al.*, 2005; Deshmukh *et al.*, 2013; Izaguirre-Mayoral *et al.*, 2017). While few studies have investigated the role of silicon in herbivore defence of legumes, positive effects reported on monocots provide useful insights since plants have similar defence mechanisms (Ma, 2004; Frew *et al.*, 2018). Silicon improves resistance to pests by enhancing the plant's mechanical, biochemical and induced defences (Reynolds *et al.*, 2009).

2.7.3.1 Physical defence

Silicon supplementation has been found to increase the rigidity and abrasiveness of plant tissues through deposition of silica in the lumen of epidermal cells, where it reinforces the strength of cell walls along with cellulose and lignin (Kvedaras *et al.*, 2010). Several studies have shown that silicon fertilised plants sustain less herbivory damage, likely due to the physical barrier developed by silicon deposition (Kaufman *et al.*, 1985; Ma *et al.*, 2001; Massey *et al.*, 2006). A study by Keeping *et al.* (2009) of sugarcane highlights how silicon can act as a physical barrier by using scanning electron microscopy and X-ray analysis to show increased silicon deposition in areas that are typical entry points for the Lepidopteran stem borer *Eldana saccharina* Walker.

There is evidence to suggest that silicon reduces plant digestibility as silicon enriched grasses have a higher retention of chlorophyll after passing through the gut of locusts (*Schistocerca gregaria* Forsk.) (Orthoptera: Acrididae). Although not conclusive, a small number of studies have demonstrated increased larval mandibular wear after feeding on silicon enriched plant tissue (Ranger *et al.*, 2019). Silicon increases the bulk density of plant tissue while reducing its digestibility, feed conversion ratio, growth rates and fecundity in herbivorous insects (Massey *et al.*, 2006; Zadda *et al.*, 2007). Furthermore, delayed penetration of silicon enriched plants and extended feeding period as a consequence of toughened tissue and poor dietary quality, exposes herbivore insects to other mortality factors such as insecticides and natural enemies.

Absorption and accumulation of silicon in plants is facilitated, at least in part, by herbivore feeding intensity (Reynolds *et al.*, 2016). A laboratory study on species of African grasses indicated that plants subjected to increased intensity of leaf damage and defoliation accumulated higher levels of silica in their leaves compared to control plants, suggesting that silica deposition is an induced response against herbivory (McNaughton & Tarrants, 1983).

2.7.3.2 Biochemical defence

Sasamoto (1958) was among the first to demonstrate that resistance of rice to insect herbivory is not only dependent on physical traits of the plant but also its chemistry. Using a laboratory host-preference study, Sasamoto (1958) showed that larvae of rice stem borer, *Chilo suppressalis* Walker (Lepidoptera: Pyralidae), preferred silicon deprived plants over treated ones, suggesting the presence of repellent compounds in silicon treated plants. Furthermore, a study on wheat and *Schizaphis graminum* R. (Homoptera: Aphididae) demonstrated that although silicon treatment did not impede stylet penetration, the authors observed increased stylet withdrawal from the phloem and reduced probing compared to silicon deprived control plants, reflecting plant chemistry changes (Goussain *et al.*, 2005). Several other studies have further implicated the role of silicon in mediating plant chemistry for defence against herbivory (Kvedaras *et al.*, 2010; Pereira *et al.*, 2010; Costa *et al.*, 2011) and its negative effects on the performance of pathogens and arthropods (Zadda *et al.*, 2007; Stanley *et al.*, 2014).

As previously stated, plant defence against herbivores is regulated by antiherbivore phytohormones (JA, SA and ET) that serve as primary signals (Aljbory & Chen, 2018). Silicon fertilisation has been found to increase synthesis of antiherbivore phytohormones resulting in increased defence responses in plants (Frew *et al.*, 2018). Many studies have shown that increased production of antiherbivore phytohormones increases the activities of defence enzymes (i.e. chitinases, peroxidase, phenylalanine ammonia lyase, polyphenoloxidase, β-1,3 glucanase) that are involved in the production of defence compounds (Cai *et al.*, 2008; Chain *et al.*, 2009; Elsharkawy & Mousa, 2015). A study comparing silicon fertilised rice genotypes indicated that a wild line was more resistant to a borer (*Cnaphalocrocis medinalis* G.) (Lepidoptera: Pyralidae) than a mutant line in which the JA biosynthesis pathway was silenced (Ye *et al.*, 2013). In addition, the study showed that elevation of JA in the wild line increased the activities of defence enzymes, indicating a positive interaction between silicon, JA biosynthesis and plant defence.

2.7.3.3 Indirect defence

Herbivore induced defence is linked to the production of several volatile organic compounds, often referred to as herbivore induced plant volatiles (HIPVs) (Leroy *et al.*, 2019). Under herbivore attack, HIPVs are used as a warning signal from damaged part(s) to the rest of the plant or to nearby plants, eliciting induction of defence for upcoming insect attack (Dudareva *et al.*, 2006). Herbivore induced plant volatiles can also serve as a deterrent preventing herbivores from further feeding or ovipositing on the plant (Dudareva *et al.*, 2006). Silicon fertilisation has been found to increase and/or alter HIPVs production in plants, providing a stronger prey location cue to natural enemies (Arimura *et al.*, 2009). The influence of silicon on the quality and/or quantity of HIPVs is likely due to its ability to prime the JA mediated defence system, including its interaction with other antiherbivore phytohormones (Ye *et al.*, 2013). For example, silicon treated cucumber plants infested by a cucumber beetle, *Diabrotica balteata* LeConte (Coleoptera: Chrysomelidae), emitted more indole compared to silicon deprived control plants (Callis-Duehl *et al.*, 2017). Similar results have been reported for a generalist predator, *Dicranolaius bellulus* (Gue´rin-Me´neville) (Coleoptera: Melyridae), which was strongly attracted to the odour of silicon fertilised cucumber plants infested by a bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae) compared to silicon deprived plants (Kvedaras *et al*., 2010). These results suggest that alteration of HIPVs by silicon results in increased attraction of natural enemies. For example, reduced production of hexanal 2-ethyl, α-bergamotene, β-sesquiophellandrene and cedrol, in silicon treated rice plants infested by *C. medinalis* resulted in increased attraction of the parasitoid *Microplitis mediator* compared to untreated plants (Liu *et al*., 2017). Similarly, De Oliveira *et al*. (2020) established that silicon treated wheat plants infested with *R. padi* produced significantly higher amounts of geranyl acetone and were more attractive to the parasitoid *Lysiphlebus testaceipes* compared to silicon deprived plants.

2.7.4 Use of silicon in agriculture

Large scale use of silicon in agriculture was first recorded in Asia during the 1950s, particularly Japan and South Korea where it was used to improve rice productivity (Takahashi *et al.*, 1990). Silicon use in agriculture remains a common practice in most Asian countries as well as the Americas (Liang *et al.*, 2015). Beneficial effects of elemental silicon to plant resistance against abiotic and biotic stresses has been the main determinant factor for its commercialisation (Yan *et al.*, 2018).

A variety of silicon fertilisers are available for agricultural use, which are mainly derived from natural minerals, metallurgic by-products and chemical products (Liang *et al.*, 2015). Silicon fertiliser choice is largely dependent on their cost, efficacy and environmental impact associated with their use (Yan *et al.*, 2018). Metallurgic by-product based silicon fertilisers are cheaper than other fertilisers but their use poses a risk of heavy metal accumulation in the soil (Yan *et al.*, 2018). On the other hand, natural mineral based fertilisers are slow releasing and thus relatively more environmentally benign but intensive processing results in higher cost (Liang *et al.*, 2015; Yan *et al.*, 2018). Potassium and sodium silicate fertilisers are usually applied as foliar sprays due to their high solubility in water but also as a more cost effective approach compared to soil applications (Liang *et al.*, 2015). For example, a foliar spray of sodium silicate in wheat increased silicification of trichomes and resistance to the grain aphid *Sitobion avenae* F. and rose-grain aphid *Metopolophium dirhodum* Walker compared to untreated plants. Similarly, a study on maize plants demonstrated that two foliar sprays or one soil application of silicon was enough to induce resistance to the corn aphid *Rhopalosiphum maidis* Fitch (Moraes *et al.*, 2005).

2.8 Mutagenesis derived aphid resistance

Mutagenesis refers to heritable alterations in the genetic material that gives rise to individuals with modified phenotypic traits and provides a source of unique germplasm to facilitate crop improvement (Figure 2.7). Such genetic alterations can be induced by exposing a plant or its propagules to physical or chemical materials with mutagenic properties (Viana *et al.*, 2019) (Table 2.2). Mutations in the DNA are described based on the alteration of gene functions (Mba, 2013). The common types of mutation induced in the DNA that are relevant to crop improvement include: single base substitutions, point mutations, insertions and deletions (Mba, 2013) (Table 2.2). Induced mutagenesis has played a key role in the genetic improvement of crops for decades, with the joint Food and Agriculture Organization (FAO) and the International Atomic Energy Agency (IAEA) database containing 3,275 mutant crop varieties derived from 225 plant species (FAO/IAEA, 2019). Mutation derived varieties are now cultivated in most parts of the world, including: Asia, Europe and North America (Horn *et al*., 2015). Use of induced mutagenesis in plant breeding programs has increased in recent years due to the development of efficient and cost effective mutation-detection techniques such as Targeting Induced Local Lesions in Genomes (TILLING) (Viana *et al.*, 2019). As a technique, induced mutagenesis has been widely adopted by plant breeders targeting pathogen resistance and other abiotic stresses (Oladosu *et al*., 2016). Few studies, however, have considered using this approach to develop aphid resistant plant varieties. Reasons for why induced mutagenesis may be considered as a means of developing aphid resistant crops in sub-Saharan Africa are here classified into the following themes: (1) those related to technological issues (i.e., accessibility and legislation) associated with other accelerated approaches to plant breeding, (2) similarities between aphid and pathogen resistance mechanisms, (3) aphid and pathogen resistant genes often being found close together on chromosomes, and (4) improved screening of mutagenised plant population.

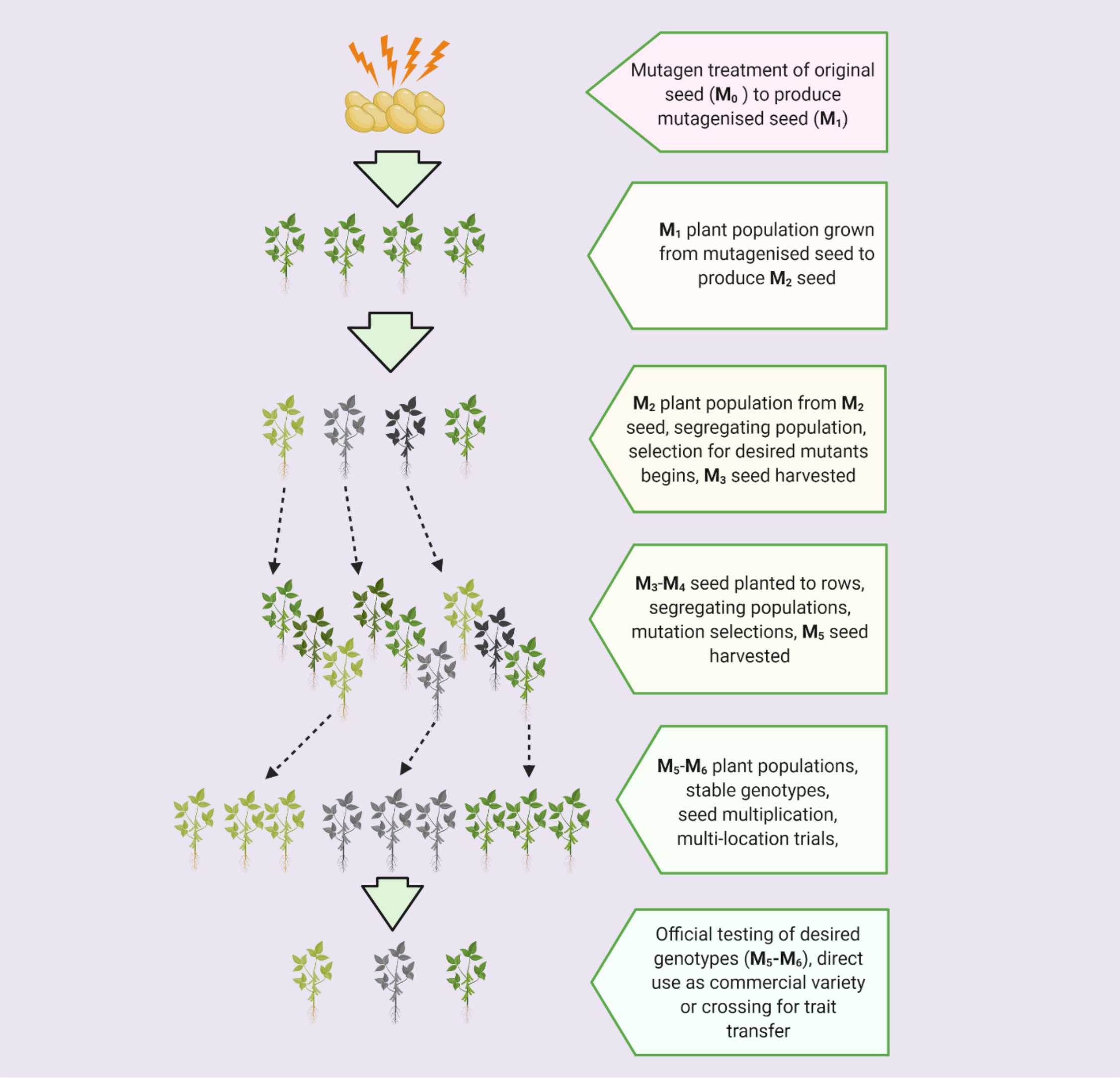

Figure 2.7: Illustration of a generalised procedure for induced mutagenesis. Seed is denoted as 'M_0' before mutagen treatment and '$M_{1\text{-}n}$' for generations following mutagenesis. 'M' = meiotic generation. After mutagenesis, M_1 seed is planted to produce M_1 plants and M_2 seed. Due to heterozygosity of M_1 plants, mutations are not yet visible at this stage. Seed is harvested, bulked and planted to produce M_2 plants and M_3 seed. Mutations begin to appear in the M_2 population due to genetic recombination and segregations which marks the beginning of screening and selection of desired mutants. From M_3 onwards, seed is harvested from individual plants and planted as single-plant progenies to facilitate detailed screening of mutants. Several generations (e.g. $M_{3\text{-}6}$) are often required for mutant genotypes to reach homogeneity. Homogenous mutants (i.e. $M_{5\text{-}6}$)

with desired traits can be directly used as a variety or as parents in breeding programmes

Table 2.2 Major chemical and physical mutagens used for induction of random mutations in plants

Mutagens	Type of mutation	Mutation derived traits	References
Chemical agents			
Ethyl Methanesulfonate	Guanine alkylation, G/C to A/T transitions or G/C to C/G or G/C to T/A transversions	Plant development and metabolism	Feldman *et al.*, 2017
		Abiotic stress tolerance	Xu *et al.*, 2017
N-methyl-N-nitrosourea	Guanine and cytosine alkylation, G/C to T/A transitions	Biotic stress tolerance	Busungu *et al.*, 2016
		Nutritional quality	Kim *et al.*, 2018
		Yield and quality improvement	Long *et al.*, 2017
Sodium azide	Generates azidoalanine causing G/C to A/T transitions	Abiotic stress tolerance	Hussain *et al.*, 2012
		Nutritional improvement	Jeng *et al.*, 2012
		Yield and quality improvement	Lin *et al.*, 2014
Colchicine	Chromosome doubling, affects the microtubules promoting symmetric cell division.	Nutritional improvement	Viana *et al.*, 2019
		Abiotic stress tolerance	Tu *et al.*, 2014
		Yield and quality improvement	Guo *et al.*, 2017
Physical agents			

Gamma-Rays	Single nucleotide substitution, inversion and deletion	Plant development and metabolism	Smillie *et al.*, 2012
		Abiotic stress tolerance	Song *et al.*, 2012
		Nutritional improvement	Hwang *et al.*, 2014
Ion Beam Radiation	Point mutation (deletion), inversion, translocation and insertion	Plant development and metabolism	Phanchaisri *et al.*, 2007
		Nutritional quality	Ishikawa *et al.*, 2012
Fast-Neutron Irradiation	A/T to G/C	Abiotic stress tolerance	Ruengphayak *et al.*, 2015
	transition, insertion, inversion, duplication and deletion	Biotic stress resistance	Chern *et al.*, 2016

2.8.1 Prospects for the use of induced mutagenesis as a tool for developing aphid resistant crop varieties

2.8.1.1 Technological issues associated with other accelerated approaches to plant breeding

Several crop improvement technologies such as genetic engineering, marker assisted selection and targeted mutagenesis have been developed and may help to accelerate plant breeding for aphid resistance (Bhattacharya, 2019; Voss-Fels *et al.*, 2019; Wang *et al.*, 2019). Despite the potential that these modern plant breeding tools offer to plant breeding, their practical use in sub-Saharan Africa is limited. The use of external DNA in genetically engineered crops, for example, has led to strict biosafety regulation for their use in most sub-Saharan African countries (Zaidi *et al.*, 2019). In countries like Kenya, for example, where use of genetically engineered crops has been approved, environmental exposure as well as trade of these crops and their products is still prohibited (Botha *et al.*, 2020). Sub-Saharan African countries are largely dominated by smallholder farmers with less financial capacity to annually purchase genetically engineered crop seed (Fischer *et al.*, 2015). There have also been concerns regarding perceived potential risks of genetically engineered crops on domestic agricultural biodiversity (Jacobsen *et al.*, 2013). In contrast to genetic engineering, targeted mutagenesis involves alteration of endogenous genes (Arora & Narula, 2017). Despite the non-integration of external DNA, there is increasing pressure to subject gene-edited crops to the same regulations as crops that are genetically engineered, perhaps due to uncertainty around the intended effects of artificially manipulating plants in this way (Callaway, 2018). In addition, the costs associated with new genomic tools, lack of skilled scientific personnel and laboratories hinder the use of modern molecular approaches to plant breeding in sub-Saharan Africa (Botha *et al.*, 2020). In comparison to these modern plant breeding tools, induced mutagenesis is more widely used and accepted as a breeding tool with a long history of safe use. The non-involvement or use of external DNA in induced mutagenesis exempts mutation derived plants from the often expensive and long regulatory procedures that genetically engineered plants are subjected to (Mba, 2013). This simplified regulatory regime for release of mutation derived varieties coupled with the robustness, simplicity and low operation costs make induced mutagenesis especially suitable for countries in sub-Saharan Africa (Mba, 2013).

2.8.1.2 Similarities between aphid and pathogen resistance mechanisms

Based on the partial overlap between plant-resistance mechanisms against aphids and microbial pathogens (Kaloshian & Walling, 2005), the production of genetic material with disease resistance provides hope for developing aphid resistant cultivars through induced mutagenesis. Plants recognise pathogen-effector proteins (e.g., flagellin, peptidoglycan, lipopolysaccharides in bacteria and chitins in fungi) using receptors on cell walls that trigger defence responses known as pathogen associated molecular pattern (PAMP)-triggered immunity (PTI) (Chisholm *et al.*, 2006). Pathogens, however, have evolved effector proteins that can suppress PTI in plants (Louis *et al.*, 2012). In response, plants have equally evolved additional *R* proteins that can recognise these pathogen effectors leading to effector-triggered immunity (ETI) (Chisholm *et al.*, 2006). Plants perceive and recognize aphids by detecting specific effector proteins in aphid saliva (e.g., pectinases, cellulases) in a similar way to the detection of pathogens (Dogimont *et al*., 2010). During feeding, aphids inject watery saliva containing proteins and other metabolites into sieve elements (Louis *et al.*, 2012). The protein molecules in aphid saliva are similar to pathogen associated molecular patterns (PAMPs) that are recognised by pattern recognition receptors (PRRs) in plants to trigger PTI (Rodriguez & Bos, 2013). To counteract PTI, aphids deliver effector proteins in their host plant to suppress this defence promoting effector-triggered susceptibility (ETS) (Jaouannet *et al.*, 2014). In return, some plant species may carry receptors or *R* proteins that can recognise effectors in aphid saliva leading to ETI in plants (Jaouannet *et al.*, 2014). Detection of pathogens or aphid species both result in activation of the salicylic acid (SA) signalling pathway due to the limited physical damaged to foliage during feeding (Züst & Agrawal, 2016). Indeed, there is evidence that aphids are negatively affected by the activation of the SA pathway. For example, mutation derived genotypes of *Arabidopsis thaliana* (L.) Heynh with increased SA signalling have been shown to be less susceptible to peach-potato aphid (*Myzus persicae* Sulzer) (Kerchev *et al*., 2013). Similarly, growth rate and population growth of the potato aphid (*Macrosiphum euphorbiae*) is adversely affected by the SA signaling pathway mediated by the *Mi*-1 gene in tomato (Li *et al.*, 2006). Therefore, it is likely that disease resistant mutation derived genotypes could also resist species of aphid that are vulnerable to the SA signalling pathway.

2.8.1.3 Aphid and pathogen resistant genes often being found close together on chromosomes

Aphid and pathogen resistance genes are often clustered on the same region of the chromosomes (Seah *et al.*, 2007; Stewart *et al.*, 2009; Dogimont *et al.*, 2010). In apple (*Malus domestica* Borkh), for example, woolly apple aphid (*Eriosoma lanigerum*) resistance genes (*Er1* and *Er2*), on chromosomes 8 and 17 respectively, are located on the same genomic regions with genes for resistance to powdery mildew (Bus *et al.*, 2008). The *Ra* gene on chromosome 2 that mediates resistance in lettuce against the lettuce root aphid (*Pemphigus bursarius* L.) is clustered together with downy mildew resistance genes on the same chromosome (Christopoulou *et al*., 2015). Similarly, the potato aphid (*M. euphorbiae*) resistance gene (*Mi*-1) on chromosome 6 in tomato shares the same location (chromosomal region) with disease resistance genes (Seah *et al*., 2007). Due to this common genomic locale of aphid and pathogen resistance genes, chromosomal alterations due to induced mutagenesis are likely to induce genetic variations for both pathogen and aphid resistance traits.

2.8.1.4 Screening of mutagenised plant population

Induced mutagenesis often introduces random changes in the target organism's genome, making it difficult to precisely target specific genes controlling a desired trait. This lack of specificity requires labour intensive screening of large mutant populations (approximately 5,000 to 10,000 genotypes) to optimise chances of finding desirable mutations. To overcome this limitation, techniques such as TILLING (Targeted Induced Local Lesions IN Genomes) have been developed to enhance the detection of useful mutations in mutagenised plant populations (Penna & Jain, 2017). The TILLING technique combines mutagenesis and polymerase chain reaction (PCR) technology to identify point mutations such as single nucleotide polymorphisms (SNPs) in target genes (Irshad *et al.*, 2020). In particular, TILLING allows for identification of variations in mutant genome providing a criteria for shortlisting mutation derived genotypes with potential aphid resistance to include in phenotypic screening (Viana *et al.*, 2019). This molecular approach for identifying mutations, as opposed to whole plants in conventional screening, makes TILLING a high throughput and cost effective screening method. The improved capability of genomic tools in recent years offers more thorough investigations of gene structure and function in mutation derived genotypes which could allow for easier identification, introgression and molecular characterisation of durable resistance to aphid pests.

CHAPTER THREE

3 MATERIALS AND METHODS

3.1 Characterisation of resistance to the black bean aphid, *Aphis fabae* (Scopoli) in selected and mutation derived genotypes of the common bean, *Phaseolus vulgaris* (L.)

This study aimed to identify aphid resistant genotypes from selected varieties and mutation derived genotypes as well as any mechanisms mediating resistance. Specifically, deterrence to aphid was assessed by measuring settling preference. Physical barriers to aphid feeding were assessed by nymph survival while reduction in palatability of phloem sap was evaluated by nymph development and mean relative growth rate (MRGR). Electrical penetration graph (EPG) recordings of aphid feeding behaviour were performed to localise plant resistance factors. All laboratory bioassays were carried out within Jean Jackson Entomology laboratory of Harper Adams University, United Kingdom.

3.1.1 Common bean genotypes and experimental design

A total of eleven common bean genotypes were evaluated for aphid resistance (Table 3.1). Of these genotypes five were selected from the Andean Diversity Panel (ADP) (Cichy *et al.*, 2015), four were mutation derived genotypes, one (Carioca) is a parent of the mutant lines, and one (AO-1012-29-3-3A) (AO) is a released variety.

Table 3.1: List and category of common bean (*Phaseolus vulgaris* L.) genotypes used in the study

Common bean line	Seed colour	Country of origin	Category
Rozi Koko (ADP 1)	Red-Mottled	Kenya	Variety
Mwezi Moja (ADP 466)	Purple	Kenya	Variety
Majesty (ADP 684)	Red Kidney	Canada	Variety
KK25 (ADP 765)	Red	Malawi	Landrace
AO 1012-29-3A	Red	-	Variety
Kabulangeti (KAB)	Purple	Zambia	Variety

CA 3	Brown-Mottled	Zambia	Mutant
CA 15	Brown-Mottled	Zambia	Mutant
CA 24	Brown-Mottled	Zambia	Mutant
CA 38	White	Zambia	Mutant
CA	Brown	Brazil	Parent line (for mutants)

The genotypes selected from the ADP included Rozi Koko (ADP 1), Mwezi Moja (ADP 466), Majesty (ADP 684), and KK 25 (ADP 765). The ADP genotypes were selected based on their agronomic traits (ADP 1 and ADP 684), and anecdotal evidence on their resistance to pests such as weevils (ADP 765) and bean stem maggot (ADP 466). AO is a determinate dark red kidney variety that was developed, and released cooperatively by Sokoine University of Agriculture, Oregon State University, USDA-ARS and the University of Puerto Rico (Kusolwa *et al.*, 2016). AO is resistant to common bean weevil (*Acanthoscelides obtectus*) (Kusolwa *et al.*, 2016; Kamfwa *et al.*, 2018). In addition, AO is resistant to Bean Common Mosaic Virus (Kusolwa *et al.*, 2016) and some races of anthracnose (Mungalu *et al.*, 2020). The commercial variety Kabulangeti (KAB), which is widely grown in Zambia, and Carioca (CA), a parent for the mutants were used as checks.

Two seeds of each genotype were sown in plastic pots (diameter and height: 9 cm) containing potting soil (John Innes No. 2, J. Arthur Bower’s, Westland Horticulture Limited, Cheshire, UK) and placed in an insect proof mesh cage within a controlled environment room maintained at 20 °C and 60 % relative humidity with a 16:8 photoperiod (Fitotron, Weiss Technik UK limited, Loughborough, UK). Seeds were allowed to germinate and grow until they were ten days old (BBCH growth stage 11-12) (Lancashire *et al.*, 1991), when plants were thinned to leave one seedling per pot. Irrigation was done by adding water to trays twice weekly throughout the study period. Twelve-day old common bean plants (BBCH growth stage 12) were used for the nymph development, settling preference, nymph survival, mean relative growth rate (MRGR) and feeding behaviour experiments. Twelve-day old plants were used in bioassays to match the aphid susceptible growth stage (Esmaeili-Vardanjani *et al.*, 2013). Plants for the trichome density experiment were sown and thinned as described above but cultivated in an insect rearing tent within a glasshouse at 20 °C and 60 % relative humidity with an 18:6 photoperiod. Approximately 15 days after germination, a 1 m long stake was inserted into each pot (9 cm depth) to provide support to the growing bean plants. Plants were allowed to grow for approximately 28 days (BBCH growth stage 16+) to match the timing of the trichome study.

3.1.2 Aphid culture and age-synchronised cohort production

A stock culture of black bean aphids (*Aphis fabae* Scopoli) was reared on field bean seedlings (*Vicia faba* cv. Tundra) in an insect proof mesh cage within a controlled environment room (Fitotron) maintained at 20 °C and 60 % relative humidity with a 16:8 photoperiod. The culture was maintained by transferring aphids onto new field bean seedlings weekly throughout the study period.

To produce a cohort of age-synchronised apterous adult aphids for nymph development, nymph survival, MRGR and feeding behaviour experiments, two to five apterous adult aphids were transferred onto individual broad bean seedlings within an insect proof cage. After 24 hours, adult aphids were removed from the plants using a paintbrush to leave only first instar nymphs. To prevent escape of nymphs and plants becoming infested with other insects, each plant was covered with a fine light-transmitting mesh bag, secured around the pot using an elastic band (Figure 3.1). Plants were maintained in a controlled environment room at 20 °C and 60 % relative humidity with a 16:8 photoperiod until the nymphs moulted into adults (approximately seven to eight days).

To produce winged adult aphids for the settling preference experiment, ten to fifteen apterous adult aphids were transferred onto individual 2-week-old field bean seedlings within an insect proof mesh cage. After 24 hours, adult aphids were removed from the plants using a paintbrush to leave only first instar nymphs. As described above, each plant was covered with a fine light-transmitting mesh bag. Development of winged aphids was stimulated by the higher population of nymphs on seedlings and consequent rapid deterioration of plant nutrition quality (Blackman & Eastop, 2000). Plant nutritional quality was further reduced by irrigating bean plants only once per week. Field bean seedlings were maintained in a controlled environment room at 20 °C and 60 % relative humidity with a 16:8 photoperiod until the majority of nymphs moulted into winged adults (approximately seven to eight days).

Figure 3.1: Bean plants covered with a fine light-transmitting mesh bags

3.1.3 Assessment of nymph development

Development of black bean aphid nymphs was assessed using a procedure adapted from Soffan and Aldawood (2014). Using a paint brush, age-synchronised adult apterous aphids were individually placed onto common bean plant leaves in an insect proof mesh cage within a controlled environment room at 20 °C and 60 % relative humidity with a 16:8 photoperiod. After 24 hours, all aphids were removed, leaving a single first instar nymph per plant. Each plant was covered with a fine light-transmitting mesh bag and returned to the controlled environment room. Development through the immature nymph life cycle stages was monitored by the presence of exuviae, which were removed using a paintbrush and the date recorded. The total number of days between birth and adult emergence was also recorded simultaneously for each bean genotype within a replicate. Ten plants from each genotype were tested in this bioassay.

3.1.4 Determination of trichome density

Glandular and hooked trichome density on common bean leaves was determined using a procedure adapted from Dahlin *et al*. (1992). A fully expanded middle leaflet was excised using a pair of scissors from the third trifoliate on each bean plant. Using a surgical blade, a 1 cm^2 section of leaf was removed from between the lateral veins at the widest region of the leaflet. Leaf sections were affixed onto scanning electron microscope (SEM) stubs using a double-sided adhesive tape, with the lower leaf surface facing up. Only trichomes on the lower leaf surface were considered in this study as it is the primary feeding site for black bean aphid (Prado & Tjallingii, 1997) (Figure 3.2b). Leaf samples were placed in a SEM specimen holder and dried in a desiccator for 48 hours. Dry leaf samples were then coated with a gold film using a sputter coater (Edwards S 150, Edwards High Vacuum, Crawley, Sussex, England). Hooked and glandular trichomes were counted and photographed using the scanning electron microscope (Cambridge Stereoscan 200, Cambridge Instruments Ltd, Cambridge, UK) (Figure 3.2a). Four plants from each common bean genotype were evaluated.

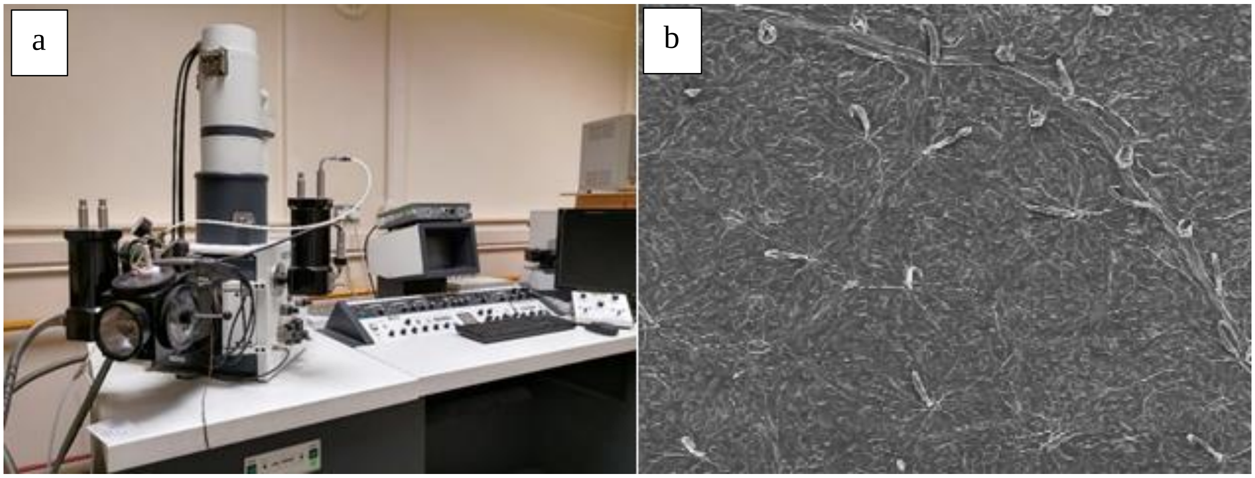

Figure 3.2: (a) Scanning electron microscope (SEM) and (b) SEM image of trichomes on lower bean leaf surface

3.1.5 Settling preference of aphids on bean genotypes

Settling preference of black bean aphid was evaluated as described by Laamari *et al*. (2008) and Kamphuis *et al*. (2012). A single plant of each genotype was placed into a cage within a controlled environment room at 20 °C and 60 % relative humidity with a 16:8 photoperiod. Plants were placed in a circular pattern such that the pots were equidistant from each other and spaced as far apart as possible (approximately 15 cm) to prevent leaves of adjacent plants touching one another (Figure 3.3). Preliminary experiments showed that the spacing of plants within the cage as well as lighting (phototaxis) did not affect the behaviour of winged aphid. A plastic 90 mm Petri dish containing a cohort of 120 age-synchronised alate aphids was placed at the centre of the cage, approximately 20 cm from each plant. Aphids were allowed to select plants on which to land and settle for a period of 72 hours. The number of aphids settling on each plant were recorded at 24, 48 and 72 hours after being released. Numbers of aphids settling on bean plants 24 hours after release indicated whether immediate deterrent factors (i.e. trichomes) were present or absent in bean genotypes while numbers of aphids recorded from 24 to 72 hours (through 48 hours) provided information whether the numbers of aphids settling on plants increased or decreased. The position of each genotype in the cage was randomly allocated in each replicate. Ten plants from each genotype were tested in this bioassay.

Figure 3.3: Aphid settling preference bioassay set-up showing (a) bean plants arranged in a circular pattern within a cage and (b) vials containing alate aphids

3.1.6 Assessment of nymph survival

Survival of black bean aphid nymphs was as described by Obopile & Ositile (2010). Using a paintbrush, two age-synchronised apterous adult aphids were placed on each common bean plant in an insect proof mesh cage within a controlled environment room at 20 °C and 60 % relative humidity with a 16:8 photoperiod. After 24 hours, aphids were removed from plants using a paintbrush, leaving ten first instar nymphs per plant. Each plant was covered with a fine light-transmitting mesh bag and returned to the controlled environment room. Nymph survival was estimated as the number of aphids found on each plant after seven days, expressed as a proportion of the initial count on each bean plant. Ten plants from each genotype were tested in this bioassay.

3.1.7 Mean relative growth rate of nymphs on bean genotypes

Mean relative growth rate of aphids was evaluated as described by van Emden & Bashford (1969). Using a paintbrush, two age-synchronised apterous adult aphids were placed on each common bean plant in an insect proof mesh cage within a controlled environment room at 20 °C and 60 % relative humidity with a 16:8 photoperiod. After 24 hours, all aphids were removed except ten first instar nymphs per plant which were weighed using a microbalance to record the initial mean weight. After weighing, nymphs were placed back onto respective plants and covered with a fine light-transmitting mesh bag. Plants were maintained in the controlled environment room for five days, when a single nymph from each plant was re-weighed to record the final weight. Mean relative growth rate was calculated using:

$$MRGR\ (\mu g/\mu g/day) = \frac{(\log(W_2) - \log(W_1))}{(t_2 - t_1)}$$

Where W_1 = initial mean weight of nymphs, W_2 = weight of a single nymph after five days and $(t_2 - t_1)$ = period (days) between the first (t_1) and final weighing (t_2) (Castle & Berger, 1993). Ten plants from each genotype were tested in this bioassay.

3.1.8 Monitoring of aphid feeding behaviour

Direct-current (DC) electrical penetration graph (EPG) recording was used to monitor probing and feeding behaviour of apterous adult aphids (Tjallingii, 1978) (Figure 3.4). Since mutation derived common bean genotypes did not have a negative biological effect on aphids in preceding experiments (nymph development, settling preference, nymph survival and MRGR), only breeding lines were subjected to EPG recording. A plant probe, soldered to an electrical wire, was inserted into the moist soil of a potted plant while the free end of the wire was connected to the out-put voltage socket of the Giga-8 EPG device (EPG Systems, Wageningen, The Netherlands) (Figure 3.4d). An aphid probe was assembled by attaching a 3-4 cm piece of gold wire (diameter 20 µm) to the copper electrode end of brass pin using conductive silver glue (EPG Systems). Using this glue, the other end of the gold wire was attached onto the aphid dorsum. Brass pins with wired aphids were then inserted into the EPG probes (Figure 3.4c) mounted on retort stands. The EPG probes were carefully lowered to allow aphids contact with leaves of wired individual plants (Figure 3.4b). Feeding behaviour of eight aphids was monitored simultaneously over a four-hour period using a Giga-8-EPG device connected to a laptop computer (Figure 3.4e). A total of fourteen successful recordings were carried out for individual aphids feeding on each bean genotype. Plants and aphids were contained in a grounded faraday cage (Figure 3.4a) during EPG recording.

Data was acquired using the stylet+ D software (EPG Systems) while waveforms; non-probing (np), pathway phase (pp), sieve element phase (SEP) and xylem ingestion (G) were annotated using the stylet+ A software (EPG Systems) based on the wave categories described by Tjallingii (1988). Annotated waveforms were transformed into time-series data using the Excel macro software developed by Sarria *et al*. (2009).

Figure 3.4: Electrical penetration graph (EPG) set-up showing the (a) faraday cage, (b) bean plants, (c) EPG probes, (d) Giga-8-EPG device and (e) a laptop computer

3.1.9 Experimental design and data analysis

Due to the homogeneity of environmental conditions in the controlled environment room and glasshouse as well as potting soil, a complete randomised design (CRD) was used for all experiments.

Statistical analyses were carried out using R version 4.0.2 (R Core Team, 2020). Prior to analysis, key assumptions for parametric statistical tests were checked. Normality of distribution was checked using the Shapiro-Wilk test while homogeneity of variance was assessed by the Bartlett test. Data that satisfied parametric test assumptions were analysed using one-way analysis of variance (ANOVA). Data that was neither normally distributed nor with homogenous variance, such as for settling preference and feeding behaviour, were log-transformed to meet parametric assumptions before analysis using one-way ANOVA and pairwise comparisons with the Holm-Sidak method. Data that did not meet parametric assumptions following log-transformation were analysed with Kruskal-Wallis rank-sum tests. For each experiment the common bean varieties and corresponding mutation derived genotypes were analysed separately, except for the settling preference experiment where all varieties and mutation derived genotypes were analysed together. Levels of significance were established at $p < 0.05$.

3.2 Characterisation of resistance to cowpea aphid, *Aphis craccivora* (Koch) in mutation derived cowpea genotypes, *Vigna unguiculata* (L.) Walp genotypes

This study identified aphid resistant genotypes after gamma irradiation of three susceptible cowpea varieties widely grown in Zambia and the mechanism(s) underpinning this resistance. All the laboratory bioassays were carried out within Jean Jackson Entomology laboratory of Harper Adams University, United Kingdom.

3.2.1 Plants

A total of eleven genotypes derived from three susceptible cowpea varieties were evaluated for aphid resistance. Susceptible cowpea varieties were Bubebe, Lutembwe and Msandile. Six genotypes BB 3-9-7-5, BB 7-9-7-5, BB 8-1-7-5, BB 10-4-2-3, BB 14-16-2-2 and BBVN1 were derived from Bubebe, three LT 3-8-4-1, LT 4-2-4-1 and LT 11-3-3-12 from Lutembwe, and two MS 1-8-1-4 and MS 10-7-2-1 from Msandile. Seed from the susceptible varieties Bubebe and Lutembwe were treated with 150 gray of gamma rays while Msandile was treated at a lower dose of 100 gray because it was more sensitive to radiation. Radiation of seed was carried out using a Co60 source at the National Institute for Scientific and Industrial Research (NISIR), Plant Science Centre, Zambia. Resulting mutation derived genotypes were then advanced to stable generations 8 -10 (M_8 – M_{10}) before agronomic traits were evaluated. Genotypes used in this study were selected based on potential pesticidal traits including resistance to cowpea bruchid (*Callosobruchus maculatus* F.) (BB 7-9-7-5 and BB 14-16-2-2) (Tembo *et al.*, 2017), anecdotal evidence of resistance to cowpea aphid (BB 10-4-2-3, BB 14-16-2-2 and LT 3-8-4-1) and cowpea leaf blight (*Ascochyta* spp.) (BB 8-1-7-5, LT 11-3-3-12 and LT 4-2-4-1).

Three seeds of each cowpea genotype were sown in plastic pots (diameter and height: 9 cm) (LBS worldwide Ltd., Lancashire, UK) containing potting soil (John Innes No. 2, J. Arthur Bower's, Westland Horticulture Limited, Cheshire, UK) and placed in an insect proof mesh cage (60 x 60 x 60 cm, BugDorm-6S610, MegaView Science Co. Ltd, Taichung, Taiwan) within a controlled environment room maintained at 20 °C and 60 % relative humidity with a 16:8 photoperiod (Fitotron, Weiss Technik UK limited, Loughborough, UK). Seeds were allowed to germinate and grow (Figure 3.1) until they were eight days old (BBCH growth stage 10) (Lancashire *et al.*, 1991) before being thinned to leave one seedling per pot. No fertiliser was applied to the plants and irrigation was done by adding water to trays twice weekly throughout the study period. Plants used for each of the bioassays completed were 10-15 days old (BBCH growth stage 11-15).

3.2.2 Aphid culture and age-synchronised cohort production

A stock culture of cowpea aphid (*Aphis craccivora* Koch) was reared on cowpea seedlings in an insect proof mesh cage (47.5 x 47.5 x 47.5 cm, BugDorm-4S4545, MegaView Science Co. Ltd, Taichung, Taiwan) within a controlled environment room maintained at 20 °C and 60 % relative humidity with a 16:8 photoperiod. The culture was maintained by transferring aphids onto new cowpea seedlings (Figure 3.5) weekly throughout the study period.

Figure 3.5: Newly emerged cowpea plants for aphid rearing and bioassays

To produce a cohort of age-synchronised apterous adult aphids for use in bioassays, two to five apterous adult aphids were transferred onto individual cowpea seedlings within an insect proof mesh cage. After 24 hours, adult aphids were removed from the plants using a size 000 paintbrush to leave only first instar nymphs. To prevent escape of nymphs and plants becoming infested with other insects, each plant was covered with a fine light-transmitting mesh bag (0.3 x 0.4 m large organza bags; mesh size 0.5 mm, TtS Ltd, UK), secured around the pot using an elastic band.

Plants were maintained in a controlled environment room at 20 °C and 60 % relative humidity with a 16:8 photoperiod until the nymphs moulted into adults (approximately seven to eight days).

3.2.3 Cowpea aphid colony growth

Colony growth of cowpea aphid nymphs was assessed using a procedure adapted from Soffan and Aldawood (2014). Using a size 000 paintbrush, 1-2-day old age-synchronised adult apterous aphids were individually placed onto cowpea plant leaves in an insect proof mesh cage (as described before) within a controlled environment room at 20 °C and 60 % relative humidity with a 16:8 photoperiod. After 24 hours, all aphids were removed, leaving three first instar nymphs per plant. Each plant was covered with a fine light-transmitting mesh bag and returned to the controlled environment room. Fourteen days after infestation, total numbers of aphids (adults and nymphs) were counted and recorded. This experiment was replicated ten times for each cowpea genotype.

3.2.4 Individual cowpea aphid performance

Performance of individual cowpea aphids was evaluated as described by Hu *et al*. (2018). Aphids were individually placed onto cowpea plant leaves and maintained as described for the colony growth experiment. After 24 hours, all aphids were removed, leaving a single first instar nymph per plant. Each plant was covered with a fine light-transmitting mesh bag and returned to the controlled environment room. Nymphs were monitored daily to record development time, fecundity, intrinsic rate of natural increase (r_m), and population doubling time (DT). Measurement and calculation of each biological parameter was carried out as described in Table 3.2. A replicate was regarded as a single nymph placed on each cowpea genotype. This experiment was replicated fifteen times.

Table 3.2: Description of aphid parameters measured in the study

Aphid parameter	**measurement**
Nymph development (d)	Duration from birth to onset of reproduction
Fecundity (M_d)	Total number of nymphs born from an individual aphid after adult emergence within a duration equivalent to d

Intrinsic rate of natural increase (r_m)	$\frac{0.738\ \text{In}\ (Md)}{d}$
Population doubling time (DT)	$\frac{\text{In}\ (2)}{rm}$
Mean relative growth rate (MRGR)	$\frac{(\log(W_2) - \log(W_1)}{(t_2 - t_1)}$

W_1 = initial mean weight of nymphs, W_2 = weight of a single nymph after four days, ($t_2 – t_1$) = period (days) between the initial (t_1) and final weighing (t_2).

Mean relative growth rate of aphids was evaluated as described by Thieme and Heimbach, (1996). Aphids were individually placed onto cowpea plant leaves and maintained as described for the colony growth experiment. After 24 hours, all aphids were removed except ten first instar nymphs per plant which were weighed using a microbalance (XPR10 Ultra-microbalance, Mettler Toledo, Greifensee, Switzerland) to record the initial mean weight. After weighing, nymphs were placed back onto their respective plants and covered with a fine light-transmitting mesh bag. Plants were maintained in the controlled environment room for four days, when a single nymph from each plant was re-weighed to record the final weight. Mean relative growth rate was calculated as described in Table 3.1. Ten replications for each genotype were completed for the MRGR experiment.

3.2.5 Feeding behaviour

Direct-current (DC) electrical penetration graph (EPG) recording was used to monitor probing and feeding behaviour of apterous adult aphids (Tjallingii, 1978). A plant probe, soldered to an electrical wire, was inserted into the moist soil of a potted plant while the free end of the wire was connected to the out-put voltage socket of the Giga-8-EPG device (EPG Systems, Wageningen, The Netherlands). An aphid probe was assembled by attaching a 3-4 cm piece of gold wire (diameter 20 µm, EPG Systems) to the copper electrode end of brass pin using conductive silver glue (EPG Systems). Using this glue, the other end of the gold wire was attached onto the aphid dorsum. Brass pins with wired aphids were then inserted into the EPG probes mounted on retort stands. The EPG probes were carefully lowered to allow aphids contact with leaves of wired individual plants. Feeding behaviour of eight aphids was monitored simultaneously over a four-hour period using a Giga-8-EPG device connected to a laptop computer. Twenty recordings were carried out for each cowpea genotype. Plants and aphids were contained in a grounded faraday cage during EPG recording.

Data was acquired using the stylet+ D software (EPG Systems) while waveforms; non-probing (np), pathway phase (pp), sieve element phase (SEP) and xylem ingestion (G) were annotated using the stylet+ A software (EPG Systems) based on the wave categories described by Tjallingii (1978). Annotated waveforms were transformed into time-series data using the Excel macro software developed by (Sarria *et al.*, 2009).

3.2.6 Cowpea aphid colony growth under field conditions

A field study was conducted at Liempe farm of the University of Zambia (UNZA) which is located in the region II of the Zambian agro-ecological zones. Three sites, UNZA-Liempe (site 1), UNZA-Agricultural Technology Demonstration Center (site 2), and UNZA-Field Research Station (site 3), were selected for the study based on preliminary experiments that indicated high and consistent cowpea infestations by the cowpea aphid. Given the laboratory screening results which showed potential aphid resistance, four cowpea genotypes namely, Lutembwe, LT 3-8-4-6, LT 4-2-4-1 and LT 11-3-3-12 were selected for this study. Standard cowpea production practices such as land preparation, sowing, and weeding were followed systematically. The land was ploughed and harrowed to a fine tilth using a tractor. A plot at each site measured 30 m long x 30 m wide and was demarcated into sixteen sub-plots, each measuring 3 m long x 3 m wide. In the sub-plot, two seeds at each station were planted at 0.45 m between rows and 0.15 m within rows. A total of five rows per sub-plot were established. Four sub-plots for each genotype were established in a plot. During seeding, basal dressing fertiliser (D-compound 10% N: 20% P_2O_5: 10% K_2O: + 6% S) was applied at a rate of 150 kg/ha. The field was manually weeded twice during the study period while irrigation was facilitated by rainfall. About thirty days after planting, total numbers of aphids, both adults and nymphs, on five randomly selected plants within the three middle rows of each sub-plot were counted and recorded.

3.2.7 Experimental design and data analysis

Due to the homogeneity of environmental conditions in the controlled environment room as well as soil used to grow plants, a complete randomised design (CRD) was used for all laboratory experiments while a randomised complete block design (RCBD) was adopted for the field study. Statistical analyses were carried out using R version 4.0.2 (R Core Team, 2020). Prior to analysis, key assumptions for parametric statistical tests were checked. Data distributions were checked using the Shapiro-Wilk test while homogeneity of variance was assessed by the Bartlett test. Data that satisfied parametric test assumptions were analysed using one-way analysis of variance (ANOVA). Non-Gaussian data that had non-homogenous variance were log-transformed to meet parametric assumptions before analysis using one-way ANOVA and pairwise comparisons with the Holm-Sidak method. Data that did not meet parametric assumptions following log-transformation such as for colony growth and feeding behaviour were analysed with Kruskal-Wallis rank-sum tests. Levels of significance were established at $p < 0.05$.

3.3 Evaluation of cowpea aphid, *Aphis craccivora* (Koch) performance on silicon treated cowpea genotypes, *Vigna unguiculata* (L.) Walp under laboratory and field conditions

This study aimed to evaluate silicon accumulation capacity of mutation derived cowpea genotypes and aphid performance under laboratory conditions. A field experiment was carried out to evaluate aphid colony growth on silicon treated cowpea plants under commercial conditions. All the laboratory bioassays were carried out within Jean Jackson Entomology laboratory of Harper Adams University, United Kingdom.

3.3.1 Aphid culture and age-synchronised cohort production

Cowpea aphids (*Aphis craccivora* Koch) were reared on cowpea seedlings in plastic pots (diameter and height: 9 cm, Teku Pöppelmann, Lohne, Germany) filled with potting soil (John Innes No. 2, J. Arthur Bower's, Westland Horticulture Limited, Cheshire, UK) in an insect proof mesh cage (47.5 x 47.5 x 47.5 cm, BugDorm-4S4545, MegaView Science Co. Ltd, Taichung, Taiwan) within a controlled environment room (Fitotron, Weiss Technik, Loughborough, UK) set to a constant 20°C and 60 % relative humidity and with a 16:8 photoperiod. The culture was maintained by transferring aphids on to new cowpea seedlings weekly throughout the study period.

To produce a cohort of age-synchronised apterous adult aphids for use in bioassays, two to five apterous adult aphids were transferred onto individual cowpea seedlings within an insect proof mesh cage. After 24 hours, adult aphids were removed from the plants using a fine paintbrush (size 000) to leave only first instar nymphs. To prevent escape of nymphs and plants becoming infested with other insects, each plant was covered with a fine light-transmitting mesh bag (30 x 40 cm large organza bags; mesh size 0.5 mm, TtS Ltd, UK), secured around the pot using an elastic band. Plants were maintained under the same controlled environment room conditions as described for the aphid culture until the nymphs moulted into adults (approximately seven to eight days).

3.3.2 Plant growth and application of silicon

A total of four cowpea genotypes namely, Lutembwe, LT 3-8-4-6, LT 4-2-4-1 and LT 11-3-3-12 were used. Lutembwe is an aphid susceptible commercial variety widely grown in Zambia while LT 3-8-4-6, LT 4-2-4-1 and LT 11-3-3-12 are mutant genotypes derived from Lutembwe using gamma irradiation and have promising aphid resistance (Zimba *et al.*, 2022b).

Three seeds of each cowpea genotype were sown in plastic pots (diameter and height: 9 cm) containing potting soil and placed in an insect proof mesh cage (60 x 60 x 60 cm, BugDorm-6S610, MegaView Science Co. Ltd, Taichung, Taiwan), which were placed into a controlled environment room set to 20 °C and 60 % relative humidity with a 16:8 photoperiod (Fitotron). Seeds were allowed to germinate and grow until they were eight days old (BBCH growth stage 10) (Lancashire *et al.*, 1991) before being thinned to leave one seedling per pot. Irrigation was done by adding water to trays twice weekly.

Ten-day old plants (BBCH growth stage 11-15) of each cowpea genotype were used for the silicon study. For each genotype, half of the plants were treated with silicon (Si+) while the remainder were used as controls. Silicon treated plants were each drenched with 50 ml of 0.25 % silicon solution (Sirius®, Orion Future Technology, Kent, UK) using a 100 ml conical flask, followed by a second application applied seven days later. Control plants received no supplementary silicon (Si-) but were each supplied with tap water equivalent to the volume of the silicon solution. Plants were placed on plastic Petri dishes when the silicon solution or water were applied in order to allow uptake of any liquid initially draining through the pot. Treatment and control plants were then placed in separate insect proof mesh cages, within a controlled environment room set to 20 °C and 60 % relative humidity with a 16:8 photoperiod (Fitotron). Plants were assessed for silicon accumulation or used in the aphid performance bioassays approximately three to four days after the second silicon application.

3.3.3 Evaluation of silicon accumulation capacity of cowpea genotypes

Using a secateur, Si- and Si+ plants were cut at the base of the stem and placed in oven bags (0.20 x 0.25 m perforated polypropylene bags, Cater for you Ltd, UK). To meet the minimum requirement for analysis of 0.5 g silicon present in dried samples, three to four plants of each genotype and treatment (Si- or Si+) were pooled within each replicate and placed into oven bags. These were then placed in the oven (Genlab Ltd, Tanhouse lane, Cheshire, UK) and dried at 60 °C for 48 hours. Ten replicates were performed for each genotype and treatment. Dry plant samples were then ground to a powder using a stainless steel mortar and pestle, and were later placed into labelled 50 ml plastic vials. Analysis of silicon in dry plant samples was conducted by the University of Salford (Manchester, United Kingdom), using a method similar to that of Ranger *et al.* (2019).

3.3.4 Laboratory assessment of aphid colony growth

Cowpea aphid nymph colony growth was assessed using a procedure adapted from Soffan and Aldawood (2014). Using a size 000 paintbrush, age-synchronised adult apterous aphids that had completed their final moult one-two days before were placed individually onto 19-day old Si- and Si+ cowpea plants. These plants were then placed into insect proof cages (47.5 x 47.5 x 47.5 cm), within a controlled environment room set to 20 °C and 60 % relative humidity with a 16:8 photoperiod (Fitotron). After 24 hours, all aphids were removed, leaving three first instar nymphs per plant. Each plant was covered with a fine light-transmitting mesh bag (TtS Ltd) secured around the pot using an elastic band and later returned to the insect proof cages within the controlled environment room set to 20 °C and 60 % relative humidity with a 16:8 photoperiod. Fourteen days after infestation, total numbers of aphids (adults and nymphs) on Si- and Si+ plants were counted and recorded. Ten Si+ and 10 Si- plants of each genotype were tested in this bioassay.

3.3.5 Evaluation of nymph mean relative growth rate

Aphid mean relative growth rate was evaluated as described by van Emden & Bashford (1969). Using a fine paintbrush (size 000), age-synchronised adult apterous aphids that had completed their final moult one-two days before were placed individually onto 19-day old Si- and Si+ cowpea plants. These plants were then placed into insect proof cages within a controlled environment room set to 20 °C and 60 % relative humidity with a 16:8 photoperiod (Fitotron). After 24 hours, all aphids were removed except ten first instar nymphs per plant which were weighed using a microbalance (XPR10 Ultra-microbalance, Mettler Toledo, Greifensee, Switzerland) to record the initial mean weight. After weighing, nymphs were placed back onto the same plant from where they were taken and the plant was covered with a fine light-transmitting mesh bag. Plants were maintained in the controlled environment room (set to 20 °C and 60 % relative humidity with a 16:8 photoperiod) for a further five days, when a single nymph from each plant was re-weighed to record the final weight. Mean relative growth rate was calculated using:

$$MRGR\ (\mu g/\mu g/day)\ = \frac{(\log(W_2) - \log(W_1))}{(t_2 - t_1)}$$

Where W_1 = initial mean weight of nymphs, W_2 = weight of a single nymph after five days and $(t_2 - t_1)$ = period (days) between the first (t_1) and final weighing (t_2) (Castle & Berger, 1993). Ten Si- and 10 Si+ plants of each genotype were tested in this bioassay.

3.3.6 Recording aphid feeding behaviour

Direct-current electrical penetration graph (EPG) recording was used to monitor probing and feeding behaviour of apterous adult aphids (Tjallingii, 1978). A plant probe, soldered to an electrical wire, was inserted into the moist soil of a potted plant while the free end of the wire was connected to the output voltage socket of the Giga-8 EPG device (EPG Systems, Wageningen, The Netherlands). An aphid probe was assembled by attaching a 3-4 cm piece of gold wire (diameter 20 µm) to the copper electrode end of brass pin using conductive silver glue (EPG Systems). Using this glue, the other end of the gold wire was attached onto the dorsal surface of age-synchronised adult apterous cowpea aphid that had completed their final moult. Brass pins with wired aphids were then inserted into the EPG probes mounted on retort stands. The EPG probes were carefully lowered to allow aphids contact with leaves of wired individual plants. Feeding behaviour of eight aphids was monitored simultaneously over a four-hour period using a Giga-8-EPG device connected to a laptop computer. A total of ten successful recordings were carried out for individual aphids feeding on each plant. Plants and aphids were contained in a grounded faraday cage during EPG recording.

Data was acquired using the stylet+ D software (EPG Systems) while waveforms; non-probing (np), pathway phase (pp), sieve element phase (SEP) and xylem ingestion (G) were annotated using the stylet+ A software (EPG Systems) based on the wave categories described by Tjallingii (1988). Annotated waveforms were transformed into time-series data using the Excel macro software developed by Sarria *et al.* (2009).

3.3.7 Field experiment

Field assessment of aphid colony growth was carried out at the University of Zambia – Agricultural Technology Demonstration Center (15º 23'S, 28º 20'E). This study site was selected based on the prevalence of cowpea aphid in this area. Four cowpea genotypes (Lutembwe, LT 3-8-4-6, LT 4-2-4-1 and LT 11-3-3-12) were used in this study. Land preparation was undertaken following standard cowpea production practices as described by (Simunji *et al.*, 2019). The land was ploughed and harrowed to a fine tilth. Three plots each measuring 30 m long x 15 m wide and separated by 10 m were marked out. Each plot was demarcated into sixteen sub-plots, with each side measuring 4 m long x 3 m wide. The sub-plot was further demarcated into two 'split-plots' (~ 0.5 m apart) measuring 2 m long and 3 m wide. Two seeds at each station were planted with a spacing of 0.45 m between and 0.15 m within rows. Each sub-plot was planted with a single genotype consisting of five rows. During planting, basal dressing fertiliser (D-compound 10 % N: 20 % P_2O_5: 10 % K_2O: + 6 % S) was applied at a rate of 200 kg/ha.

Within the sub-plot, plants in one split-plot were treated with Si (Si+) while those from the other (Si-) were treated with equivalent volume of water as a control. Silicon was applied two days after plant emergence, followed by a second and third application seven and fourteen days after the first application respectively. Silicon solution was prepared by diluting 1.25 ml of Si product in 1 L of water. A knapsack sprayer (Jacto-XP16, Pompeia, Brazil), with a nozzle removed, was calibrated to deliver approximately 50 ml of Si mixture (~ 0.5 L/ha) per second, by drenching the soil around the plant. Since the natural seedling infestation by cowpea aphid was very sporadic in the field by ten to fourteen-day post emergence, it was necessary to artificially infest a sample of cowpea plants. Using a paint brush (size 000), age-synchronised adult apterous aphids that had completed their final moult one to two days before, were placed individually onto cowpea plants within the middle row of each split-plot. Fourteen days after artificial infestation, total numbers of aphids, both adults and nymphs, on ten randomly selected plants within the three middle rows of each split-plot were counted and recorded.

3.3.8 Experimental design and data analysis

Due to the homogeneity of environmental conditions in the controlled environment room and compost, a complete randomised design was used for all laboratory experiments while a split-plot design was adopted for the field study. Statistical analyses were carried out using R version 4.0.2 (R Core Team, 2020). Prior to analysis, key assumptions for parametric statistical tests were validated. Normality of distribution was validated using the Shapiro-Wilk test while homogeneity of variance was assessed by the Bartlett test. Differences in silicon content, aphid colony growth, MRGR and aphid feeding activities between Si+ and Si- plants of each genotype were analysed using a Welch two sample t-test. Data that did not meet parametric assumptions after log-transformation, such as for EGP parameters and colony growth, were analysed with Wilcoxon rank sum test. Levels of significance were established at $p < 0.05$.

CHAPTER FOUR

4 RESULTS

4.1 Characterisation of resistance to black bean aphid (*Aphis fabae*) in selected varieties and mutation derived common bean (*Phaseolus vulgaris*) genotypes

4.1.1 Results summary

Aphid performance bioassays were undertaken to assess the relative resistance of common bean genotypes in comparison with susceptible commercial cultivars. Among the parameters measured, significant differences in aphid performance were observed on nymph development, trichome density and feeding behaviour (phloem ingestion). No significant differences were detected for settling preference, nymph survival and MRGR.

1.1.1.1 Assessment of nymph development

Nymph development on common bean varieties ranged from 9.4 (ADP 684) to 13.6 (AO) days with a mean of 10.8 days. Significant differences in nymph development were detected between common bean varieties (one-way ANOVA: $F = 12.58$, $df = 5$, $p = 0.001$) (Figure 4.1A). Nymph development was significantly longer on AO-1012-29-3A compared to the commercial variety (KAB) (Holm-Sidak test, $p = 0.006$). Nymph development on mutation derived lines ranged from 11.3 (CA 3) to 13.7 (CA 38) days with a mean of 12.4 days. Significant differences in nymph development were observed between mutation derived lines (one-way ANOVA: $F = 4.26$, $df = 4$, $p = 0.005$). However, none of the mutation derived lines differed significantly from the parent (Holm-Sidak test, $p > 0.05$) (Figure 4.1B).

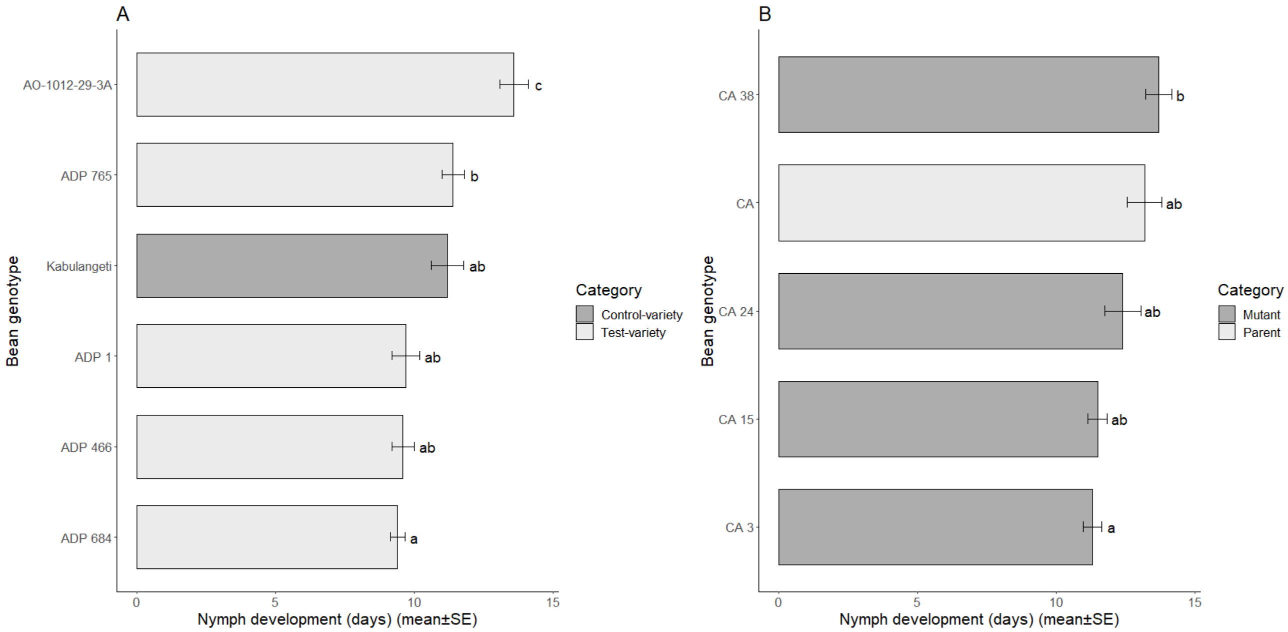

Figure 4.1: Black bean aphid nymph development on (A) common bean breeding lines and (B) mutation derived genotypes (*n* =10). Bars followed by different letters are significantly different (Holm-Sidak post-hoc test). Among the common bean breeding lines, nymph development was significantly longer on AO-1012-29-3A compared to the commercial variety Kabulangeti (Holm-Sidak test, $p = 0.006$). Among mutation derived genotypes, none of the mutants differed significantly from the parent (CA) (Holm-Sidak test, $p > 0.05$). Error bars represent standard error of the mean (SE). Common bean breeding lines and corresponding mutation derived genotypes were analysed separately. Genotype name; ADP 1 = Rozi Koko, ADP 466 = Mwezi Moja, ADP 684 = Majesty, ADP 765 = KK25

4.1.1.1 Determination of trichome density

Mean hooked trichome density on common bean varieties ranged from 9 (AO) to 39.7 (ADP 466) with a mean of 21.8. Significant differences were detected between common bean varieties (one-way ANOVA: $F = 25.59$, $df = 5$, $p = 0.001$). Genotypes ADP 466 (Holm-Sidak test, $p < 0.0001$) and ADP 765 (Holm-Sidak test, $p = 0.002$) had 26.3 and 17.0 more hooked trichomes respectively compared to the commercial variety (KAB) (Figure 4.2A). Densities of hooked trichomes in mutation derived genotypes ranged from 12.5 (CA 24) to 21.5 (CA) with a mean of 15.8. Significant differences were detected between mutation derived genotypes (one-way

ANOVA: $F = 3.69$, $df = 4$, $p = 0.03$) (Figure 2.6B). Genotype CA 24 had lower numbers of hooked trichomes compared to the parent (Holm-Sidak test, $p = 0.03$) (Figure 4.2B).

In the case of glandular trichomes, densities on common bean varieties ranged from 0.25 (AO) to 3.75 (ADP 684 and KAB) with a mean of 2.4. Significant differences were observed between bean varieties (one-way ANOVA: $F = 7.92$, $df = 5$, $p < 0.001$). Genotypes ADP 1 (Holm-Sidak test, $p = 0.049$) and AO (Holm-Sidak test, $p = 0.001$) had 2.3 and 3.5 less glandular trichomes respectively compared to the commercial variety (KAB) (Figure 4.2C). No significant differences were observed between mutation derived genotypes and the parent (one-way ANOVA: $F = 2.84$, $df = 4$, $p > 0.05$) (Figure 4.2D).

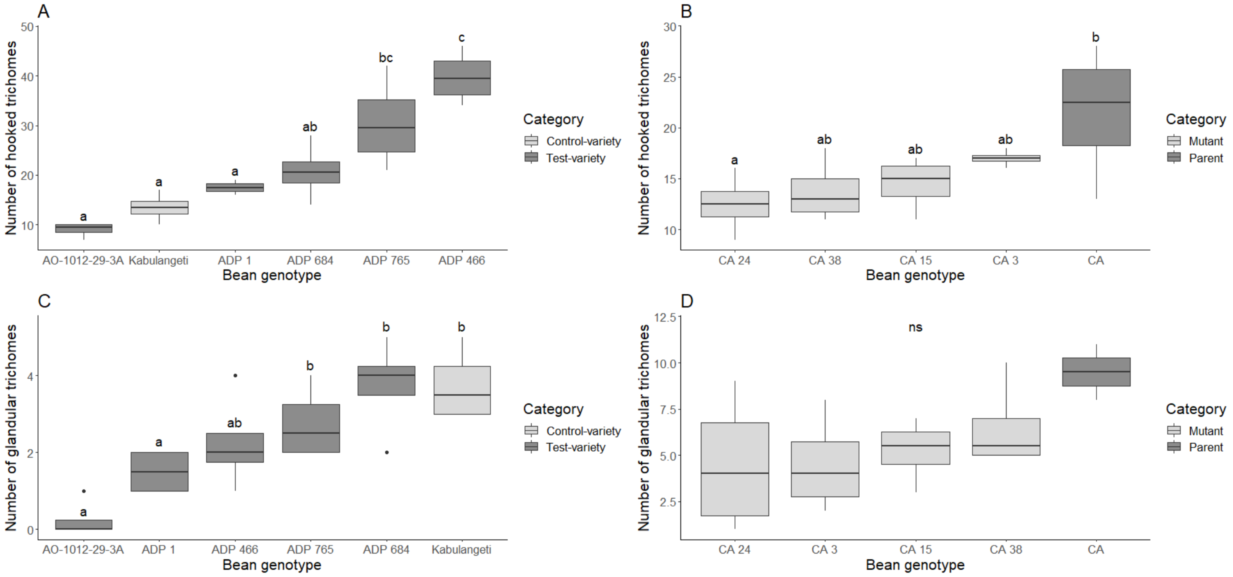

Figure 4.2: Median densities and confidence intervals of (A) hooked trichomes on common bean breeding lines (B) hooked trichomes on mutation derived genotypes (C) glandular trichomes on common bean breeding lines and (D) glandular trichomes on mutation derived genotypes, on lower leaf surfaces ($n = 4$). Among common bean breeding lines, ADP 765 (Holm-Sidak test, $p = 0.002$) as well as ADP 466 (Holm-Sidak test, $p < 0.0001$) had significantly higher numbers of hooked trichomes compared to the commercial variety. For mutation derived genotypes, CA 24 had smaller numbers of hooked trichomes compared to the parent (Holm-Sidak test, $p = 0.03$). For glandular trichomes on common bean breeding lines, AO-1012-29-3A (Holm-Sidak test, $p =$

0.001) and ADP 1 (Holm-Sidak test, *p* = 0.049) had fewer trichomes compared to the commercial variety Kabulangeti. Common bean breeding lines and corresponding mutation derived genotypes were analysed separately. Groups followed by different letters are significantly different (Holm-Sidak post-hoc test), ns = non-significant differences between groups. Genotype name; ADP 1 = Rozi Koko, ADP 466 = Mwezi Moja, ADP 684 = Majesty, ADP 765 = KK25

4.1.1.2 Settling preference of aphids on bean genotypes

The number of alate aphids settling on plants did not differ significantly between the common bean genotypes (one-way ANOVA: *F* = 0.82, *df* = 10, *p* = 0.59) 24 hours after being released (Table 2.2). Alate aphid numbers did not change significantly either 48 hours (one-way ANOVA: *F* = 1.03, *df* = 10, *p* = 0.38) or 72 hours (one-way ANOVA: *F* = 1.03, *df* = 10, *p* = 0.40) after release (Table 4.1).

Table 4.1: Settling preference of adult winged aphid black bean aphid (*Aphis fabae*) on common bean genotypes 24, 48 and 72 hours after being released in the cage (SE = standard error; *n* = 10). Common bean breeding lines and mutation derived genotypes were analysed together.

		No. aphids per plant (mean ± SE)		
Genotype	**Category**	**24 hrs**	**48 hrs**	**72 hrs**
Rozi Koko (ADP 1)	Variety	2.2 ± 0.4	2.4 ± 0.5	2.6 ± 0.5
Mwezi Moja (ADP 466)	Variety	6.9 ± 1.4	7.7 ± 1.5	7.3 ± 1.5
Majesty (ADP 684)	Variety	5.4 ± 1.7	5.2 ± 1.6	5.1 ± 1.4
KK25 (ADP 765)	Landrace	4.9 ± 1.4	4.9 ± 1.4	4.5 ± 1.3
AO 1012-29-3A	Variety	5.5 ± 1.6	6.1 ± 1.9	5.7 ± 1.9
Kabulangeti (KAB)	Variety	5.2 ± 1.6	5.7 ± 1.0	5.6 ± 0.9

CA 15	Mutant	6.3 ± 1.4	6.7 ± 1.9	5.5 ± 1.3
CA 24	Mutant	4.6 ± 1.1	5.2 ± 1.1	4.9 ± 1.1
CA 3	Mutant	8.0 ± 2.3	7.5 ± 2.0	6.7 ± 1.8
CA 38	Mutant	3.6 ± 0.8	3.4 ± 0.7	2.9 ± 0.5
CA	Parent line (for mutants)	3.8 ± 0.9	3.4 ± 0.9	3.0 ± 0.7
P-value		0.355	0.290	0.313

4.1.1.3 Assessment of nymph survival

Nymph survival on mutation derived genotypes ranged from 33 (CA) to 70 % (CA 38) with a mean of 49.4 %. Significant differences were observed between mutation derived genotypes and the parent (one-way ANOVA: *F* = 7.09, *df* = 4, *p* = 0.0002). On genotype CA 38, 37 % more aphids survived compared to the parent (CA) (Holm-Sidak test, *p* = 0.0001) (Figure 4.3B). In the case of common bean varieties, there were no significant differences in nymph survival between common bean varieties and the commercial variety (KAB) (one-way ANOVA: *F* = 1.42, *df* = 5, *p* = 0.23) (Figure 4.3A).

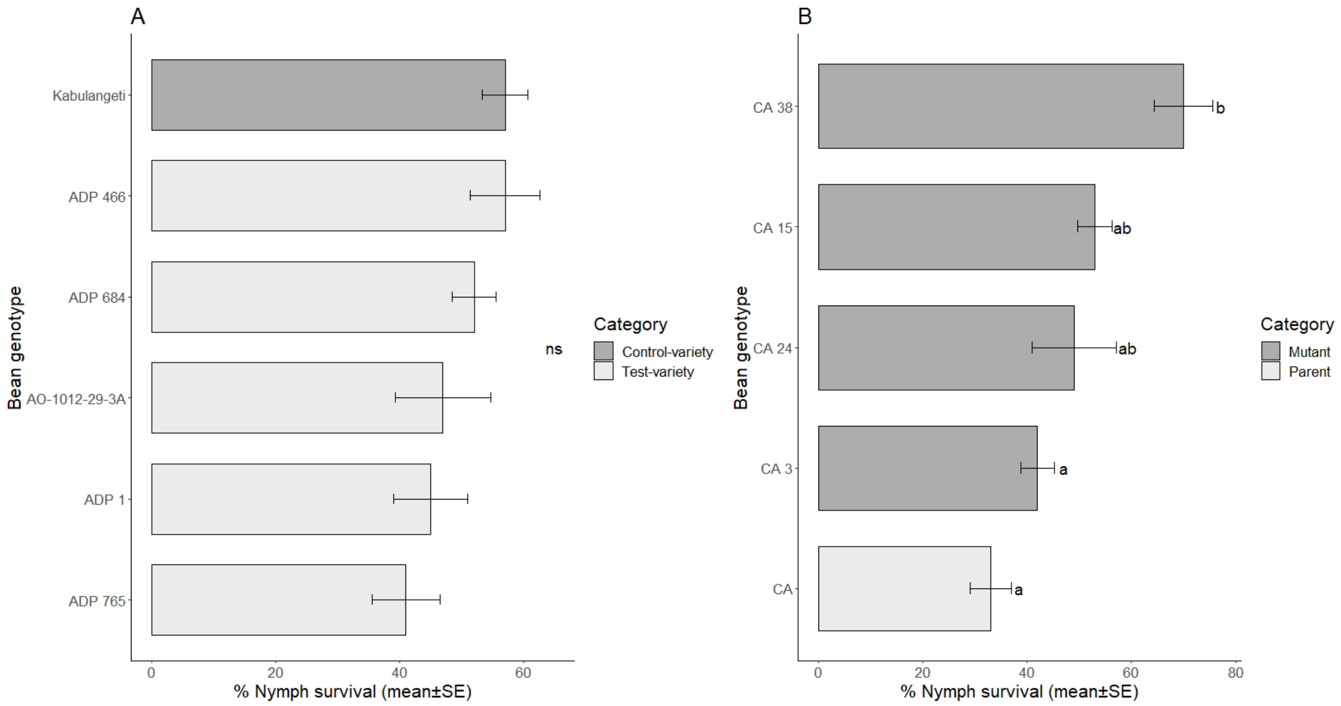

Figure 4.3: Black bean aphid nymph survival on (A) common bean breeding lines and (B) mutation derived genotypes (*n* = 10). Among the mutation derived genotypes, more aphids survived on CA 38 compared to the parent (CA) (Holm-Sidak test, *p* = 0.0001). Bars followed by different letters are significantly different (Holm-Sidak post-hoc test). ns = non-significant differences among bars. Error bars represent standard error of the mean (SE). Common bean varieties and corresponding mutation derived genotypes were analysed separately. Genotype name; ADP 1 = Rozi Koko, ADP 466 = Mwezi Moja, ADP 684 = Majesty, ADP 765 = KK25

4.1.1.4 Mean relative growth rate of nymphs on bean genotypes

When MRGR was assessed, significant differences were detected between common bean varieties (one-way ANOVA: *F* = 2.52, *df* = 5, *p* = 0.04). However, none of the bean varieties differed significantly from the commercial variety (Figure 4.4A). In the case of mutation derived genotypes, no significant differences were detected between mutation derived genotypes (one-way ANOVA: *F* = 1.55, *df* = 4, *p* = 0.20) (Figure 4.4 B).

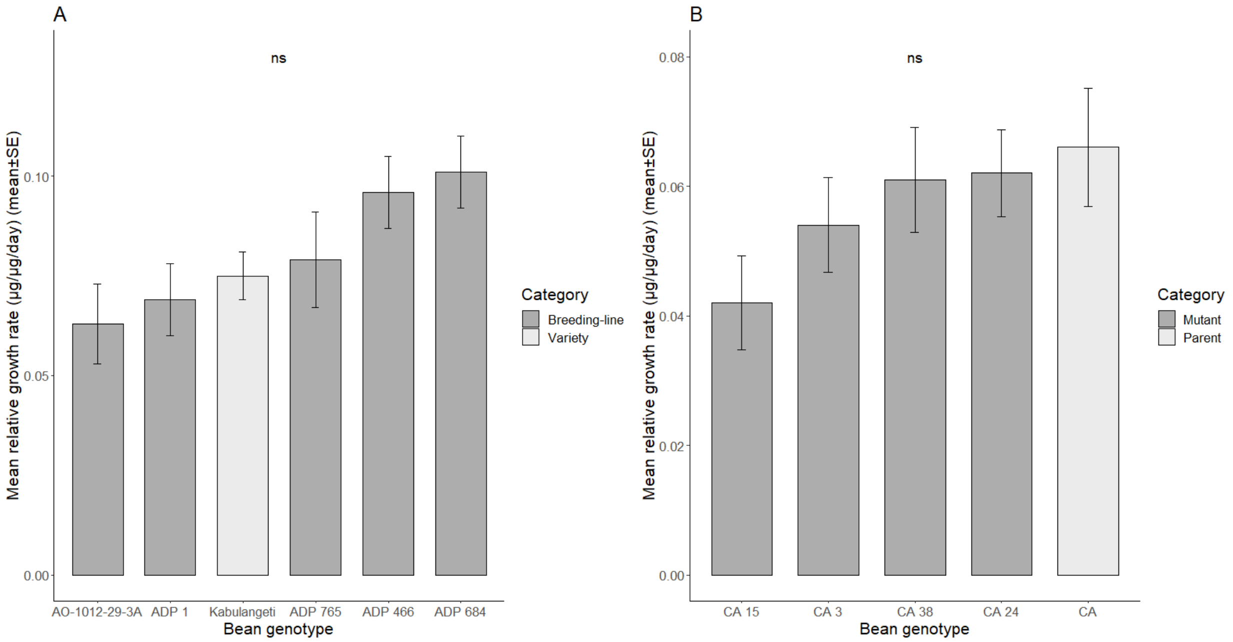

Figure 4.4: Black bean aphid mean relative growth rate on (A) common bean breeding lines and (B) mutation derived genotypes (*n* = 10). ns = non-significant differences among bars. Error bars represent standard error of the mean (SE). Common bean varieties and corresponding mutation derived genotypes were analysed separately. Genotype name; ADP 1 = Rozi Koko, ADP 466 = Mwezi Moja, ADP 684 = Majesty, ADP 765 = KK25

4.1.1.5 Monitoring of aphid feeding behaviour

Total duration of phloem ingestion ranged from 2.5 (ADP 1) to 22.4 minutes (ADP 684) with a mean of 6.9 minutes. Significant differences in the phloem ingestion were observed between the common bean varieties and the commercial variety (KAB) (one-way ANOVA: $F = 4.39$, $df = 5$, $p = 0.002$) (Figure 4.5F). Aphids fed on ADP 684 for 18.2 more minutes compared to the commercial variety (Holm-Sidak test, $p = 0.01$) (Figure 4.5F). There were no significant differences between bean varieties in the duration of the non-probing phase (Kruskal-Wallis: $X^2 = 7.96$, $df = 5$, $p = 0.15$) (Figure 4.5A), period to first probe from beginning of EPG recording (one-way ANOVA: $F = 1.07$, $df = 5$, $p = 0.28$) (Figure 4.5B), pathway phase (one-way ANOVA: $F = 1.06$, $df = 5$, $p = 0.39$) (Figure 4.5C), period from first probe to phloem ingestion (one-way ANOVA: $F = 0.79$, $df = 5$, $p = 0.55$) (Figure 4.5D), and phloem salivation (one-way ANOVA: $F = 0.72$, $df = 5$, $p = 0.51$) (Figure 4.5E).

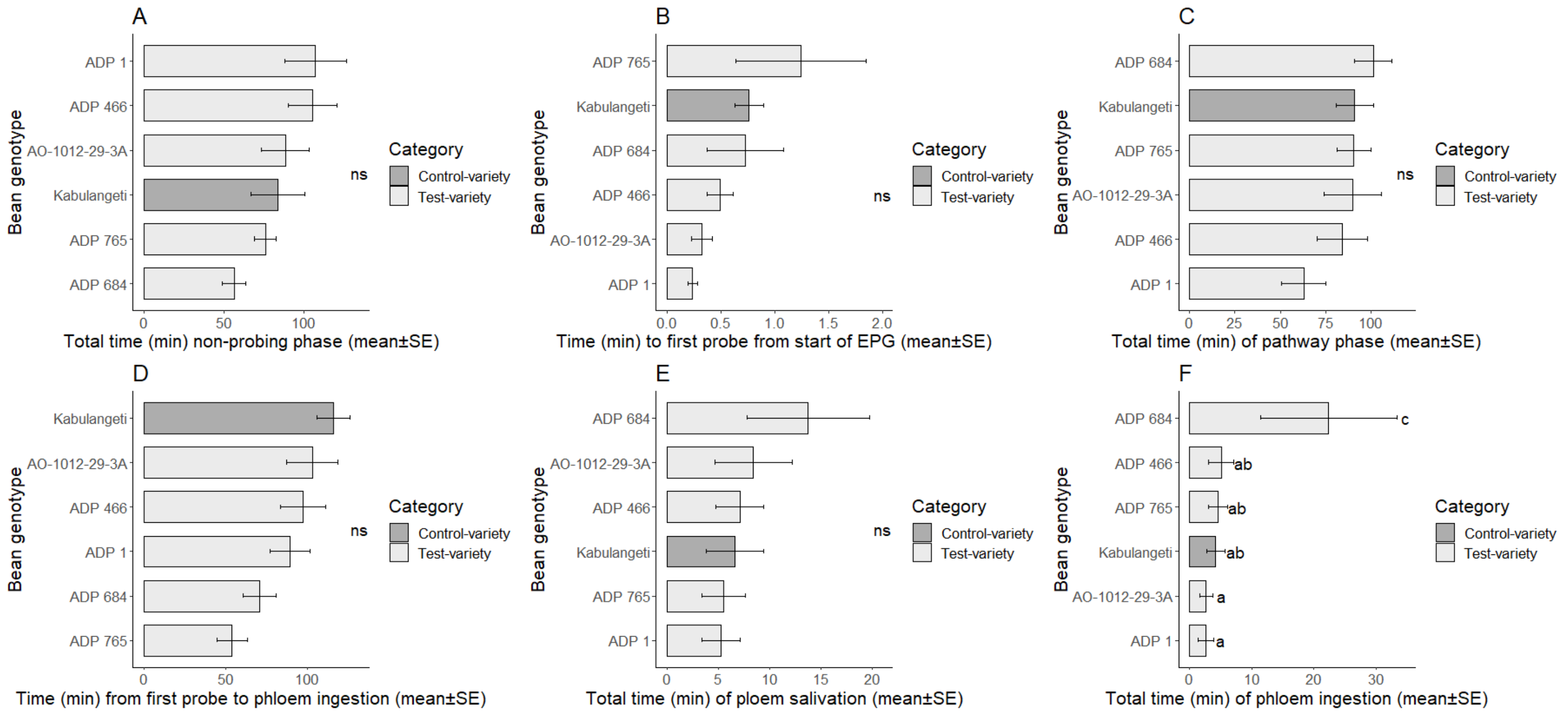

Figure 4.5: Aphid probing and feeding behaviour on common bean breeding lines. (A) total time of non-probing phase, (B) time to first probe from beginning of EPG recording, (C) total time of pathway phase, (D) time from first probe to sustained phloem ingestion, (E) total time of phloem salivation and, (F) total time of phloem ingestion (*n* = 14). In the case of phloem ingestion (F), aphids fed longer on ADP 684 compared to the commercial variety Kabulangeti (Holm-Sidak test, *p* = 0.01), Common bean genotypes in figures A-F were analysed separately. Error bars represent standard error of the mean (SE), Bars followed by different letters are significantly different (Holm-Sidak post-hoc test), ns = non-significant differences among bars. Genotype name; ADP 1 = Rozi Koko, ADP 466 = Mwezi Moja, ADP 684 = Majesty, ADP 765 = KK

4.2 Characterisation of resistance to cowpea aphid (*Aphis craccivora*) in mutation derived cowpea (*Vigna unguiculata*) genotypes

4.2.1 Cowpea aphid colony growth

The total number of aphids after fourteen days of colony development was influenced by plant genotype (Figure 4.6). Significant differences in aphid colony growth were identified between the parent Bubebe and its associated genotypes derived through mutagenesis (Kruskal-Wallis: $X^2 = 19.67$, $df = 6$, $p = 0.003$) (Figure 4.6A), with colony size reduced by 48.5 % on BB 7-9-7-5, 31.6 % on BB 14-16-2-2, 17.2 % on BB 10-4-2-3 and, 5.8 % on BB 3-8-4-1 (Figure 4.6A). Similarly, significant reductions in aphid colony growth were observed between mutation derived genotypes and the parent Lutembwe (Kruskal-Wallis: $X^2 = 18.16$, $df = 3$, $p = 0.0004$) (Figure 4.6B), with colony size reduced by 78.7 % on LT 11-3-3-12, 69.3 % on LT 3-8-4-1 and, 67.5 % on LT 4-2-4-1 (Figure 4.6B). Mutagenesis had no impact in reducing colony growth in genotypes derived from the parent Msandile (one-way ANOVA: $F = 2.19$, $df = 2$, $p > 0.05$) (Figure 4.6C).

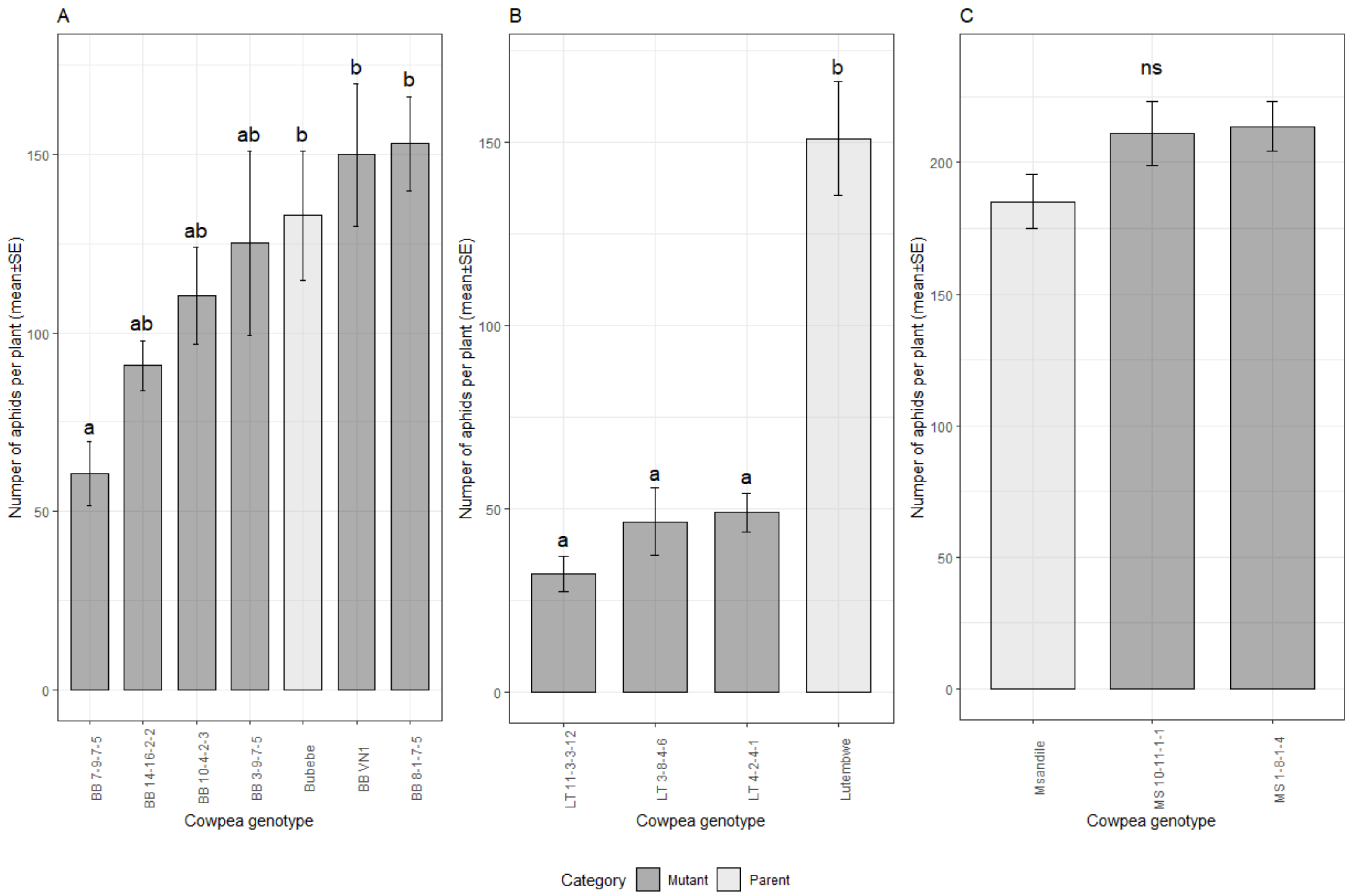

Figure 4.6 Aphid colony growth on (A) Bubebe, (B) Lutembwe and, (C) Msandile genotypes. *n* = 10. Error bars represent standard error of the mean (SE), Bars followed by different letters are significantly different (Holm-Sidak post-hoc test), ns = non-significant differences among bars

4.2.2 Performance of individual cowpea aphids

Aphid fecundity was significantly reduced on three genotypes (BB 3-9-7-5, BB 7-9-7-5 and BB 10-4-2-3) derived from Bubebe through mutagenesis (one-way ANOVA: $F = 8.23$, $df = 6$, $p < 0.001$) (Table 4.2). Aphids reared on genotypes BB 7-9-7-5, BB 3-9-7-5 and BB 10 had lower fecundity compared to the parent (Table 4.2). Aphid intrinsic rate of natural increase (r_m) differed between Bubebe and its derived genotypes (one-way ANOVA: $F = 4.44$, $df = 6$, $p < 0.001$) (Table 4.2). However, none of the genotypes reduced aphid r_m significantly when compared to the parent Bubebe (Table 4.2). Where aphid population doubling time was calculated, differences were identified between Bubebe and its associated genotypes derived through mutagenesis (one-way ANOVA: $F = 4.41$, $df = 6$, $p < 0.001$) (Table 4.2). None of the genotypes derived from Bubebe, however, increased aphid population doubling time compared to the parent (Table 4.2). Aphid mean relative growth rate (MRGR) differed between Bubebe and its derived genotypes produced through mutagenesis (one-way ANOVA: $F = 3.92$, $df = 6$, $p < 0.01$) (Table 4.2). However, none of the other genotypes affected aphid MRGR when compared to their parent (Bubebe) (Table 4.2). There were no differences in nymph development time between Bubebe and its derived genotypes (one-way ANOVA: $F = 0.80$, $df = 6$, $p > 0.05$) (Table 4.2).

Significant differences in aphid fecundity were observed between the parent Lutembwe and its derived genotypes LT 3-8-4-1, LT 4-2-4-1 and LT 11-3-3-12 (one-way ANOVA: $F = 33.73$, $df = 3$, $p < 0.001$) (Table 4.3). Reduced fecundity was observed in aphids feeding on LT 3-8-4-1, LT 4-2-4-1 and LT 11-3-3-12 compared to their parent (Table 4.3). Aphid r_m differed between Lutembwe and its derived genotypes (one-way ANOVA: $F = 11.75$, $df = 3$, $p < 0.001$) (Table 4.3). Genotypes LT 3-8-4-1, LT 4-2-4-1 and LT 11-3-3-12 resulted in lower aphid r_m compared to their parent (Table 4.3). Where aphid population doubling time was calculated, differences were detected between Lutembwe and its derived genotypes (one-way ANOVA: $F = 10.76$, $df = 3$, $p < 0.001$) (Table 4.3). Genotypes LT 3-8-4-1, LT 4-2-4-1 and LT 11-3-3-12 resulted in longer aphid population doubling time compared to the parent (Table 4.3). Differences in MRGR were identified between Lutembwe and its derived genotypes (one-way ANOVA: $F = 14.61$, $df = 3$, $p < 0.001$) (Table 4.3). On genotypes LT 3-8-4-1, LT 4-1-4-2 and LT 11-3-3-12, recorded MRGRs were significantly lower than the parent (Table 4.3). No differences in nymph development time were detected between Lutembwe and derived genotypes (one-way ANOVA: $F = 1.60$, $df = 3$, $p > 0.05$) (Table 4.3).

Table 4.2 Performance of cowpea aphid on Bubebe genotypes (mean ± SE). SE = standard error. *n* = 10. Means followed by the same letter in the same row are not significantly different (Holm-Sidak post-hoc test). *d* = nymph development, M_d = fecundity, r_m = intrinsic rate of natural increase, DT = development time and, MRGR = mean relative growth rate

Parameters	**Cowpea genotypes**						
	Bubebe	**BB 3-9-7-5**	**BB 7-9-7-5**	**BB 8-1-7-5**	**BB 10-4-2-3**	**BB 14-16-2-2**	**BB VN1**
d	7.73 ± 0.118 a	7.87 ± 0.192 a	7.73 ± 0.153 a	7.60 ± 0.131 a	7.67 ± 0.126 a	7.60 ± 0.131 a	7.47 ± 0.133 a
M_d	62.00 ± 3.925 c	42.67 ± 2.499 a	42.53 ± 1.518 a	60.73 ± 2.726 bc	48.53 ± 3.458 ab	51.27 ± 3.078 abc	62.00 ± 2.926 c
r_m	0.39 ± 0.006 abc	0.36 ± 0.010 ab	0.36 ± 0.009 a	0.40 ± 0.010 bc	0.37 ± 0.010 abc	0.38 ± 0.009 abc	0.41 ± 0.009 c
DT	1.78 ± 0.031 ab	1.94 ± 0.051 b	1.94 ± 0.046 b	1.75 ± 0.043 ab	1.89 ± 0.052 ab	1.83 ± 0.040 ab	1.71 ± 0.040 a
MRGR	0.16 ± 0.013 ab	0.14 ± 0.010 a	0.13 ± 0.014 a	0.15 ± 0.005 b	0.14 ± 0.009 a	0.19 ± 0.009 ab	0.18 ± 0.016 ab

Table 4.3 Performance of cowpea aphid on Lutembwe genotypes (mean ± SE). SE = standard error. *n* = 10. Means followed by the same letter in the same row are not significantly different (Holm-Sidak post-hoc test). *d* = nymph development, M_d = fecundity, r_m = intrinsic rate of natural increase, DT = development time and, MRGR = mean relative growth rate

Parameters	**Cowpea genotypes**			
	Lutembwe	**LT 3-8-4-6**	**LT 4-2-4-1**	**LT 11-3-3-12**
d	7.53 ± 0.133 a	7.40 ± 0.131 a	7.53 ± 0.215 a	7.87 ± 0.133 a
M_d	65.60 ± 1.740 b	39.33 ± 2.464 a	42.73 ± 2.357 a	37.00 ± 2.430 a
r_m	0.41 ± 0.008 b	0.36 ± 0.007 a	0.37 ± 0.010 a	0.34 ± 0.010 a
DT	1.70 ± 0.032 b	1.91 ± 0.036 a	1.90 ± 0.059 a	2.08 ± 0.057 a
MRGR	0.18 ± 0.009 b	0.11 ± 0.012 a	0.10 ± 0.007 a	0.09 ± 0.015 a

Mutagenesis had no impact on promoting aphid resistant genotypes derived from the Msandile parent (Table 4.4).

Table 4.4 Performance of cowpea aphid on Msandile genotypes (mean ± SE). SE = standard error. n = 10. Means followed by the same letter in the same row are not significantly different (Holm-Sidak post-hoc test). d = nymph development, M_d = fecundity, r_m = intrinsic rate of natural increase, DT = development time and, MRGR = mean relative growth rate

Parameters	**Cowpea genotypes**		
	Msandile	**MS 1-8-1-4**	**MS 10-11-1-1**
d	7.60 ± 0.163	7.40 ± 0.131	7.60 ± 0.163
M_d	72.33 ± 2.425	72.87 ± 2.569	75.67 ± 2.259
r_m	0.42 ± 0.007	0.43 ± 0.005	0.42 ± 0.008
DT	1.67 ± 0.031	1.62 ± 0.020	1.65 ± 0.034
MRGR	0.20 ± 0.011	0.17 ± 0.013	0.20 ± 0.010

4.2.3 Feeding behaviour

Cowpea aphid showed differences in feeding and probing behaviour within the leaf epidermis and mesophyll tissues (Figure 4.7). Significant differences in the duration of the first aphid probe were observed between the parent Lutembwe and genotypes derived from this parent through mutagenesis (one-way ANOVA: F = 3.12, df = 3, p = 0.03) (Figure 4.7A). The first aphid probe on the parent was longer compared to the genotype LT 11-3-3-12 (Figure 4.7A). The pathway phase (duration until first phloem puncture) differed significantly between the parent and respective genotypes LT 3-8-4-1, LT 4-1-4-2 and LT 11-3-3-12 (Kruskal-Wallis: X^2 = 25.28, df = 3, $p < 0.001$) (Figure 4.7B). Pathway phase duration was longer on genotype LT4 compared to the parent (Figure 4.7B). There were no differences between parent genotype and its derived genotypes in the duration of phloem salivation (Kruskal-Wallis: X^2 = 7.96, df = 5, $p > 0.05$) (Figure 4.7C) or phloem ingestion (one-way ANOVA: F = 1.07, df = 5, $p > 0.05$) (Figure 4.7D).

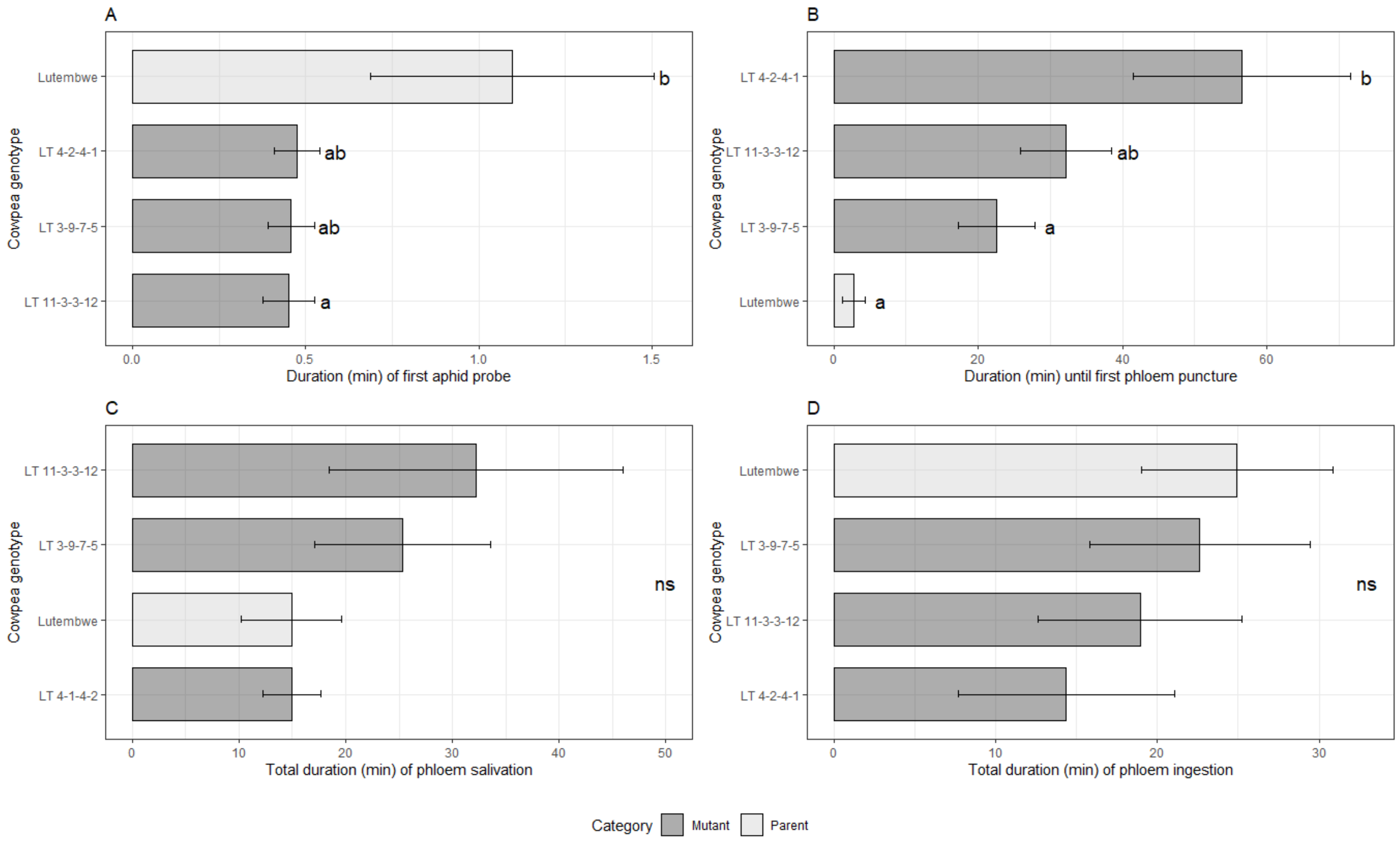

Figure 4.7 Aphid probing and feeding behaviour on cowpea genotypes. (A) duration of first aphid probe, (B) duration until first phloem puncture, (C) total duration of phloem salivation and, (D) total duration of phloem ingestion. Error bars represent standard error of the mean (SE), Bars followed by different letters are significantly different (Holm-Sidak post-hoc test), ns = non-significant differences among bars. *n* = 12.

4.2.4 Cowpea aphid colony growth under field conditions

The cowpea aphid population after thirty days of colony development was influenced by plant genotype (Figure 4.8). Aphid colony growth on mutation derived genotypes (LT 3-8-4-1, LT 4-2-4-1 and LT 11-3-3-12) were significantly lower than the parent Lutembwe throughout, site one (Kruskal-Wallis: $X^2 = 21.51$, $df = 3$, $p < 0.0001$) (Figure 4.8-site1), site two (Kruskal-Wallis: $X^2 = 16.39$, $df = 3$, $p < 0.001$) (Figure 4.8-site2), and site three (Kruskal-Wallis: $X^2 = 8.89$, $df = 3$, $p < 0.05$) (Figure 4.8-site3). Compared to the parent Lutembwe, colony size reduced by 48.33 % on LT 3-8-4-1, 45.73 % on LT 11-3-3-12, and 45.63 % on LT 4-2-4-1 on site one and, 78.45 % on LT 3-8-4-1, 83.27 % on LT 11-3-3-12, and 62.42 % on LT 4-2-4-1 on site two and, 62.02 % on LT 3-8-4-1, 64.21 % on LT 11-3-3-12, and 47.35 % on LT 4-2-4-1 on site three.

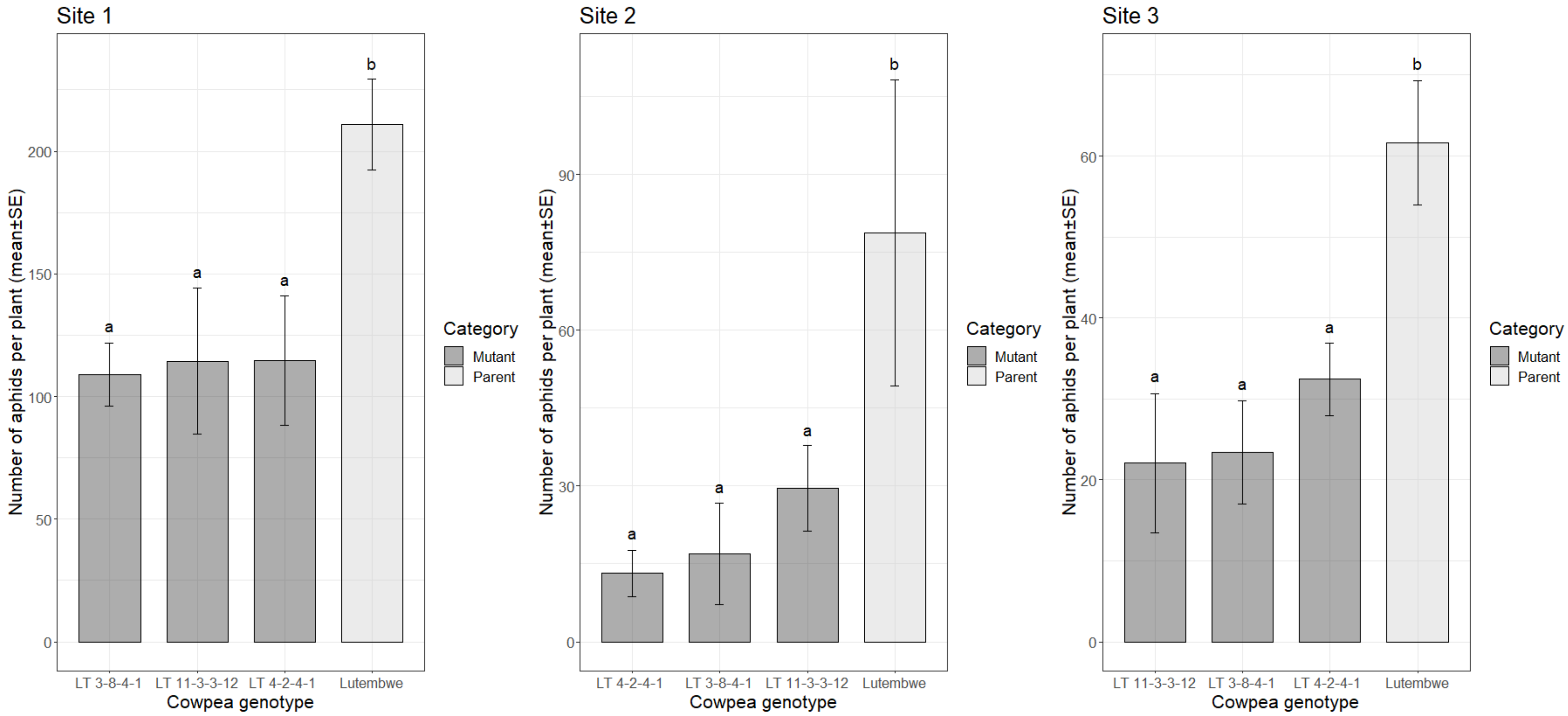

Figure 4.8 Aphid colony growth under field conditions on sites 1 (UNZA-Liempe), 2 (UNZA-Agricultural Technology Demonstration Center) and 3 (UNZA-Field Research Station) within the University of Zambia farm. *n* = 4. Error bars represent standard error of the mean (SE), Bars followed by different letters are significantly different (Holm-Sidak post-hoc test)

4.3 Evaluation of cowpea aphid performance on silicon treated cowpea genotypes under laboratory and field conditions

4.3.1 Evaluation of silicon accumulation capacity of cowpea genotypes

Silicon concentration ranged from 0.028 ppm (LT 11-3-3-12) to 0.035 ppm (LT 4-2-4-1) and 0.041 ppm (LT 11-3-3-12) to 0.047 ppm (Lutembwe) in Si- and Si+ plants respectively. The mean (±SE) silicon concentration in Si- and Si+ plants was 0.031 ± 0.002 and 0.045 ± 0.002 seconds, respectively, which equates to ~ 45 % increase in treated plants. Silicon concentration was significantly higher in Si+ plants of LT 11-3-3-12 (Welch Two Sample t-test: $t = -6.25$, $df = 2.21$, $p = 0.019$) and Lutembwe (Welch Two Sample t-test: $t = -3.91$, $df = 2.33$, $p = 0.046$) genotypes compared to respective Si- plants (Figure 4.9). In the case of LT 3-8-4-6 and LT 4-2-4-1 genotypes, no significant differences in silicon concentration were observed between Si- and Si+ plants, although the same trend was apparent.

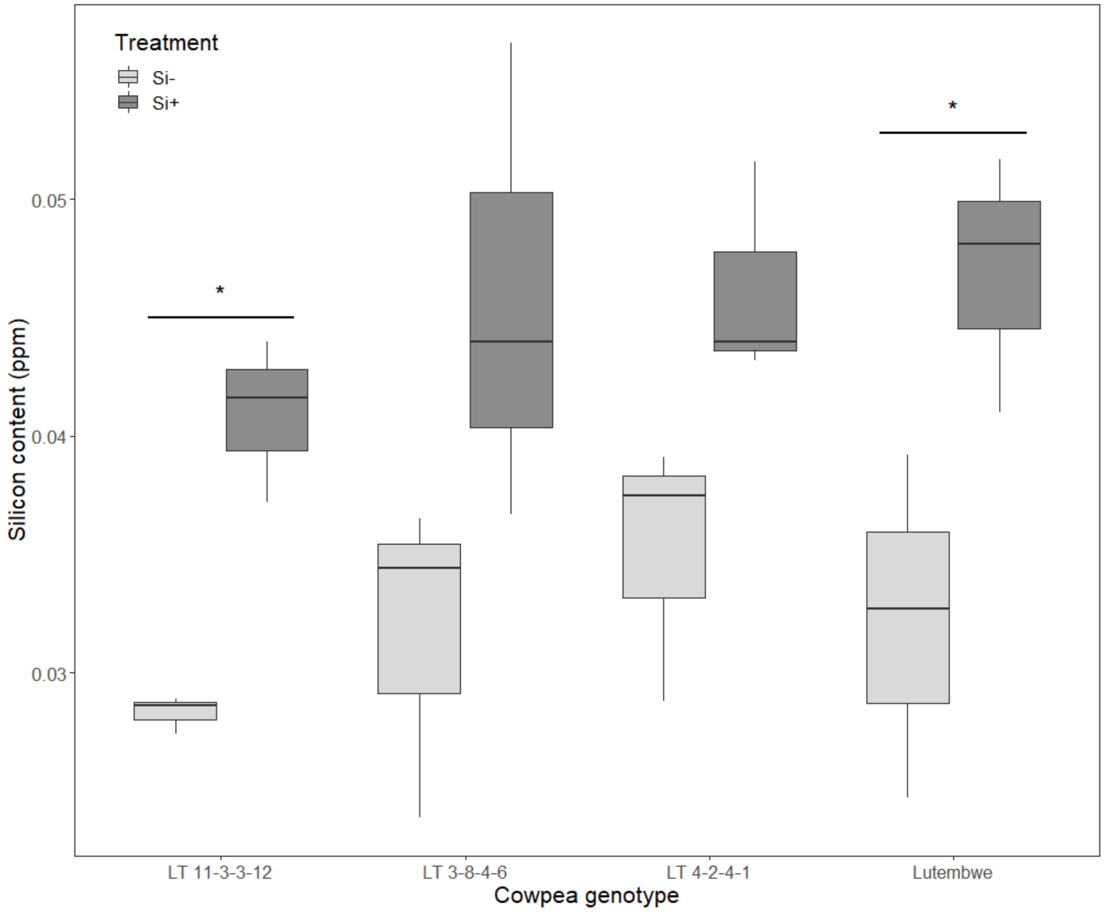

Figure 4.9 Silicon concentration in treated (Si+) and untreated (Si-) plants of cowpea genotypes. *n* = 10. Boxplots show median and confidence intervals; Boxplots with a horizontal line and asterisk above indicate significant differences between Si- and Si+ plants of the cowpea genotype (Welch two sample t-test).

4.3.2 Laboratory assessment of aphid colony growth

Aphid colony growth was significantly reduced where silicon was applied to Lutembwe plants compared to plants where silicon was not applied (Welch Two Sample t-test: *t* = 2.30, *df* = 17.56, *p* = 0.033) (Figure 4.10). The mean (±SE) numbers of aphids were 106.3 ± 17.4 and 53.7 ± 14.7 on Si- and Si+ plants, respectively (Figure 4.10). No significant differences in aphid numbers were observed between Si- and Si+ plants of LT 3-8-4-6, LT 4-2-4-1 and LT 11-3-3-12 genotypes (Figure 4.10).

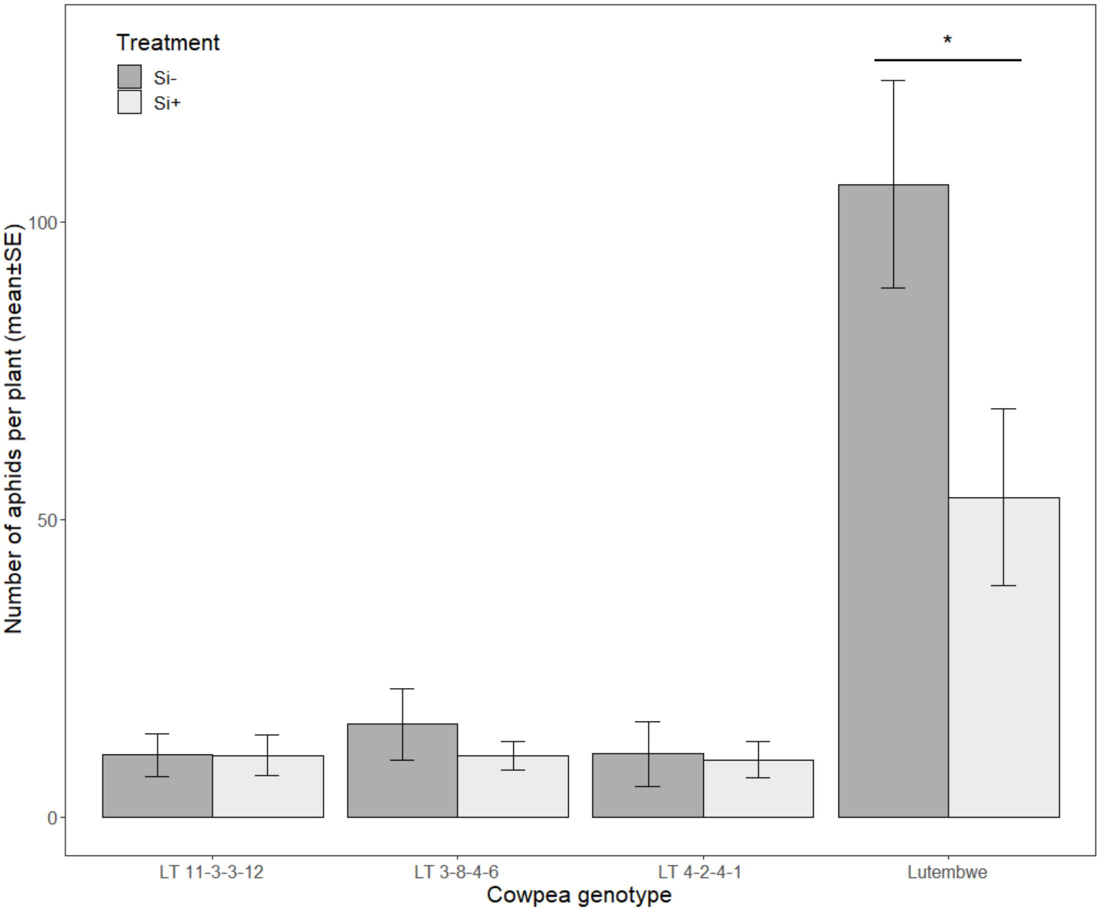

Figure 4.10 Aphid colony growth on silicon treated (Si+) and silicon deprived (Si-) plants of cowpea genotypes. *n* = 10. Error bars represent standard error of the mean (SE). Bars with a line and asterisk above show significant difference between Si- and Si+ plants of the cowpea genotype (Welch two sample t-test)

4.3.3 Evaluation of nymph mean relative growth rate

On the parent Lutembwe, aphid MRGR (±SE) was significantly reduced by the application of silicon compared to plants where silicon was not applied ($t = 3.01$, $df = 14.93$, $p = 0.009$) (Figure 4.11). Aphid MRGRs (±SE) were 0.193 ± 0.009 and 0.141 ± 0.015 µg/µg/day on to Si- and Si+ plants, respectively. There were no significant differences in aphid MRGR between Si- and Si+ plants of LT 3-8-4-6, LT 4-2-4-1 and LT 11-3-3-12 genotypes (Figure 4.11).

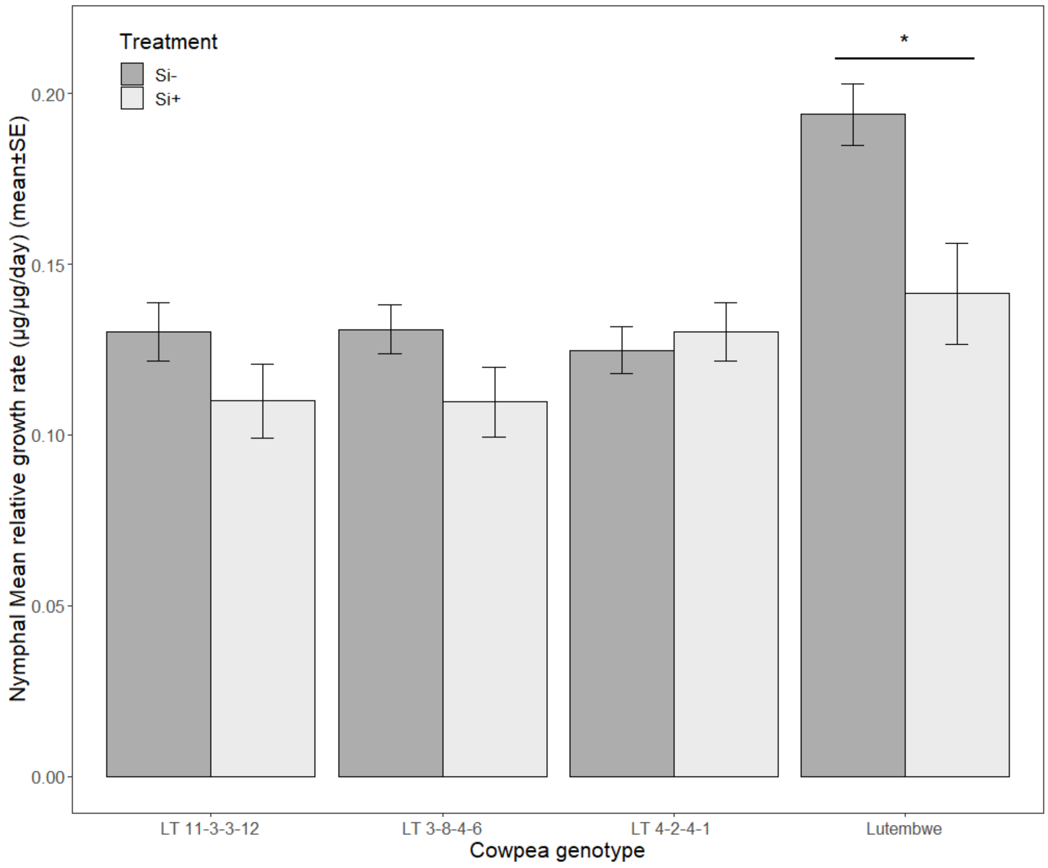

Figure 4.11 Aphid mean relative growth rate on silicon treated (Si+) and silicon deprived (Si-) plants of cowpea genotypes. $n = 10$. Error bars represent standard error of the mean (SE). Bars with a line and asterisk above are significantly different (Welch two sample t-test)

4.3.4 Recording of aphid feeding behaviour

Mean (±SE) duration of aphid phloem ingestion on Si- and Si+ Lutembwe plants was 492.14 ± 135.08 and 116.83 ± 11.26 seconds respectively, and represented a significant reduction in phloem ingestion duration between Si- and Si+ plants (Wilcoxon rank sum test: $W = 15$, $p = 0.035$) (Figure 4.12). Significant differences in aphid pathway duration were observed between Si- and Si+ plants of LT 4-2-4-1 genotype (Welch Two Sample t-test: $t = -3.21$, $df = 16.80$, $p = 0.005$). Aphid pathway duration was 18.2 minutes longer in LT 4-2-4-1 Si+ plants compared to Si- plants (Figure 4.12). In addition, time to 1st stylet probe on Si+ plants of LT 4-2-4-1 was significantly longer compared to Si- plants (Wilcoxon rank sum test: $W = 13.5$, $p = 0.011$) (Figure 4.12). The 1st stylet probe duration on Si+ plants was ~ 10 minutes longer compared to Si- plants. The application of silicon did not affect aphid feeding parameters on LT 3-8-4-6 and LT 11-3-3-12 genotypes (Figure 4.12).

4.3.5 Field experiment

Cowpea aphid population after thirty days of colony development was influenced by the application of silicon (Figure 4.13). Aphid colony growth was significantly lower on Si+ plants than Si- plants of Lutembwe genotype in field plots A (Welch Two Sample t-test: $t = 2.51$, $df = 18.03$, $p = 0.021$) (Figure 4.13A) and B (Welch Two Sample t-test: $t = 2.36$, df = 18.71, $p = 0.029$) (Figure 4.13B). In plot A, mean aphid (± SE) numbers on Lutembwe were 109.56 ± 2.55 and 42.06 ± 8.16 on Si- and Si+ plants, respectively, which equates to ~ 60 % aphid population reduction on Si+ plants. In the case of plot B, aphid mean (± SE) numbers on Lutembwe genotype were 171.75 ± 32.52 and 93.62 ± 21.27 on Si- and Si+ plants, respectively, a ~ 45 % aphid population reduction on Si+ plants. Although a similar trend in aphid population on treated Lutembwe plants was seen in plot C, no significant differences in aphid numbers were observed between Si- and Si+ plants (Welch Two Sample t-test: $t = 1.88$, $df = 19.96$, $p = 0.074$) (Figure 4.13C). Across the plots, the application of silicon did not affect aphid colony growth on LT 3-8-4-6, LT 4-2-4-1 and LT 11-3-3-12 genotypes (Figure 4.13A, B and C).

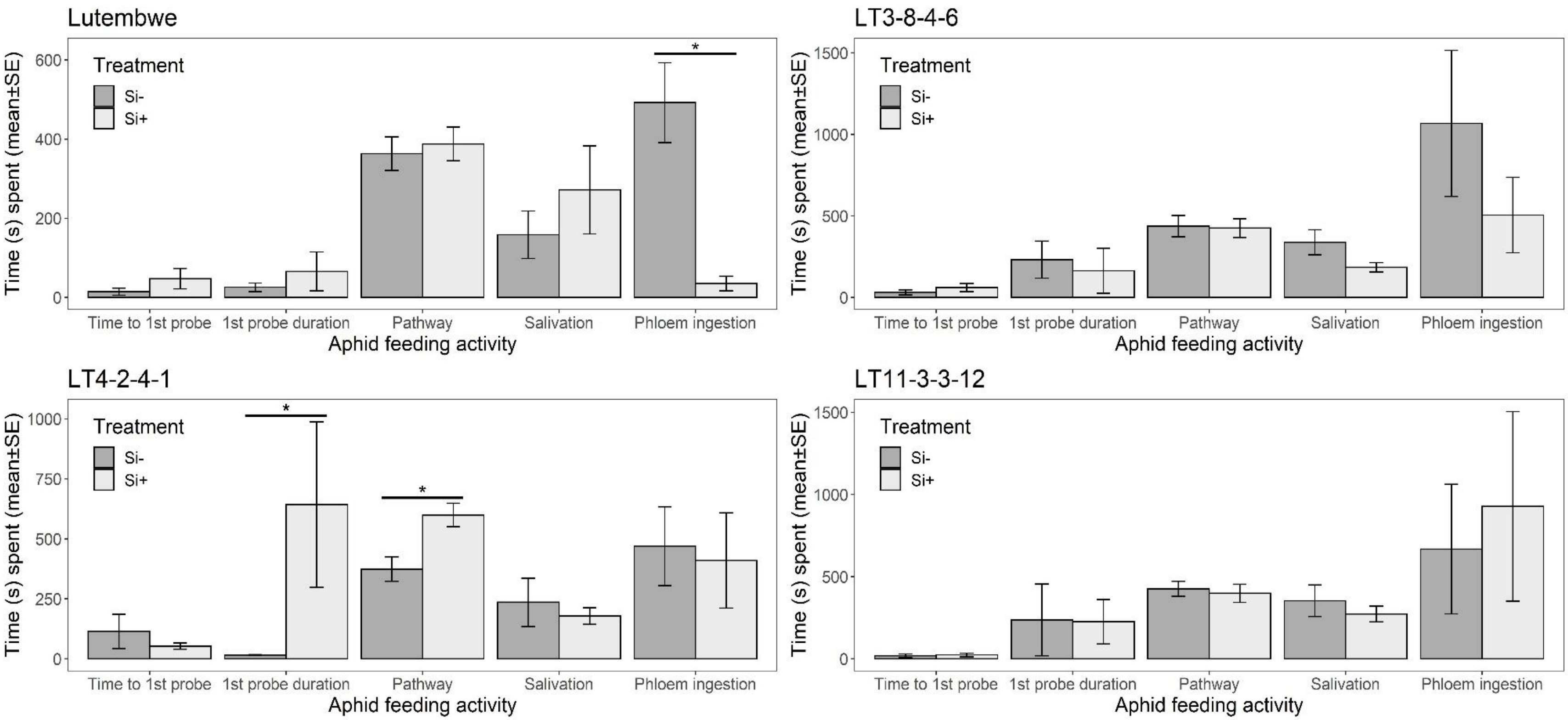

Figure 4.12 Electrical penetration graph recordings of cowpea aphid on silicon treated (Si+) and untreated (Si-) plants of cowpea genotypes (Lutembwe, LT 3-8-4-6, LT 4-2-4-1 and LT 11-3-3-12). Aphid feeding activities are indicative of epidermal, mesophyll and phloem resistance factors. Error bars represent standard error of the mean (SE); Bars with a line and asterisk

above indicate significant difference in feeding activity between Si- and Si+ plants of the cowpea genotype (Welch two sample t-test)

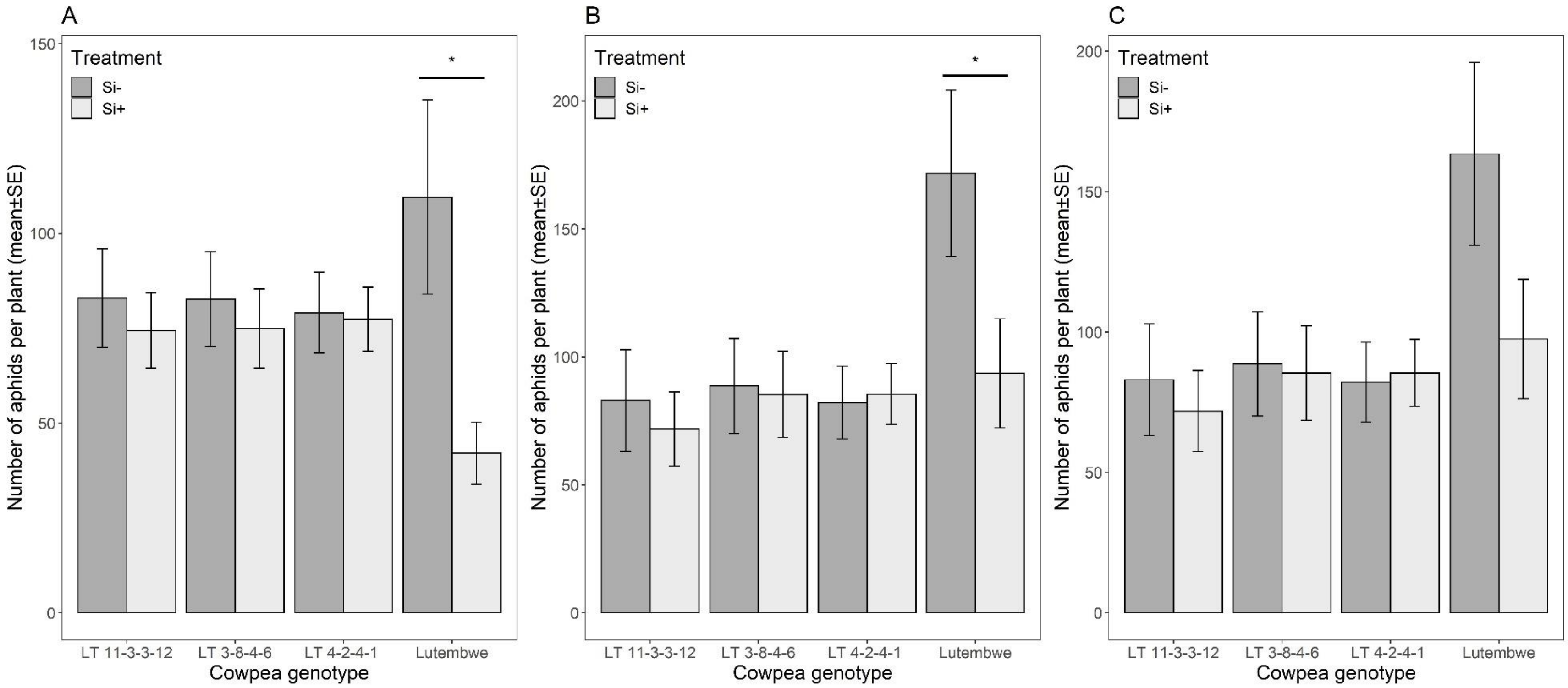

Figure 4.13 Aphid colony growth on silicon treated (Si+) and silicon deprived (Si-) plants of cowpea genotypes within field plots A, B and C at University of Zambia-Agricultural Technology Demonstration Center. *n* = 10. Error bars represent standard error

of the mean (SE). Bars with a line and asterisk above show significant difference between Si+ and Si- plants of the cowpea genotype (Welch two sample t-test)

CHAPTER FIVE

5 DISCUSSION

5.1 Characterisation of resistance to black bean aphid (*Aphis fabae*) in selected varieties and mutation derived common bean (*Phaseolus vulgaris*) genotypes

A greater understanding of the mechanisms mediating plant-aphid interactions is an important preliminary step in breeding aphid resistant crop varieties. This study demonstrated that some biological parameters of black bean aphid were significantly influenced by common bean genotypes. Nymph development was significantly longer on AO compared to the commercial cultivar KAB, indicating a level of host plant resistance (Obopile & Ositile, 2010). Longer nymph development may suggest reduced nutritional quality of phloem sap, which could have resulted in poor nourishment and therefore slower development (Leybourne *et al.*, 2019). Indeed, although statistically insignificant, EPG analysis indicated reduced phloem feeding by aphids on AO compared to aphids on KAB. This adverse effect of AO on aphid nymph development may suggest that reduced palatability is the major resistant trait against the cowpea aphid. Characterisation of amino acids and defence compounds in AO should be considered in future studies in order to establish the modality of resistance.

Studies in other legume-aphid systems have also pointed to the importance of mesophyll- and phloem-based resistance traits (Kamphuis *et al.*, 2012; Leybourne *et al.*, 2019). Resistance to pea aphid (*Acyrthosiphon pisum* Harris) in pea cultivars (*Pisum sativum* L.), for example, has been partly attributed to imbalances in essential amino acid composition in the phloem sap (Sandström & Pettersson, 1994). The variety AO is resistant to common bean weevil (*A. obtectus*) (Kusolwa *et al.*, 2016) and this resistance has been attributed to insecticidal activity of three proteins including arcelins, phytohemagglutinin and alpha-amylase (Kusolwa & Myers, 2011). There is a possibility of cross-resistance to black bean aphid since the aphicidal effects of lectins and protease inhibitors are well known (Nalam *et al.*, 2019). However, biochemical characterisation of essential amino acids and their role in cross-resistance to common bean weevil and black bean aphid, should be considered in future studies. Results presented here reflect the interaction between a range of plant lines but only a single aphid clone. As such it would be useful to repeat this work using other aphid clones in order to establish if the results reported here are consistent for a wider range of aphid genotypes.

Common bean plants are known to possess trichomes that serve as physical defences against aphid attack (Xing *et al*., 2017). Hooked trichomes, for example, may trap or impale aphids while glandular trichomes may exude toxic compounds or adhesive fluids that trap insects (Saska *et al.*, 2020). In this study, significantly fewer glandular trichomes were detected on lines ADP 1 and AO compared to the commercial cultivar. Since low glandular trichome densities did not reflect an increased survival of aphids on ADP 1 and AO, it is likely that glandular trichomes were not a primary modality of resistance to black bean aphid in these genotypes. Nymph survival is among other factors often associated with high trichome density (Saska *et al.*, 2020). This study showed that nymph survival did not vary significantly between the bean genotypes. Indeed, no significant differences were detected in the number of hooked trichomes between the bean genotypes that usually affect nymph survival on common bean (Xing *et al.*, 2017). Given the presence of other resistance mechanisms, low trichome density on bean genotypes would benefit the performance of natural enemies which could complement aphid control (Riddick & Simmons, 2014). Although the adverse impacts of hooked trichomes to black bean aphid are well studied for common bean (Xing *et al.*, 2017), the impact of glandular trichomes is poorly understood and would warrant further study.

Alate aphids often use visual and volatile cues to locate their host plants (Powell *et al.*, 2006; Webster *et al.*, 2008; Döring, 2014). Observations of black bean aphid settling behaviour showed that their preference for the tested common bean genotypes did not vary significantly between the genotypes 24 hours after release, suggesting similarities in aphid host location cues. Between 24 and 72 hours, there was little movement of aphids between plants, indicating lack of host preference. Black bean aphid is known to discriminate host plants based on colour and semiochemicals. For example, using a wind tunnel and monochromatic light, Hardie (1989) showed that black bean aphids were preferentially attracted to the green region of the spectrum, indicating a preference for green coloured plants. In olfactometer experiments, Nottingham *et al.* (1991) showed that black bean aphids were able to discriminate between cultivars ("Sutton Dwarf" and "Tick Bean") of field bean, suggesting the role of semiochemicals in host location.

Nymph MRGR on common bean breeding lines did not vary significantly when compared to the commercial cultivar. However, the general trend showed lowest and highest weight gain of nymphs on AO and ADP 684, respectively, which may be a consequence of phloem nutrition quality of these genotypes. Feeding analysis by EPG indicated significantly prolonged phloem feeding on ADP 684 compared to the commercial cultivar, suggesting higher susceptibility through reduced resistance in the phloem. Mean relative growth rate is often a good predictor of aphid performance since lower weight gains are associated with reduced fecundity and population growth (Dixon & Wratten, 1971). Based on the trend, lower MRGR of black bean aphid on AO may be attributed to reduced food quality (Obopile & Ositile, 2010).

This study showed that black bean aphid nymphs feeding on AO developed more slowly than other lines tested or KAB. Although not significant, AO further reflected reduced aphid weight gain and phloem feeding. Lower glandular trichome density was also recorded on AO, which could benefit natural enemy performance and consequently enhance biological control of aphids (Riddick & Simmons, 2014). AO is therefore a promising variety that should be further evaluated for useful genetic attributes that may be used to develop aphid resistant common bean varieties. On the other hand, mutagenesis did not generate resistance to black bean aphid in the tested mutation derived lines. Future studies should consider screening a wider range of mutation derived lines in order to increase chances of finding aphid resistant genotypes. However, based on other grower preferred attributes (i.e., seed size and colour) associated with the mutation derived lines tested, further studies are needed to establish if mutagenesis generated other useful traits that may be beneficial to aphid natural enemies.

5.2 Characterisation of resistance to cowpea aphid (*Aphis craccivora*) in mutation derived cowpea (*Vigna unguiculata*) genotypes

This study highlights the potential role that induced mutagenesis has in generating novel sources of resistance for breeding aphid resistant crop varieties. It is evident from this study that the population and biological parameters of the cowpea aphid were significantly influenced by mutation derived cowpea genotypes. A bioassay was initially conducted to screen cowpea genotypes for potential resistance traits. Colony growth on BB 7-9-7-5, LT 3-8-4-1, LT 4-2-4-1 and LT 11-3-3-12 were lower when compared to their respective parents Bubebe and Lutembwe, indicating the presence of aphid resistance traits in these genotypes. In a follow-up field study, lower aphid colony growth rates were recorded on LT 3-8-4-1, LT 4-2-4-2 and LT 11-3-3-12 compared to the parent Lutembwe, indicating a similar trend to laboratory studies. Morphological (*e.g.*, trichomes) and biochemical (*e.g.*, alkaloids, phenols, flavonoids) traits are known to influence cowpea aphid performance on cowpea (Ofuya, 1997). Lower aphid colony growth on BB 7-9-7-5, LT 3-8-4-1, LT 4-2-4-2 and LT 11-3-3-12 reflects reduced host quality of these genotypes (Soffan & Aldawood, 2014), likely due to mutagenesis derived resistance traits (Viana *et al.*, 2019). Results obtained from the colony development bioassay, however, need to be tested under field conditions to establish whether they can be replicated outside of the laboratory.

Although nymph development was not found to be lower on any cowpea genotypes produced through mutagenesis, there was a general trend of extended nymph development on BB 3-9-7-5, BB 8-1-7-5, BB 10-4-2-3 and LT 11-3-3-12 compared to their respective parents. Extended nymph development on these genotypes may indicate host resistance since aphid resistant traits are often associated with delayed adult emergence (Zimba *et al.*, 2022a). However, it will be important to screen the promising genotypes with a range of widespread aphid clones to establish if the results reported here are consistent for a wider range of aphid biotypes. Particularly, such studies would help to assess if the promising genotypes in this study are effective against the previously reported resistance breaking aphid biotypes.

Cowpea aphid fecundity was reduced on genotypes BB 7-9-7-5, BB 3-9-7-5, BB 10-4-2-3, LT 3-8-4-1, LT 4-2-4-2 and LT 11-3-3-12 compared to their respective parents, possibly indicating the presence of resistance factors including reduced nutrition quality of these genotypes. Genetic effects of induced mutagenesis such as base substitution and gene deletion (Viana *et al.*, 2019) may have led to changes in the composition of amino acids and secondary metabolites (*i.e.*, polyphenols and flavonoids) in these genotypes, which could have led to poor nutrition and therefore lower fecundity of aphids. Indeed, Douglas and Prosser (1992) showed that exclusion of essential amino acids such as tryptophan in artificial diets reduced the fitness of the pea aphid (*Acyrthosiphon pisum*). Lattanzio *et al.* (2000) also demonstrated that high levels of flavonoids, such as quercetin and isorhamnetin, in cowpea genotypes inhibited cowpea aphid reproduction. Mean relative growth rate (MRGR) is often used as a predictor of aphid reproductive performance since lower weight gains are correlated with reduced fecundity (Obopile & Ositile, 2010). Lower aphid MRGRs on LT 3-8-4-1, LT 4-2-4-2, and LT 11-3-3-12 were also associated with lower fecundity compared to the parent, which may suggest reduced food quality of these genotypes due to potential resistant factors described above. Phytochemicals and low nutritional values associated with resistant crop cultivars may reduce fitness of omnivorous natural enemies of aphids (Lundgren *et al.*, 2008). Therefore further studies are needed to assess if promising aphid resistant genotypes are compatible with natural enemies within IPM systems (Michereff *et al.*, 2015).

Intrinsic rate of natural increase (r_{m}) is a function of nymph development (d) and fecundity (M_{d}) (Wyatt & White, 1977). This development metric is a useful summary parameter that provides an estimate of aphid performance when reared on different host plants and has been widely used to evaluate aphid resistance in crop cultivars (Obopile & Ositile, 2010; Leybourne *et al.*, 2019). Higher r_{m} values indicate greater growth potential when aphid populations are reared on susceptible host plants (Dixon, 1998). Genotypes LT 3-8-4-1, LT 4-2-4-2 and LT 11-3-3-12 resulted in lower aphid r_{m} compared to their parent, indicating host plant resistance and an inhibition of aphid population growth. This corroborates with findings by Obopile and Ositile (2010) and Soffan and Aldawood (2014) who reported significantly lower values of aphid r_{m} when reared on resistant genotypes of cowpea and broad bean (*Vicia faba* L.) respectively. Indeed, aphid colony growth on genotypes LT 3-8-4-1, LT 4-2-4-2 and LT 11-3-3-12 was reduced suggesting poor aphid performance on these lines.

Population doubling time (DT) is the time it takes for the aphid population to double in size. Aphid populations took, on average, 0.3 days longer to double on genotypes LT 3-8-4-1, LT 4-2-4-2 and LT 11-3-3-12 compared to their parent genotype. Feeding analysis by EPG indicated a reduced first probe (duration of first stylet movement within the leaf epidermal layer) duration on LT 11-3-3-12, suggesting the presence of epidermal barriers (*i.e.*, epicuticular chemical compounds) to leaf penetration by the aphid stylet (Leybourne *et al.*, 2019). Moreover, the longer pathway phase (duration of stylet movement from leaf surface until phloem puncture) in these genotypes may further suggest the presence of resistance factors in the mesophyll. Previous studies have demonstrated the contribution of epidermal (Leybourne *et al.*, 2019) and mesophyll (Kamphuis *et al.*, 2012) based aphid resistance factors in plants. However, biochemical and morphological characterisation of cowpea leaves would be useful in future studies.

Genotypes BB 7-9-7-5 and BB 14-16-2-2 are resistant to cowpea bruchid (*C. maculatus*) (Tembo *et al.*, 2017). Although BB 14-16-2-2 did not affect colony growth or most indicators of individual cowpea aphid performance, reduced aphid fecundity observed on BB 7-9-7-5 suggests cross-resistance to cowpea aphid. BB 7-9-7-5 resistance to cowpea aphid and cowpea bruchid may be mediated by biochemical compounds that have broader insecticidal activity, such as alpha-amylase inhibitors, tannins, phenolic compounds, lectins and protease inhibitors. Previous field observations on genotypes BB 8-1-7-5, LT 11-3-3-12 and LT 4-2-4-2 indicated low incidences of the leaf blight (*Ascochyta* spp.) (unpublished). While BB 8-1-7-5 did not affect cowpea aphid biology, several parameters (nymph development, fecundity, MRGR, r_m and DT) were adversely affected by genotypes LT 11-3-3-12 and LT 4-2-4-2, which may further indicate resistance to both leaf blight and cowpea aphid. Aphid and pathogen resistance genes are often clustered on the same region of the chromosomes (Dogimont *et al.*, 2010). For example, the *Ra* gene on chromosome 2 in lettuce, which mediates resistance against the lettuce root aphid (*Pemphigus bursarius* L.), is clustered together with downy mildew resistance genes on the chromosome (Wroblewski *et al.*, 2007; Christopoulou *et al.*, 2015).Typically, plants respond to aphid feeding in a similar way to plant pathogens (Zimba *et al.*, 2022c). Due to this common genomic locale of aphid and pathogen resistance genes, supposed chromosomal alterations due to mutagenesis in LT 4-2-4-2 and LT 11-3-3-12 may have induced genetic variations for both pathogen and aphid resistance traits. However, genetic characterisation of genotypes BB 7, LT 4-2-4-2 and LT 11-3-3-12 requires further work to elucidate mechanisms of resistance.

In conclusion, this study shows that aphids reared on genotypes LT 3-8-4-6, LT 4-2-4-1 and LT 11-3-3-12 that were produced through mutagenesis had lower colony growth, fecundity, MRGR, r_m and DT compared to the parent. Among the genotypes derived from the parent Bubebe through mutagenesis, genotype BB7 had the effect of significantly reducing cowpea aphid colony growth compared to the parent. Characterisation of aphid probing and feeding behaviour using EPG indicates that resistance factors in genotypes LT 3-8-4-6, LT 4-2-4-1 and LT 11-3-3-12 may predominantly reside within the epidermal and mesophyll tissues of cowpea leaves. Genotypes BB 7-9-7-5, LT 3-8-4-6, LT 4-2-4-1 and LT 11-3-3-12 are therefore promising lines that should be further evaluated for useful genetic attributes that may be used to develop aphid resistant cowpea varieties. Although developing aphid resistance using induced mutagenesis is associated with several challenges as highlighted above, this study shows that using this approach could contribute to sustainable management of aphid pests in crops. Furthermore, the long history of safe use, low cost of equipment as well as wide acceptability makes induced mutagenesis an important technique that could be exploited further to speed up the delivery of aphid resistant crop varieties in SSA.

5.3 Evaluation of cowpea aphid performance on silicon treated cowpea genotypes under laboratory and field conditions

An important challenge with aphid management using resistant cultivars is the ability of aphids to develop new virulent biotypes that overcome host-plant resistance (Botha, 2021). It is well established that deploying multiple control measures such as the use of resistant varieties, biocontrol agents and insecticides within an IPM framework reduces the likelihood of aphid species evolving virulent biotypes (Jaouannet *et al.*, 2014; Botha, 2021). The main objective of this study was to establish if the application of silicon could complement mutation derived aphid resistance in cowpea to offset resistance issues.

Research on functional roles of silicon in plants has, so far, largely focused on grasses (Poaceae) while little attention has been paid to other plant groups such as legumes (Fabaceae) (Putra *et al.*, 2020). A possible explanation for this may be due to the ability of most grasses to hyper-accumulate silicon through the facilitation of transporter genes (Coomey *et al.*, 2020). The few studies on legumes, however, indicate the potential of plants to accumulate silicon (Johnson *et al.*, 2019; Putra *et al.*, 2020). Since silicon transport is genetically mediated (Sun *et al.*, 2020), it was necessary in this study to assess the accumulation capacity of mutation derived cowpea genotypes before conducting aphid performance bioassays. Analysis of dry plant tissue showed significantly higher silicon concentration in Si+ plants of LT 11-3-3-12 and Lutembwe genotypes compared to Si- plants. Although silicon concentrations in LT 3-8-4-6 and LT 4-2-4-1 genotypes were not significantly higher in Si+ plants compared to the respective Si- plants, there is a clear trend that silicon levels were higher in plants when it was applied. Results of this bioassay, indicates that mutation derived cowpea genotypes are able to take up and accumulate silicon. Since plant analysis was conducted within ten days of silicon application, results of this study further reflect the capacity of cowpea to accumulate silicon within a short period of time (7-10 days). Although a commercial silicon application rate of ~0.5 L/ha was adopted for this bioassay, establishing an optimal application rate specifically for cowpea genotypes would be useful in future studies.

Colony growth assessment provides a preliminary method for detecting the presence of aphid resistance in crop species (Zimba *et al.*, 2022b). The application of silicon to Lutembwe plants resulted in a significant colony growth reduction when compared to Si- plants in both the laboratory and field experiments, indicating the presence of silicon-induced aphid resistance. This is often expressed through enhancement of physical (i.e., trichomes, tissue abrasiveness) and biochemical (i.e., defence compounds) plant traits (Yang *et al.*, 2022). Application of silicon to mutation derived cowpea genotypes (LT 3-8-4-6, LT 4-2-4-1 and LT 11-3-3-12) did not cause reduction in aphid colony growth despite the genotypes showing a clear trend of increased silicon concentration in Si+ plants. The lack of additive or synergistic effects in reducing aphid performance from applying silicon to mutation derived cowpea genotypes, may indicate that whilst mutation derived genotypes can accumulate silicon, they cannot do this effectively to reduce susceptibility to this pest. Alternatively, the lower aphid performance on mutation derived genotypes may have reduced the apparency of additional benefit of silicon application.

Measures of MRGR are often used to assess the reproductive performance of aphids since higher weight gains are correlated with increased fecundity (Obopile & Ositile, 2010; Zimba *et al.*, 2022b). Aphid MRGRs on Si+ Lutembwe plants were reduced by ~37 % when compared to Si- plants, which may indicate a reduction in food quality of treated plants (Johnson *et al.*, 2019). Coupled with observations on aphid colony growth, it is likely that the application of silicon to Lutembwe plants induced biochemical defences against the cowpea aphid resulting in reduced feeding and weight gain (Zimba *et al.*, 2022b). Aphid MRGRs on mutation-derived cowpea genotypes, however, were not affected by the application of silicon indicating the absence of or reduced interaction between these plants and the application of silicon. Since mutagenesis induced changes in the plant DNA may lead to loss or gain in gene function (Viana *et al.*, 2019), it is possible that silicon-defence mediating genes in mutation derived genotypes were lost or disabled during mutagenesis.

Silicon application to plants has been reported to have more adverse effects on chewing herbivores than phloem feeding insects (Rowe *et al.*, 2020). For example, higher mortalities, lower weight gain and increased wear of mouthparts in fall armyworm (*Spodoptera frugiperda*) larvae has been correlated to abrasiveness and low digestibility of silicon enriched plant tissue (Verma *et al.*, 2021). Aphid phloem feeding was negatively affected by silicon application to the parent genotype Lutembwe. A reduction in phloem feeding associated with an increase in salivation was observed on Lutembwe Si+ plants compared to Si- plants, suggesting the presence of phloem-based resistance factors. Indeed, feeding behaviour studies with *Schizaphis graminum*, for example, showed that phloem-based defence compounds on silicon treated wheat were more important for aphid resistance than physical traits (Gomes *et al.*, 2005; Goussain *et al.*, 2005).

Aphid EPG recordings on Si+ Lutembwe plants further suggests that silicon accumulation in the epidermis and inter-cellular spaces of cowpea did not impede aphid stylet movement. Aphids are thought to be less affected by silicon-induced physical defences due to the ability of these insects to manoeuvre their stylets around silicon particles in the plant tissue (Rowe *et al.*, 2020). However, it would be useful in future studies to consider analysing the profile of phloem-based defence compounds in Si+ plants of Lutembwe genotype. Elevated levels of phenolic compounds such as 5-caf-feoylquinic acid and p-coumaroylquinic acid has been associated with silicon-induced defence against aphids (Ranger *et al.*, 2009). In the case of mutation derived genotypes, silicon application did not affect aphid colony growth, MRGR or feeding behaviour. Although LT 4-2-4-1 mutation derived line did not significantly accumulate silicon, increased duration of both 1st stylet probe duration and pathway phase was observed on Si+ plants, which is often indicative of epidermal and mesophyll based resistance. Silicon application to LT 4-2-4-1, however, did not translate into apparent reduction in aphid performance. It is not clear why significant silicon accumulation in LT 11-3-3-12 mutation derived genotype did not impact aphid performance, but the absence of interaction between silicon and other plant aphid defence mechanisms is one possible explanation. It would be useful to consider genetic characterisation of tested mutation derived cowpea genotypes to ascertain if genetic differences may account for the reduced effect of silicon on cowpea aphid resistance.

This study showed a positive interaction between silicon application and aphid resistance, but only in the susceptible parent genotype Lutembwe. Silicon application to mutation derived genotypes (LT 3-8-4-1, LT 4-2-4-1 and LT 11-3-3-12) did not show any observable reduction in aphid performance even though these genotypes were shown to increase silicon levels when silicon was applied to these. plants when it was applied. This may suggest that mutations have somehow stopped the plants being able to benefit from silicon application. Alternatively, since aphid performance is lower in mutation derived genotypes, the additional contribution of silicon application to aphid resistance may have been less apparent. This study further showed that cowpea is able to take up and accumulate silicon within a relatively shorter period (7-10 days), and is potentially a crop that could benefit from silicon-induced defence against insect pests. This study also highlights the potential antagonism between mutagenesis and induced plant-defence through the application of silicon against insect pests. Since the application of silicon may have adverse effects on higher trophic levels such as parasitoids (Hall *et al.*, 2021), it would be useful to evaluated the impacts on aphid natural enemies in future studies. Aphid performance on Si+ plants of the susceptible parent Lutembwe is comparable to aphid-resistant mutation derived lines (LT 3-8-4-6, LT 4-2-4-1 and LT 11-3-3-12), suggesting diverse range of tools with which to manage cowpea aphid. However, results presented here indicate that there is no benefit in combining these approaches within future IPM programmes.

CHAPTER SIX

6 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

The aim of this study was to contribute to the development of sustainable management of aphid pests through identification and characterisation of resistance to aphids in common bean and cowpea genotypes, while also assessing the potential of silicon to complement mutation derived cowpea resistance to aphids. Realising this aim involved pursuing several objectives including: identifying and characterising resistance to black bean aphid in selected breeding lines and mutation derived common bean genotypes; identifying and characterising resistance to cowpea aphid in mutation derived cowpea genotypes under laboratory and field conditions; and evaluating the performance of cowpea aphid on silicon treated mutation derived cowpea genotypes under laboratory and field conditions. All the objectives have been achieved to a large extent. This chapter, therefore, aims to highlight the significant findings and implications for management of aphid pests on common bean and cowpea.

6.1.1 Black bean aphid performance on selected common bean genotypes

Evidence for occurrence of black bean aphid resistance in one of the evaluated common bean lines is presented in chapter four (section 3.1) of this thesis. Aphid nymphal development was significantly longer on the common bean genotype AO-1012-29-3-3A (AO) compared to the reference cultivar Kabulangeti (KAB) or other genotypes tested, reflecting a level of host plant resistance. longer nymphal development on AO suggests reduced nutritional quality of phloem sap which could have resulted in reduced feeding and therefore slower development (Zimba *et al.*, 2022a). The EPG recordings of aphid feeding behaviour on AO indicated a modest reduction in phloem feeding duration compared to KAB, which may indicate the presence of resistance factors in the phloem sap. Resistance to pea aphid in pea, for example, has been attributed to imbalances in essential amino acid composition in the phloem sap (Sandström & Pettersson, 1994). Interestingly, AO is resistant to the common bean weevil (*A. obtectus*) (Kusolwa *et al.*, 2016) and this resistance has been attributed to insecticidal proteins such as arcelins, phytohemagglutinin and alpha-amylase (Kusolwa & Myers, 2011). Arcelins, phytohemagglutinin and alpha-amylase constitute a family of lectins in common bean germplasm and protects seed against insect herbivory (Mojica & Mejía, 2015). Alpha-amylase reduces the activity of insect amylases and carbohydrate digestion, while arcelins and phytohemagglutinin bind to glycoproteins on insect gut membranes and interfere with protein digestion and nutrient absorption (Paes *et al.*, 2000). Higher levels of these proteins in common bean has often been associated with restricted growth of seed feeding insects (Carlini & Grossi-De-Sá, 2002; Kusolwa *et al.*, 2016). Aphicidal activities of phloem based lectins such as alpha-amylase in plants are well known (Rahbé *et al.*, 1995; Nalam *et al.*, 2019), and may have contributed to the cross-resistance of these proteins to the black bean aphid in AO line. Studies on biochemical characterisation of phloem sap in AO, however, are required to ascertain the source of aphid resistance factors. Lower glandular trichome density was also recorded on AO, which could benefit natural enemy performance and consequently enhance biological control of aphids (Riddick & Simmons, 2014). The common bean breeding line AO-1012-29-3A can therefore be used in genetic improvement of common bean for aphid resistance.

6.1.2 Cowpea aphid performance on mutation derived cowpea genotypes

Studies conducted in chapter three (section 3.2) of this thesis further highlights the potential role of induced mutagenesis in breeding for aphid resistant cowpea varieties (Zimba *et al.*, 2022b). Population and biological parameters of the cowpea aphid were adversely affected by the mutation derived cowpea genotypes. Aphid population growth was significantly inhibited on genotypes BB 7-9-7-5, LT 3-8-4-1, LT 4-2-4-1 and LT 11-3-3-12 compared to their respective parents Bubebe and Lutembwe. In the case of intrinsic rate of natural increase (r_m), genotypes LT3-8-4-1, LT4-2-4-1 and LT11-3-3-12 further reflected lower aphid performance compared to the parent. The r_m is a useful summary parameter that is used to estimate aphid performance when reared on different host plants and has been widely used to evaluate aphid resistance in crop cultivars (Obopile & Ositile, 2010; Leybourne *et al.*, 2019). Reduced aphid performance on BB 7-9-7-5, LT 3-8-4-1, LT 4-2-4-1 and LT 11-3-3-12 suggests the presence of mutation derived aphid resistance. Assessment of aphid feeding behaviour on LT3-8-4-1, LT4-2-4-1 and LT11-3-3-12 using electrical penetration graph (EPG) recording showed that resistance to cowpea aphid is mediated by epidermal and mesophyll-based resistance factors. Trichomes and biochemical compounds such as alkaloids, phenols and flavonoids are known to influence aphid performance on cowpea (Ofuya, 1997). Further studies, therefore, are needed to assess epidermal (i.e., wax, trichomes) and mesophyll (i.e., chemical compounds) aphid resistance traits associated with the mutation derived cowpea genotypes. Additionally, further studies are needed to establish whether the resistance traits in mutation derived cowpea genotypes are not detrimental to aphid natural enemies

(Michereff *et al.*, 2015). Genotypes BB 7-9-7-5, LT 3-8-4-1, LT 4-2-4-1 and LT 11-3-3-12 are therefore promising lines that should be further evaluated for useful genetic attributes that may be used to develop aphid resistant cowpea varieties.

This growing number of reported successes of using induced mutagenesis to produce genotypes with resistance to aphid pests indicate the potential of this approach. Despite this, the far larger number of successes in breeding for disease resistant crops using induced mutagenesis (Jung *et al.*, 2005; Busungu *et al.*, 2016; Oladosu *et al.*, 2016) and the overlap between aphid and pathogen resistance mechanisms indicate that breeding for aphid resistance is a comparatively under exploited use for this technique. This suggestion is further supported by the common location of pathogen and aphid resistant genes on chromosomes. Furthermore, the long history of safe use, low cost of equipment as well as wide acceptability makes induced mutagenesis an important technique that could be exploited further to speed up the delivery of aphid resistant cowpea varieties in sub-Saharan Africa. This is emphasised by a current lack of policy frameworks to regulate the use of modern breeding tools in most countries of sub-Saharan Africa. Although developing aphid resistance using induced mutagenesis is associated with several challenges, this approach provides a practical means through which to develop sustainable management programmes for aphid pests in crops throughout regions such as sub-Saharan Africa.

6.1.3 Cowpea aphid performance on silicon treated cowpea mutant genotypes

Chapter three (section 3.3) of this thesis investigated whether silicon applied as a drench could complement mutation-derived resistance to cowpea aphid. Initial assessment of silicon accumulation capacity indicated a general trend of increased silicon levels in mutation derived cowpea plants that were treated with silicon compared to those that were not treated. This observation highlights the capacity of these cowpea genotypes to take up and accumulate silicon (Shedeed *et al.*, 2018), as well as the potential of these genotypes to benefit from silicon derived resistance to abiotic and abiotic stresses. Application of silicon to the parent genotype Lutembwe resulted in a significant reduction in aphid colony growth, MRGR and phloem feeding when compared to silicon deprived plants, reflecting the occurrence of silicon induced aphid resistance. Silicon-induced resistance to insects is often expressed through enhancement of physical (i.e., trichomes, tissue abrasiveness) and biochemical (i.e., defence compounds) plant traits. Recording of aphid feeding behaviour using the EPG showed reduced duration of phloem feeding on silicon treated Lutembwe plants compared to silicon deprived plants. This observation suggests the presence of phloem-based aphid resistance factors in silicon treated Lutembwe plants, and is consistent with previous studies on silicon-induced aphid resistance (Gomes *et al.*, 2005; Goussain *et al.*, 2005). Elevated levels of phenolic compounds such as 5-caf-feoylquinic acid and p-coumaroylquinic acid has been associated with silicon-induced defence against aphids (Ranger *et al.*, 2009). Biochemical characterisation of phloem sap in silicon treated Lutembwe plants would be useful to identify which factors are responsible for the observed aphid resistance.

Silicon application to mutation derived cowpea genotypes (LT 3-8-4-1, LT 4-2-4-1 and LT 11-3-3-12), however, did not reduce aphid performance despite these genotypes showing a clear trend of higher silicon levels in silicon treated plants. The lack of silicon-based complementary effects, in reducing aphid performance, on mutation derived cowpea genotypes, may indicate that whilst mutation derived genotypes can accumulate silicon, they cannot do this effectively to reduce susceptibility to cowpea aphid. Alternatively, the lower aphid population and performance on mutation derived genotypes may have reduced the apparency of additional benefits of silicon application. However, it would be useful to consider genetic characterisation of the tested mutation-derived genotypes to establish if mutagenesis may account for the reduced interaction between silicon application and aphid resistance.

6.2 Recommendations

Based on the findings of the present study, the following recommendations are made:

i. Characterisation of amino acids and defence compounds in AO should be considered in future studies in order to establish the modality of resistance.
ii. The impact of glandular trichomes on black bean aphid is poorly understood and would warrant further study.
iii. It would be important to screen the promising cowpea genotypes with a range of aphid clones to establish if the results reported here are consistent for a wider range of aphid biotypes.
iv. Biochemical and morphological characterisation of promising aphid resistant cowpea genotypes would be useful in future studies.
v. Studies are needed to assess if promising aphid resistant cowpea genotypes are compatible with natural enemies within IPM systems.

vi. Future studies should consider evaluating profiles of mesophyll and phloem-based defence compounds in Lutembwe parent genotype.

vii. Genetic characterisation of Lutembwe parent line and its mutation derived cowpea genotypes is required to ascertain if genetic differences may account for the reduced silicon derived aphid resistance in mutation derived lines.

7 REFERENCES

Abate, T. & Ampofo, J.K.O. (1996) Insect Pests of Common Bean in Africa: Their Ecology and Management. *Annual Review of Entomology*, **41**, 45–73.

Abraham, A.D., Menzel, W., Lesemann, D.E., Varrelmann, M. & Vetten, H.J. (2006) Chickpea chlorotic stunt virus: A new polerovirus infecting cool-season food legumes in Ethiopia. *Phytopathology*, **96**, 437–446.

Abrol, D.P. & Shankar, U. (2012) *Integrated Pest Management Principles and Practice*. CABI International. pp 137-142.

Adekola, O.F. & Oluleye, F. (2007) Influence of mutation induction on the chemical composition of cowpea *Vigna unguiculata* (L.) Walp. *African Journal of Biotechnology*, **6**, 2143–2146.

Agbicodo, E.M., Fatokun, C.A., Muranaka, S., Visser, R.G.F. & Linden Van Der, C.G. (2009) Breeding drought tolerant cowpea: Constraints, accomplishments, and future prospects. *Euphytica*, **167**, 353–370.

Ajlan, A.M. & Potter, D.A. (1992) Lack of effect of tobacco mosaic virus-induced systemic acquired resistance on arthropod herbivores in tobacco. *Phytopathology*, **82**, 647–651.

Ali, J.G. & Agrawal, A.A. (2012) Specialist versus generalist insect herbivores and plant defense. *Trends in Plant Science*, **17**, 293–302.

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

Anda, M., Suryani, E., Husnain & Subardja, D. (2015) Strategy to reduce fertilizer application in volcanic paddy soils: Nutrient reserves approach from parent materials. *Soil and Tillage Research*, **150**, 10–20.

Arimura, G.I., Matsui, K. & Takabayashi, J. (2009) Chemical and molecular ecology of herbivore-induced plant volatiles: Proximate factors and their ultimate functions. *Plant and Cell Physiology*, **50**, 911–923.

Arora, L. & Narula, A. (2017) Gene editing and crop improvement using CRISPR-cas9 system.

Frontiers in Plant Science, **8**, 01932.

Arora, R. & Sandhu, S. (2017) *Breeding Insect Resistant Crops for Sustainable Agriculture*. Springer Nature Singapore. pp 126-133.

Atiri, G.I., Enobakhare, D.A. & Thottappilly, G. (1986) The importance of colonizing and non-colonizing aphid vectors in the spread of cowpea aphid-borne mosaic virus in cowpea. *Crop Protection*, **5**, 406–410.

Autrique, A., Stary, P. & Ntahimpera, L. (1989) Biological control of pest aphids by hymenopterous parasitoids in Burundi. *FAO Plant Protection Bulletin*, **37**, 71–76.

Awika, J.M. & Duodu, K.G. (2017) Bioactive polyphenols and peptides in cowpea (*Vigna unguiculata*) and their health promoting properties: A review. *Journal of Functional Foods*, **38**, 686–697.

Azimi, S. & Amini, R. (2015) Population density of Aphis fabae Scopoli (Hemiptera: Aphididae) and its natural enemies in intercropping of faba bean (*Vicia faba* L.) and dragonhead (*Dracocephalum moldavica* L.). *Journal of Biodiversity and Environmental Sciences*, **6**, 380–388.

Baker, B.P., Green, T.A. & Loker, A.J. (2020) Biological control and integrated pest management in organic and conventional systems. *Biological Control*, **140**, 104095.

Barber, D.A. & Shone, M.G.T. (1966) The absorption of silica from aqueous solutions by plants. *Journal of Experimental Botany*, **17**, 569–578.

Basu, R.C., Chakrabarti, S. & Raychaudhuri, D.N. (1968) Record of the sexuales of *Aphis craccivora* Koch (Homoptera : Aphididae) from India. *Oriental Insects*, **2**, 349–351.

van Bel, A.J.E. & Will, T. (2016) Functional evaluation of proteins in watery and gel saliva of aphids. *Frontiers in Plant Science,* **7**, 1–19.

Benchasri, S., Bairaman, C. & Nualsri, C. (2011) Investigation of Cowpea and Yardlong Bean for Resistance to Bean Aphids (*Aphis craccivora* Koch), **22**, 2–6.

Beneteau, J., Renard, D., Marché, L., Douville, E., Lavenant, L., Rahbé, Y., Dupont, D., Vilaine, F., Dinant, S. (2010) Binding properties of the N-acetylglucosamine and high-mannose

N-glycan PP2-A1 phloem lectin in Arabidopsis. *Plant Physiology*, **153**, 1345–1361.

Bhattacharya, S. (2019) Brassica-aphid interaction: Challenges and prospects of genetic engineering for integrated aphid management. *Physiological and Molecular Plant Pathology*, **108**, 101442.

Biondi, A., Desneux, N., Siscaro, G. & Zappalà, L. (2012) Using organic-certified rather than synthetic pesticides may not be safer for biological control agents: Selectivity and side effects of 14 pesticides on the predator *Orius laevigatus*. *Chemosphere*, **87**, 803–812.

Blackman, R. (1974) *Aphids*. Ginn & Company Limited, London. pp 100-112.

Blackman, R.L. & Eastop, V.F. (2000) *Aphids on the World's Crops: An Identification and Information Guide*. Second Edi. John Wiley & Sons, Chichester. pp 97-107.

Borowiak-Sobkowiak, B., Durak, R. & Wilkaniec, B. (2017) Morphology, biology and behavioral aspects of *Aphis craccivora* (Hemiptera: Aphididae) on *Robinia pseudoacacia*. *Acta Scientiarum Polonorum Hortorum Cultus*, **16**, 39–49.

Botha, A. (2021) Fast developing Russian wheat aphid biotypes remains an unsolved enigma. *Current Opinion in Insect Science*, **45**, 42–52.

Botha, A.M., Kunert, K.J., Maling'a, J. & Foyer, C.H. (2020) Defining biotechnological solutions for insect control in sub-Saharan Africa. *Food and Energy Security*, **9**, 1–21.

Botha, C.E.J., Malcolm, S.B. & Evert, R.F. (1977) An investigation of preferential feeding habit in four Asclepiadaceae by the Aphid, *Aphis nerii*. *protoplasma*, **92**, 1–19.

Botha, C.E.J. & Matsiliza, B. (2004) Reduction in transport in wheat (*Triticum aestivum*) is caused by sustained phloem feeding by the Russian wheat aphid (*Diuraphis noxia*). *South African Journal of Botany*, **70**, 249–254.

Boukar, O., Belko, N., Chamarthi, S., Togola, A., Batieno, J., Owusu, E., Haruna, M., Diallo, S., Umar, M.L., Olufajo, O. & Fatokun, C. (2019) Cowpea (*Vigna unguiculata*): Genetics, genomics and breeding. *Plant Breeding*, **138**, 415–424.

Brown, J., McCaffrey, J.P., Harmon, B.L., Davis, J.B., Brown, A.P. & Erickson, D.A. (1999) Effect of late season insect infestation on yield, yield components and oil quality of

Brassica napus, *B. rapa*, *B. juncea* and *Sinapis alba* in the Pacific Northwest region of the United States. *Journal of Agricultural Science*, **132**, 281–288.

Buruchara, R., Ampofo, K. & Mukankusi, C. (2010) Bean Disease and Pest. *Ciat,* **371**, 6–8.

Buruchara, R., Chirwa, R., Sperling, L., Mukankusi, C., Rubyogo, J.C. & Muthoni, R. (2011) Development and delivery of bean varieties in Africa: The Pan-Africa bean research alliance (PABRA) model. *African Crop Science Journal*, **19**, 227–245.

Bus, V.G.M., Chagné, D., Bassett, H.C.M., Bowatte, D., Calenge, F., Celton, J.M., Durel, C. E., Malone, M.T., Patocchi, A., Ranatunga, A.C., Rikkerink, E.H.A., Tustin, D. S., Zhou, J. & Gardiner, S. E. (2008) Genome mapping of three major resistance genes to woolly apple aphid (*Eriosoma lanigerum* Hausm.). *Tree Genetics and Genomes*, **4**, 223–236.

Busungu, C., Taura, S., Sakagami, J.I. & Ichitani, K. (2016) Identification and linkage analysis of a new rice bacterial blight resistance gene from XM14, a mutant line from IR24. *Breeding Science*, **66**, 636–645.

Cai, K., Gao, D., Luo, S., Zeng, R., Yang, J. & Zhu, X. (2008) Physiological and cytological mechanisms of silicon-induced resistance in rice against blast disease. *Physiologia Plantarum*, **134**, 324–333.

Callaway, E. (2018) CRISPR plants now subject to tough GM laws in European Union. *Nature*, **560**, 16–17.

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.

Cammell, M.E., Way, M.J. & Heathcote, G.D. (1978) Distribution of Eggs of the Black Bean Aphid, *Aphis fabae* Scop., on the Spindle Bush, *Euonymus europaeus* L., with Reference to Forecasting Infestations of the Aphid on Field Beans. *Plant Pathology*, **27**, 68–76.

Cardona, C. & Karel, A.K. (1990) Key insects and other invertebrate pests of beans. In *Insect Pests of Tropical Food Legumes* (ed. by S.R. Singh). John Wiley & Sons, West Sussex, pp. 157–191.

Cardona, C., Kornegay, J. & Clement, S. (2019) Bean Germplasm Resources for Insect Resistance. In *Global Plant Genetic Resources for Insect-Resistant Crops* (ed. by Clement, S.L. & Quisenberry, S.S.). Taylor & Francis, London, pp. 85–96.

Carlini, C.R. & Grossi-De-Sá, M.F. (2002) Plant toxic proteins with insecticidal properties. A review on their potentialities as bioinsecticides. *Toxicon*, **40**, 1515–1539.

Carrillo, L., Martinez, M., Álvarez-Alfageme, F., Castañera, P., Smagghe, G., Diaz, I. & Ortego, F. (2011) A barley cysteine-proteinase inhibitor reduces the performance of two aphid species in artificial diets and transgenic Arabidopsis plants. *Transgenic Research*, **20**, 305–319.

Carvalho, F.P. (2017) Pesticides, environment, and food safety. *Food and Energy Security*, **6**, 48–60.

Castle, S.J. & Berger, P.H. (1993) Rates of growth and increase of *Myzus persicae* on virus-infected potatoes according to type of virus-vector relationship. *Experimentalis et Applicata*, **69**, 51–60.

Chain, F., Côté-Beaulieu, C., Belzile, F., Menzies, J.G. & Bélanger, R.R. (2009) A comprehensive transcriptomic analysis of the effect of silicon on wheat plants under control and pathogen stress conditions. *Molecular Plant-Microbe Interactions*, **22**, 1323–1330.

Chandler, D., Bailey, A.S., Mark Tatchell, G., Davidson, G., Greaves, J. & Grant, W.P. (2011) The development, regulation and use of biopesticides for integrated pest management. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **366**, 1987–1998.

Chapoto, A., Chisanga, B. & Kabisa, M. (2019) *Zambia Agriculture Status Report 2018*. Lusaka.

Chekanai, V., Chikowo, R. & Vanlauwe, B. (2018) Response of common bean (*Phaseolus vulgaris* L.) to nitrogen, phosphorus and rhizobia inoculation across variable soils in Zimbabwe. *Agriculture, Ecosystems and Environment*, **266**, 167–173.

Chern, M., Xu, Q., Bart, R.S., Bai, W., Ruan, D., Sze-To, W.H., Canlas, Patrick E., Jain, R., Chen, X. & Ronald, P.C. (2016) A Genetic Screen Identifies a Requirement for Cysteine-Rich–Receptor-Like Kinases in Rice NH1 (OsNPR1)-Mediated Immunity. *PLoS*

Genetics, **12**, 1–20.

Chisholm, S.T., Coaker, G., Day, B. & Staskawicz, B.J. (2006) Host-microbe interactions: Shaping the evolution of the plant immune response. *Cell*, **124**, 803–814.

Chougule, N.P. & Bonning, B.C. (2012) Toxins for transgenic resistance to hemipteran pests. *Toxins*, **4**, 405–429.

Christopoulou, M., McHale, L.K., Kozik, A., Wo, S.R.C., Wroblewski, T. & Michelmore, R.W. (2015) Dissection of two complex clusters of resistance genes in lettuce (*Lactuca sativa*). *Molecular Plant-Microbe Interactions*, **28**, 751–765.

Cichy, K.A., Porch, T.G., Beaver, J.S., Cregan, P., Fourie, D., Glahn, R.P., Grusak, M.A., Kamfwa, K., Katuuramu, D.N., McClean, P., Mndolwa, E., Nchimbi-Msolla, S., Pastor-Corrales, M.A. & Miklas, P.N. (2015) A *Phaseolus vulgaris* diversity panel for andean bean improvement. *Crop Science*, **55**, 2149–2160.

Cole, R.A. (1997) The relative importance of glucosinolates and amino acids to the development of two aphid pests *Brevicoryne brassicae* and *Myzus persicae* on wild and cultivated brassica species. *Entomologia Experimentalis et Applicata*, **85**, 121–133.

Coomey, J., Sibout, R. & Hazen, S. (2020) Grass secondary cell walls , *Brachypodium distachyon* as a model for discovery: Tansley review. *New Phytologist*, **227**, 1649–1667.

Costa, R.R., Moraes, J.C. & DaCosta, R.R. (2011) Feeding behaviour of the greenbug *Schizaphis graminum* on wheat plants treated with imidacloprid and/or silicon. *Journal of Applied Entomology*, **135**, 115–120.

Crowder, D.W. & Jabbour, R. (2014) Relationships between biodiversity and biological control in agroecosystems: Current status and future challenges. *Biological Control*, **75**, 8–17.

Cullen, E.M., Zalom, F.G., Flint, M.L. & Zilbert, E.E. (2000) Quantifying trade-offs between pest sampling time and precision in commercial IPM sampling programs. *Agricultural Systems*, **66**, 99–113.

Dahlin, R.M., Brick, M.A. & Ogg, J.B. (1992) Characterization and density of trichomes on three common bean cultivars. *Economic Botany*, **46**, 299–304.

Davies, J.C. (1972) Studies on the ecology of *Aphis craccivora* Koch (Hemiptera: Aphididae), the vector of rosette disease of groundnuts, in Uganda. *Bulletin of Entomological Research*, **62**, 169–182.

De Oliveira, R.S., Peñaflor, M.F.G.V., 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**, 1–13.

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.

Desneux, N., Barta, R.J., Hoelmer, K.A., Hopper, K.R. & Heimpel, G.E. (2009) Multifaceted determinants of host specificity in an aphid parasitoid. *Oecologia*, **160**, 387–398.

Desneux, N., Decourtye, A. & Delpuech, J.-M. (2006) The Sublethal Effects of Pesticides on Beneficial Arthropods. *Annual Review of Entomology*, **52**, 81–106.

Dewar, A.M. & Foster, S. (2017) Overuse of pyrethroids may be implicated in the recent BYDV epidemics in cereals. *Outlooks on Pest Management*, **28**, 7–12.

Diehl, E., Sereda, E., Wolters, V. & Birkhofer, K. (2013) Effects of predator specialization, host plant and climate on biological control of aphids by natural enemies: A meta-analysis. *Journal of Applied Ecology*, **50**, 262–270.

Dixon, A.F.G. (1998) *Aphid Ecology: an Optimization Approach, 2nd edition. Chapman and Hall, London, UK.* Second Edi. London. pp 90-102.

Dixon, A.F.G. & Wratten, S.D. (1971) Laboratory studies on aggregation, size and fecundity in the black bean aphid, Aphis fabae Scop. *Bulletin of Entomological Research*, **61**, 97.

Dogimont, C., Bendahmane, A., Chovelon, V. & Boissot, N. (2010) Host plant resistance to aphids in cultivated crops: Genetic and molecular bases, and interactions with aphid populations. *Comptes Rendus - Biologies*, **333**, 566–573.

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

Douglas, A.E. & Prosser, W.A. (1992) Synthesis of the essential amino acid tryptophan in the pea aphid (*Acyrthosiphon pisum*) symbiosis. *Journal of Insect Physiology,* **38**, 565–568.

Dreyer, D.L. & Campbell, B.C. (1987) Chemical basis of host-plant resistance to aphids. *Plant, Cell & Environment*, **10**, 353–361.

Du, L., Ge, F., Zhu, S. & Parajulee, M.N. (2009) Effect of Cotton Cultivar on Development and Reproduction of *Aphis gossypii* (Homoptera: Aphididae) and Its Predator *Propylaea japonica* (Coleoptera: Coccinellidae). *Journal of Economic Entomology*, **97**, 1278–1283.

Du, Y., Poppy, G.M., Powell, W., Pickett, J.A., Wadhams, L.J. & Woodcock, C.M. (1998) Identification of semiochemicals released during aphid feeding that attract parasitoid *Aphidius ervi*. *Journal of Chemical Ecology*, **24**, 1355–1368.

Dubey, N.K., Shukla, R., Kumar, A., Singh, P. & Prakash, B. (2010) Prospects of botanical pesticides in sustainable agriculture. *Current Science,* **98**, 479–480.

Dudareva, N., Negre, F., Nagegowda, D.A. & Orlova, I. (2006) Plant volatiles: Recent advances and future perspectives. *Critical Reviews in Plant Sciences*, **25**, 417–440.

Dunlap, R. & Beus, C. (1992) Understanding Public Concerns About Pesticides: An Empirical Examination. *The journal of consumer affairs*, **26**, 418–438.

Ehlers, J.D. & Hall, A.E. (1996) Genotypic classification of cowpea based on responses to heat and photoperiod. *Crop Science,* **36**, 673–679.

Ellis, P.R., Singh, R., Pink, D.A.C., Lynn, J.R. & Saw, P.L. (1996) Resistance to *Brevicoryne brassicae* in horticultural brassicas. *Euphytica*, **88**, 85–96.

Elowad, H.O. & Hall, A.E. (1987) Influences of early and late nitrogen fertilization on yield and nitrogen fixation of cowpea under well-watered and dry field conditions. *Field Crops Research*, **15**, 229–244.

Elsharkawy, M.M. & Mousa, K.M. (2015) Induction of systemic resistance against Papaya ring spot virus (PRSV) and its vector *Myzus persicae* by *Penicillium simplicissimum* GP17-2 and silica (Sio2) nanopowder. *International Journal of Pest Management*, **61**, 353–358.

van Emden, H.F. & Bashford, M.A. (1969) A comparison of the reproduction of *Brevicoryne brassicae* and *Myzus persicae* in relation to soluble nitrogen concentration and leaf age (leaf position) in the Brussels sprout plant. *Entomologia Experimentalis et Applicata*, **12**, 351–364.

van Emden, H.F. & Harrington, R. (2017) *Aphids as Crop Pests*. Second Edi. CABI, Oxfordshire. pp 120-132.

Epstein, E. (1994) The anomaly of silicon in plant biology. *Proceedings of the National Academy of Sciences of the United States of America*, **91**, 11–17.

Esmaeili-Vardanjani, M., Askarianzadeh, A., Saeidi, Z., Hasanshahi, G.H., Karimi, J. & Nourbakhsh, S.H. (2013) A study on common bean cultivars to identify sources of resistance against the black bean aphid, *Aphis fabae* Scopoli (Hemiptera: Aphididae). *Archives of Phytopathology and Plant Protection*, **46**, 1598–1608.

FAO/IAEA. (2019) *The Food and Agriculture Organization of the United Nations/International Atomic Energy Agency – Mutant Variety Database (FAO/ IAEA-MVD) data (2019) reports*.

FAO. (2018) *Food and Agriculture Organisation of the United Nation: Transforming Food and Agriculture To Achieve the SDGs*. Food and Agriculture Organisation of the United Nation. pp 49-54.

Farrell, J.A.K. (1976) Effects of groundnut crop density on the population dynamics of *Aphis craccivora* Koch (Hemiptera: Aphididae) in Malawi. *Bulletin of Entomological Research*, **66**, 317–329.

Fauteux, F., Rémus-Borel, W., Menzies, J.G. & Bélanger, R.R. (2005) Silicon and plant disease resistance against pathogenic fungi. *FEMS Microbiology Letters*, **249**, 1–6.

Feldman, A.B., Leung, H., Baraoidan, M., Elmido-Mabilangan, A., Canicosa, I., Quick, W.P., Sheehy, J. & Murchie, E.H. (2017) Increasing leaf vein density via mutagenesis in rice results in an enhanced rate of photosynthesis, smaller cell sizes and can reduce interveinal mesophyll cell number. *Frontiers in Plant Science*, **8**, 1–10.

Feng, X., Poplawsky, A.R., Nikolaeva, O. V., Myers, J.R. & Karasev, A. V. (2014) Recombinants of bean common mosaic virus (bcmv) and genetic determinants of bcmv involved in overcoming resistance in common bean. *Phytopathology*, **104**, 786–793.

Fischer, K., Berg, J. Van Den & Mutengwa, C. (2015) Is Bt maize effective in improving South African smallholder agriculture? *South African Journal of Science*, **111**, 1–2.

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.

Fuentes-Contreras, E. & Niemeyer, H.M. (1998) DIMBOA glucoside, a wheat chemical defense, affects host acceptance and suitability of *Sitobion avenae* to the cereal aphid parasitoid *Aphidius rhopalosiphi*. *Journal of Economic Entomology*, **24**, 371–381.

Fürstenberg-Hägg, J., Zagrobelny, M. & Bak, S. (2013) Plant defense against insect herbivores. *International Journal of Molecular Sciences*, **14**, 10242-10297.

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

Golawska, S., Leszczynski, B. & Staszewski, Z. (2005) Saponins as a source of alfalfa resistance towards pea aphid, Acyrthosiphon pisum Harris. In *Breeding for Resistance to Pests and Diseases. IOBC, Bialowieza, Poland*. IOBC, Bialowieza, pp. 45–50.

Gomes, F.B., Moraes, J.C. de, Santos, S.D. dos & Marcos, M. (2005) Resistance induction in wheat plants by silicon and aphids. *Scientia Agricola*, **62**, 547–551.

Goussain, M.M., Prado, E. & Moraes, J.C. (2005) Effect of silicon applied to wheat plants on the biology and probing behaviour of the greenbug *Schizaphis graminum* (Rond.) (Hemiptera: Aphididae). *Neotropical Entomology*, **34**, 807–813.

Grisley, W. & Shamambo, M. (1993) An analysis of the adoption and diffusion of carioca beans in Zambia resulting from an experimental distribution of seed, **29**, 379–386.

Guo, H., Mendrikahy, J.N., Xie, L., Deng, J., Lu, Z., Wu, J., Li, X.S., Muhammad, Q.L. &

Xiangdong, X. (2017) Transcriptome analysis of neo-tetraploid rice reveals specific differential gene expressions associated with fertility and heterosis. *Scientific Reports*, **7**, 1–11.

Halkier, B.A. & Gershenzon, J. (2006) Biology and Biochemistry of Glucosinolates. *Annual Review of Plant Biology*, **57**, 303–333.

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.

Hanley, M.E., Lamont, B.B., Fairbanks, M.M. & Rafferty, C.M. (2007) Plant structural traits and their role in anti-herbivore defence. *Perspectives in Plant Ecology, Evolution and Systematics*, **8**, 157–178.

Hardie, J. (1989) Spectral specificity for targeted flight in the black bean aphid, *Aphis fabae*. *Journal of Insect Physiology*, **35**, 619–626.

Henriet, C., Draye, X., Oppitz, I., Swennen, R. & Delvaux, B. (2006) Effects, distribution and uptake of silicon in banana (*Musa* spp.) under controlled conditions. *Educational Technology Research and Development*, **287**, 359–374.

Hewer, A., Becker, A. & Bel, A.J.E. Van. (2011) An aphid's Odyssey - The cortical quest for the vascular bundle. *Journal of Experimental Biology*, **214**, 3868–3879.

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.

Hogenhout, S.A. & Bos, J.I.B. (2011) Effector proteins that modulate plant-insect interactions. *Current Opinion in Plant Biology*, **14**, 422–428.

Holland, J., Bown, B., Clarke, J. & Mchugh, N. (2019) Patterns of cereal aphid infestation in autumn and implications for Barley Yellow Dwarf Virus control, **143**, 105–109.

Horn, L. & Shimelis, H. (2013) Radio-sensitivity of selected cowpea (*Vigna unguiculata*) genotypes to varying gamma irradiation doses. *Scientific Research and Essays*, **8**, 1991–1997.

Horn, L., Shimelis, H. & Laing, M. (2015) Participatory appraisal of production constraints , preferred traits and farming system of cowpea in the northern Namibia: implications for breeding, **38**, 691–700.

Horn, L.N., Ghebrehiwot, H.M. & Shimelis, H.A. (2016) Selection of novel cowpea genotypes derived through gamma irradiation. *Frontiers in Plant Science*, **7**, 1–13.

Hort, J. & Birch, N. (1984) Taxonomy, evolution and domestication of Vicia in relation to aphid resistance. *Annals of Applied Biology*, **105**, 547–556.

Howe, G.A. & Jander, G. (2008) Plant Immunity to Insect Herbivores. *Annual Review of Plant Biology*, **59**, 41–66.

Hu, X.S., Zhang, Z.F., Zhu, T.Y., Song, Y., Wu, L.J., Liu, X.F., Zhao, H.Y. & Liu, T.X. (2018) Maternal effects of the English grain aphids feeding on the wheat varieties with different resistance traits. *Scientific Reports*, **8**, 1–11.

Hussain, A.J., Ali, J., Siddiq, E.A., Gupta, V.S., Reddy, U.K. & Ranjekar, P.K. (2012) Mapping of tms8 gene for temperature-sensitive genic male sterility (TGMS) in rice (*Oryza sativa* L.). *Plant Breeding*, **131**, 42–47.

Huynh, B.L., Ehlers, J.D., Ndeve, A., Wanamaker, S., Lucas, M.R., Close, T.J. & Roberts, P.A. (2015) Genetic mapping and legume synteny of aphid resistance in African cowpea (*Vigna unguiculata* L. Walp.) grown in California. *Molecular Breeding*, **35**.

Hwang, J.E., Ahn, J.W., Kwon, S.J., Kim, J.B., Kim, S.H., Kang, S.Y. & Kim, D.S. (2014) Selection and molecular characterization of a high tocopherol accumulation rice mutant line induced by gamma irradiation. *Molecular Biology Reports*, **41**, 7671–7681.

Irshad, A., Guo, H., Zhang, S. & Liu, L. (2020) TILLING in cereal crops for allele expansion and mutation detection by using modern sequencing technologies. *Agronomy*, **10**, 1–18.

Ishikawa, S., Ishimaru, Y., Igura, M., Kuramata, M., Abe, T., Senoura, T., Hase, Y.A., Tomohito, N., Naoko, K. & Nakanishi, H. (2012) Ion-beam irradiation, gene identification, and marker-assisted breeding in the development of low-cadmium rice. *Proceedings of the National Academy of Sciences of the United States of America*, **109**, 19166–19171.

Izaguirre-Mayoral, M.L., Brito, M., Baral, B. & Garrido, M.J. (2017) Silicon and nitrate differentially modulate the symbiotic performances of healthy and virus-infected Bradyrhizobium-nodulated cowpea (*Vigna unguiculata*), Yardlong Bean (*V. unguiculata* subsp. *sesquipedalis*) and Mung Bean (*V. radiata*). *Plants*, **6**, 1–15.

Jackai, L.E.N. & Daoust, R.A. (1986) Insect pests of cowpeas. *Annual Review of Entomology*, **31**, 95–119.

Jacobsen, S.E., Sørensen, M., Pedersen, S.M. & Weiner, J. (2013) Feeding the world: Genetically modified crops versus agricultural biodiversity. *Agronomy for Sustainable Development*, **33**, 651–662.

James, K.L., Randall, N.P., Walters, K.F.A., Haddaway, N.R. & Land, M. (2016) Evidence for the effects of neonicotinoids used in arable crop production on non-target organisms and concentrations of residues in relevant matrices: a systematic map protocol. *Environmental Evidence*, **5**, 1–9.

Jaouannet, M., Rodriguez, P.A., Thorpe, P., Lenoir, C.J.G., Macleod, R., Escudero-Martinez, C. & Bos, J.I.B. (2014) Plant immunity in plant-aphid interactions. *Frontiers in Plant Science*, **5**, 1–10.

Jayathilake, C., Visvanathan, R., Deen, A., Bangamuwage, R., Jayawardana, B.C., Nammi, S. & Liyanage, R. (2018) Cowpea: an overview on its nutritional facts and health benefits. *Journal of the Science of Food and Agriculture*, **98**, 4793–4806.

Jeng, T.L., Lin, Y.W., Wang, C.S. & Sung, J.M. (2012) Comparisons and selection of rice mutants with high iron and zinc contents in their polished grains that were mutated from the indica type cultivar IR64. *Journal of Food Composition and Analysis*, **28**, 149–154.

Johnson, A.W., Sisson, V.A., Snook, M.E., Fortnum, B.A. & Jackson, D.M. (2002) Aphid Resistance and Leaf Surface Chemistry of Sugar Ester Producing Tobaccos. *Journal of Entomological Science*, **37**, 154–165.

Johnson, S.N., Rowe, R.C. & Hall, C.R. (2019) Silicon is an inducible and effective herbivore defence against *Helicoverpa punctigera* (Lepidoptera: Noctuidae) in soybean. *Bulletin of Entomological Research*, **110**, 417–422.

Jones, J.D.G. & Dangl, J.L. (2006) The plant immune system. *Nature*, **444**, 323–329.

Jones, L.H.P. & Handreck, K.A. (1967) Silica in soils, plants, and animals. *Advances in Agronomy*, **19**, 107–149.

Jung, Y.H., Lee, J.H., Agrawal, G.K., Rakwal, R., Kim, J.A., Shim, J.K., Lee, S.K., Jeon, J.S., Koh, H.J., Lee, Y.H., Iwahashi, H. & Jwa, N.S. (2005) The rice (*Oryza sativa*) Blast Lesion Mimic Mutant, blm, may confer resistance to blast pathogens by triggering multiple defense-associated signaling pathways. *Plant Physiology and Biochemistry*, **43**, 397–406.

Kaloshian, I. & Walling, L.L. (2005) Hemipterans as plant pathogens. *Annual Review of Phytopathology*, **43**, 491–521.

Kaloshian, I. & Walling, L.L. (2016) Plant Immunity: Connecting the Dots Between Microbial and Hemipteran Immune Responses. In *Management of Insect Pests to Agriculture*. Springer, pp. 217–243.

Kamfwa, K., Beaver, J.S., Cichy, K.A. & Kelly, J.D. (2018) QTL Mapping of Resistance to Bean Weevil in Common Bean. *Crop Science*, **58**, 2370–2378.

Kamphuis, L.G., Gao, L. & Singh, K.B. (2012) Identification and characterization of resistance to cowpea aphid (*Aphis craccivora* Koch) in Medicago truncatula. *BMC Plant Biology*, **12**, 2–12.

Karungi, J., Adipala, E., Ogenga-Latigo, M.W., Kyamanywa, S. & Oyobo, N. (2000) Pest management in cowpea. Part 1. Influence of planting time and plant density on cowpea field pests infestation in eastern Uganda. *Crop Protection*, **19**, 231–236.

Kataria, R. & Kumar, D. (2012) Occurrence and Infestation Level of Sucking pests : Aphids on various host plants in Agricultural Fields of. *International Journal of Scientific and Research Publications*, **2**, 1–6.

Kaufman, P.B., Dayanandan, P., Franklin, C.I. & Takeoka, Y. (1985) Structure and function of silica bodies in the epidermal system of grass shoots. *Annals of botany*, **55**, 487–507.

Keeping, M.G., Kvedaras, O.L. & Bruton, A.G. (2009) Epidermal silicon in sugarcane: Cultivar differences and role in resistance to sugarcane borer *Eldana saccharina*. *Environmental and Experimental Botany*, **66**, 54–60.

Keeping, M.G. & Meyer, J.H. (2006) Silicon-mediated resistance of sugarcane to *Eldana saccharina* Walker (Lepidoptera: Pyralidae): Effects of silicon source and cultivar. *Journal of Applied Entomology*, **130**, 410–420.

Kennedy, B.Y.J.S. & Booth, C. (1951) To the Age and Kind of Leaves. *Annals of Applied Biology*, **38**, 25–64.

Kerchev, P.I., Karpińska, B., Morris, J.A., Hussain, A., Verrall, S.R., Hedley, P.E., Fenton, B., Foyer, C.H., Hancock, R.D. (2013) Vitamin C and the abscisic acid-insensitive 4 transcription factor are important determinants of aphid resistance in arabidopsis. *Antioxidants and Redox Signaling*, **18**, 2091–2105.

Kim, B., Woo, S., Kim, M.J., Kwon, S.W., Lee, J., Sung, S.H., Koh, H.J. (2018) Identification and quantification of flavonoids in yellow grain mutant of rice (*Oryza sativa* L.). *Food Chemistry*, **241**, 154–162.

Kim, J.H. & Jander, G. (2007) *Myzus persicae* (green peach aphid) feeding on Arabidopsis induces the formation of a deterrent indole glucosinolate. *Plant Journal*, **49**, 1008–1019.

Kim, K.H., Kabir, E. & Jahan, S.A. (2017) Exposure to pesticides and the associated human health effects. *Science of the Total Environment*, **575**, 525–535.

Knight, J.D. & Camell, M.E. (1994) A decision support system for forecasting infestations of the black bean aphid, *Aphis fabae* Scop., on spring-sown field beans, *Vicia faba*. *Computers and Electronics in Agriculture*, **10**, 269–279.

Krupke, C.H., Alford, A.M., Cullen, E.M., Hodgson, E.W., Knodel, J., Mccornack, B., Potter, B.D., Spigler, M.I., Tilmon, K. & Welch, K. (2017) Assessing the value and pest management window provided by neonicotinoid seed treatments for management of soybean aphid (*Aphis glycines* Matsumura) in the Upper Midwestern United States. *Pest Management Science*, **73**, 2184–2193.

Kumar, S. & Sangha, M.K. (2013) Biochemical mechanism of resistance in some Brassica genotypes against *Lipaphis Erysimi* (Kaltenbach) (Homoptera: Aphididae). *Vegetos*,

26, 387–395.

Kusolwa, P.M. & Myers, J.R. (2011) Seed storage proteins ARL2 and its variants from the apalocus of wild bean G40199 confers resistance to Acanthocellides obtectus when expressed in common beans. *African Crop Science Journal*, **19**, 255–265.

Kusolwa, P.M., Myers, J.R., Porch, T.G., Trukhina, Y., González-Vélez, A. & Beaver, J.S. (2016) Registration of AO-1012-29-3-3A Red Kidney Bean Germplasm Line with Bean Weevil, BCMV, and BCMNV Resistance. *Journal of Plant Registrations*, **10**, 149–153.

Kvedaras, O.L., An, M., Choi, Y.S. & Gurr, G.M. (2010) Silicon enhances natural enemy attraction and biological control through induced plant defences, **61**, 367–371.

Laamari, M., Khelfa, L. & Cœur d'Acier, A. (2008) Resistance source to cowpea aphid (*Aphis craccivora* Koch) in broad bean (*Vicia faba* L.) Algerian landrace collection. *African Journal of Biotechnology*, **7**, 2486–2490.

Lancashire, P.D., Bleiholder, H., Boom, T.V.D., Langelüddeke, P., Stauss, R., Weber, E. & Witzenberger, A. (1991) A uniform decimal code for growth stages of crops and weeds. *Annals of Applied Biology*, **119**, 561–601.

Larocca, A., Fanti, P., Molinaro, A., Mattia, M.F. & Battaglia, D. (2011) Aphid performance on *Vicia faba* and two southern Italy *Phaseolus vulgaris* landraces. *Bulletin of Insectology*, **64**, 101–106.

Lattanzio, V., Arpaia, S., Cardinali, A. & Venere, D. Di. (2000) Role of Endogenous Flavonoids in Resistance Mechanism of Vigna to Aphids. *Journal of Agricultural and Food Chemistry*, **48**, 5316–5320.

van Lenteren, J.C. & De Ponti, O.M.B. (1991) Plant-leaf morphology, host-plant resistance and biological control. *Insects-plants '89. Proc. 7th symposium on insect-plant relationships, Budapest, 1989*, 365–386.

Leroy, N., Tombeur, F. De, 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*, **8,** 444.

Leybourne, D.J., Valentine, T.A., Robertson, J.A.H., Pérez-Fernández, E., Main, A.M., Karley, A.J. & Bos, J.I.B. (2019) Defence gene expression and phloem quality contribute to mesophyll and phloem resistance to aphids in wild barley. *Journal of Experimental Botany*, **70**, 4011–4026.

Li, Q., Xie, Q.G., Smith-Becker, J., Navarre, D.A. & Kaloshian, I. (2006) Mi-1-mediated aphid resistance involves salicylic acid and mitogen-activated protein kinase signaling cascades. *Molecular Plant-Microbe Interactions*, **19**, 655–664.

Liang, N., Bélanger, M.N. & Song, H.G. (2015) *Silicon in Agriculture From Theory to Practice*. springer Dordrecht Heidelberg, New York. pp 137-142.

Lin, D., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., Kong, M., Li, L., Zhang, Q., Liu, Y., Chen, H., Qin, W., Wu, H. & Chen, S. (2016) An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. *Molecules*, **21**, 1–19.

Lin, D.G., Chou, S.Y., Wang, A.Z., Wang, Y.W., Kuo, S.M., Lai, C.C., Chen, L.J. & Wang, C.S. (2014) A proteomic study of rice cultivar TNG67 and its high aroma mutant SA0420. *Plant Science*, **214**, 20–28.

Liu, J., Zhu, J., Zhang, P., Han, L. & Reynolds, O.L. (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**, 1–8.

Long, W., Dong, B., Wang, Y., Pan, P., Wang, Y., Liu, L., Chen, X., Liu, X., Liu, S., Tian, Y., Chen, L. & Wan, J. (2017) FLOURY ENDOSPERM8, encoding the UDP-glucose pyrophosphorylase 1, affects the synthesis and structure of starch in rice endosperm. *Journal of Plant Biology*, **60**, 513–522.

Louis, J., Singh, V. & Shah, J. (2012) *Arabidopsis thaliana* —Aphid Interaction . *The Arabidopsis Book*, **10**, e0159.

Lowe, H.J.B., Murphy, G.J.P. & Parker, M.L. (1985) Non-glaucousness, a probable aphid-resistance character of wheat. *Annals of Applied Biology*, **106**, 555–560.

Lundgren, J.G., Fergen, J.K. & Riedell, W.E. (2008) The influence of plant anatomy on oviposition and reproductive success of the omnivorous bug *Orius insidiosus*. *Animal Behaviour*, **75**, 1495–1502.

Ma, J.F. (2004) Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. *Soil Science and Plant Nutrition*, **50**, 11–18.

Ma, J.F., Miyake, Y. & Takahashi, E. (2001) Silicon as a beneficial element for crop plants. In *Studies in plant Science*. Elsevier, pp. 17–39.

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

Macaulay, M., Ramsay, L. & Åhman, I. (2020) Quantitative trait locus for resistance to the aphid *Rhopalosiphum padi* L. in barley (*Hordeum vulgare* L.) is not linked with a genomic region for gramine concentration. *Arthropod-Plant Interactions*, **14**, 57–65.

Macfoy, C.C.A. & Dabrowski, Z.T. (1984) Preliminary studies on cowpea resistance to *Aphis craccivora* Koch (Hom., Aphididae). *Zeitschrift für Angewandte Entomologie*, **97**, 202–209.

Machuka, J., Damme, E.J.M. Van, Peumans, W.J. & Jackai, L.E.N. (1999) Effect of plant lectins on larval development of the legume pod borer, *Maruca vitrata*. *Entomologia Experimentalis et Applicata*, **93**, 179–187.

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.

Mba, C. (2013) Induced Mutations Unleash the Potentials of Plant Genetic Resources for Food and Agriculture. *Agronomy*, **3**, 200–231.

Mba, C., Afza, R., Bado, S. & Jain, S.M. (2010) Induced mutagenesis in plants using physical and chemical agents. In *Plant cell culture: essential methods*. pp. 111–130.

McColloch, J. & Salmon, S. (1923) The resistance of wheat to the hessian fly - A progress report. *Journal of Economic Entomology,* **16**, 293-298.

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

Medina-Ortega, K.J. & Walker, G.P. (2015) Faba bean forisomes can function in defence against generalist aphids. *Plant, Cell and Environment*, **38**, 1167–1177.

Mensah, C., DiFonzo, C., Nelson, R.L. & Wang, D. (2005) Resistance to soybean aphid in early maturing soybean germplasm. *Crop Science*, **45**, 2228–2233.

Mewis, I., Appel, H.M., Hom, A., Raina, R. & Schultz, J.C. (2005) Major signaling pathways modulate Arabidopsis glucosinolate accumulation and response to both phloem-feeding and chewing insects. *Plant Physiology*, **138**, 1149–1162.

Michereff, M.F.F., Michereff Filho, M., Blassioli-Moraes, M.C., Laumann, R.A., Diniz, I.R. & Borges, M. (2015) Effect of resistant and susceptible soybean cultivars on the attraction of egg parasitoids under field conditions. *Journal of Applied Entomology*, **139**, 207–216.

Miklas, P.N., Kelly, J.D., Beebe, S.E. & Blair, M.W. (2006) Common bean breeding for resistance against biotic and abiotic stresses: From classical to MAS breeding. *Euphytica*, **147**, 105–131.

Mile, P.W. (1999) Aphid saliva. *Biological Reviews*, **74**, 41–85.

Miles, N., Manson, A.D., Rhodes, R., Antwerpen, R. van & Weigel, A. (2014) Extractable Silicon in Soils of the South African Sugar Industry and Relationships with Crop Uptake. *Communications in Soil Science and Plant Analysis*, **45**, 2949–2958.

Mkenda, P.A., Mtei, K. & Ndakidemi, P.A. (2014) Pesticidal efficacy of Tephrosia vogelii and Tithonia diversifolia against field insect pests of common beans [*Phaseolus vulgaris* L.] within African farming communities. *African Journal of Applied Agricultural Sciences and Technologies*, **2**, 9–26.

Mojica, L. & Mejía, E.G. De. (2015) Characterization and Comparison of Protein and Peptide Profiles and their Biological Activities of Improved Common Bean Cultivars (*Phaseolus vulgaris* L.) from Mexico and Brazil. *Plant Foods Human Nutrition*, **70**, 105–112.

Moraes, J.C., Carvalho, G.A. & Costa, R.R. (2005) Feeding non-preference of the corn leaf aphid *Rhopalosiphum maidis* (Fitch , 1856) (Hemiptera: Aphididae) to corn plants (*Zea mays* L.) treated with silicon. *Ciência e Agrotecnologia*, **29**, 761–766.

Morkunas, I. & Mai, V.C. (2011) Phytohormonal signaling in plant responses to aphid feeding. *Acta Physiologiae Plantarum,* **33**, 2057–2073.

Mungalu, H., Sansala, M., Hamabwe, S., Mukuma, C., Gepts, P., Kelly, J.D. & Kamfwa, K. (2020) Identification of race-specific quantitative trait loci for resistance to Colletotrichum lindemuthianum in an Andean population of common bean. *Crop Science*, **60**, 2843–2856.

Musenga, C., Mwaba, D., Kilubi, V., Bwalya, K., Mweembe, J., Siame, B., Mtawa, M., Kelly, R., Chipuluka, A.K., Chilala, C., Ndalamei, D.M., Muchula, K. & Ntenga, I. (2016) Pest Management Decision Guide: Green and Yellow List, Cowpea aphids on cowpeas. Zambia Agricultural Research Institute (ZARI)-Plantwise [WWW Document]. *CABI*. URL https://www.plantwise.org/KnowledgeBank/pmdg/20177800703 [accessed on 2016].

Mwanauta, R.W., Mtei, K.M. & Ndakidemi, P.A. (2015) Potential of Controlling Common Bean Insect Pests (Bean Stem Maggot (*Ophiomyia phaseoli*), Ootheca (*Ootheca bennigseni*) and Aphids (*Aphis fabae*)) Using Agronomic, Biological and Botanical Practices in field. *Agricultural Sciences*, **6**, 487–497.

Mwangi, S.N., Deng, A.L. & Kamau, A.W. (2009) Response of Kenyan varieties of common bean, *Phaseolus vulgaris* L., to infestation by *Aphis fabae* Scopoli. *African Entomology*, **16**, 196–202.

Mweetwa, A.M., Chilombo, G. & Gondwe, B.M. (2016) Nodulation , Nutrient Uptake and Yield of Common Bean Inoculated with Rhizobia and Trichoderma in an Acid Soil. *Journal of Agricultural Sciences*, **8**, 61–71.

Naessens, E., Dubreuil, G., Giordanengo, P., Baron, O.L., Minet-Kebdani, N., Keller, H. & Coustau, C. (2015) A Secreted MIF Cytokine Enables Aphid Feeding and Represses Plant Immune Responses. *Current Biology*, **25**, 1898–1903.

Naik, K., Mishra, S., Srichandan, H., Singh, P.K. & Sarangi, P.K. (2019) Plant growth promoting microbes: Potential link to sustainable agriculture and environment. *Biocatalysis and Agricultural Biotechnology*, **21**, 101326.

Nakata, Y., Ueno, M., Kihara, J., Ichii, M., Taketa, S. & Arase, S. (2008) Rice blast disease and susceptibility to pests in a silicon uptake-deficient mutant lsi1 of rice. *Crop Protection*, **27**, 865–868.

Nalam, V.J., Louis, J., Patel, M. & Shah, J. (2018) Arabidopsis-green peach aphid interaction: Rearing the insect, no-choice and fecundity assays, and electrical penetration graph technique to study insect feeding behavior. *bio-protocol*, **8**, 1–24.

Nalam, V.J., Louis, J. & Shah, J. (2019) Plant defense against aphids, the pest extraordinaire. *Plant Science*, **279**, 96–107.

Nanayakkara, U.N., Uddin, W. & Datnoff, L.E. (2008) Application of silicon sources increases silicon accumulation in perennial ryegrass turf on two soil types. *Plant and Soil*, **303**, 83–94.

Ndakidemi, B., Mtei, K. & Ndakidemi, P.A. (2016) Impacts of Synthetic and Botanical Pesticides on Beneficial Insects. *Agricultural Sciences*, **07**, 364–372.

Ngalamu, T., Odra, J. & Tongun, N. (2015) *Cowpea Production Handbook*. College of Natural Resources and Environmental Studies, University of Juba, IFS/AGRA, Juba.

Nikolic, M., Nikolic, N., Liang, Y., Kirkby, E.A. & Römheld, V. (2007) Germanium-68 as an adequate tracer for silicon transport in plants. Characterization of silicon uptake in different crop species. *Plant Physiology*, **143**, 495–503.

Nottingham, S.F., Hardie, J., Dawson, G.W., Hick, A.J., Pickett, J.A., Wadhams, L.J. & Woodcock, C.M. (1991) Behavioral and electrophysiological responses of Aphids to host and nonhost plant volatiles. *Journal of Chemical Ecology*, **17**, 1231–1242.

Novak, F.J. & Brunner, H. (1992) Plant breeding: Induced mutation technology for crop improvement. *IAEA Bulletin*, **4**, 24–33.

Nuessly, G.S., Hentz, M.G., Beiriger, R. & Scully, B.T. (2004) Insects Associated With Faba

Bean, *Vicia Faba* (Fabales: Fabaceae), in Southern Florida. *Florida Entomologist*, **87**, 204–211.

Obopile, M. & Ositile, B. (2010) Life table and population parameters of cowpea aphid , *Aphis craccivora* Koch (Homoptera: Aphididae) on five cowpea *Vigna unguiculata* (L. Walp.) varieties. *Journal of Pest Science*, **83**, 9–14.

Ofuya, T.I. (1997) Control of the cowpea aphid, *Aphis craccivora* Koch (Homoptera: Aphididae), in cowpea, *Vigna unguiculata* (L.) Walp. *Integrated Pest Management Reviews*, **2**, 199–207.

Ogenga-Latigo, M.W., Baliddawa, C.W. & Ampofo, J.K.O. (1993) Factors influencing the incidence of the black bean aphid, *Aphis fabae* Scop., on common beans intercropped with maize. *African Crop Science Journal*, **1**, 49–58.

Ogenga-Latigo, M.W. & Khaemba, B.M. (1985) Some aspects of the biology of the black bean aphid *Aphis fabae* Scopoli reared on the common bean *Phaseolus vulgaris* L. *International Journal of Tropical Insect Science*, **6**, 591–593.

Okoth, J.K., Ochola, S.A., Gikonyo, N.K. & Makokha, A. (2017) Development of a nutrient-dense complementary food using amaranth-sorghum grains. *Food Science and Nutrition*, **5**, 86–93.

Oladosu, Y., Rafii, M.Y., Abdullah, N., Hussin, G., Ramli, A., Rahim, H.A., Miah, G. & Usman, M. (2016) Principle and application of plant mutagenesis in crop improvement: A review. *Biotechnology and Biotechnological Equipment*, **30**, 1–16.

Olasupo, F.O., Ilori, C.O., Forster, B.P. & Bado, S. (2018) Selection for Novel Mutations Induced by Gamma Irradiation in Cowpea [*Vigna unguiculata* (L.) Walp.]. *International Journal of Plant Breeding and Genetics*, **12**, 1–12.

Ombakho, G.A., Tyagi, A.P. & Pathak, R.S. (1987) Inheritance of resistance to the cowpea aphid in cowpea. *Theoretical and Applied Genetics*, **74**, 817–819.

Omkar, Mishra, G., Srivastava, S., Gupta, A.K. & Singh, S.K. (2005) Reproductive performance of four aphidophagous ladybirds on cowpea aphid, *Aphis craccivora* Koch. *Journal of Applied Entomology*, **129**, 217–220.

Omoigui, L.O., Ekeuro, G.C., Kamara, A.Y., Bello, L.L., Timko, M.P. & Ogunwolu, G.O. (2017) New sources of aphids [*Aphis craccivora* (Koch)] resistance in cowpea germplasm using phenotypic and molecular marker approaches. *Euphytica*, **213**, 178.

Orawu, M., Melis, R., Laing, M. & Derera, J. (2013) Genetic inheritance of resistance to cowpea aphid-borne mosaic virus in cowpea. *Euphytica*, **189**, 191–201.

Ouédraogo, A.P., Batieno, B.J., Traore, F., Tignegre, J., Huynh, L., Roberts, P.A., Close, T., Ouédraogo, J.T. (2018) Screening of cowpea (*Vigna unguiculata* (L.) Walp.) lines for resistance to three Aphids (*Aphis craccivora* Koch) strains in Burkina Faso, **13**, 1487–1495.

Paes, N.S., Gerhardt, I.R., Coutinho, M. V, Yokoyama, M., Santana, E., Harris, N., Chrispeels, M.J., Grossi, M.F. & Sa, De. (2000) The effect of arcelin-1 on the structure of the midgut of bruchid larvae and immunolocalization of the arcelin protein. *Journal of Insect Physiology*, **46**, 393–402.

Painter, R.H. (1951) *Insect resistance in crop plants*. Macmillan Publishers Limited, New York. pp. 120–126.

Pare, P.W. & Tumlinson, J.H. (1999) Update on Plant-Insect Interactions Plant Volatiles as a Defense against Insect Herbivores. *Plant Physiology*, **121**, 325–331.

Penna, S. & Jain, S.M. (2017) Mutant resources and mutagenomics in crop plants. *Emirates Journal of Food and Agriculture*, **29**, 651–657.

Pereira, R.R.C., Moraes, J.C., Prado, E. & Dacosta, R.R. (2010) Resistance inducing agents on the biology and probing behaviour of the greenbug in wheat. *Scientia Agricola*, **67**, 430–434.

Perrin, R.M. & Phillips, M.L. (1978) Some Effects of Mixed Cropping on the Population Dynamics of Insect Pests. *Entomologia Experimentalis et Applicata*, **24**, 585–593.

Pertot, I., Caffi, T., Rossi, V., Mugnai, L., Hoffmann, C., Grando, M.S., Gary, C., Lafond, D., Duso, C., Thiery, D., Mazzoni, V. & Anfora, G. (2017) A critical review of plant protection tools for reducing pesticide use on grapevine and new perspectives for the implementation of IPM in viticulture. *Crop Protection*, **97**, 70–84.

Peters, W.S., Bel, A.J.E. Van & Knoblauch, M. (2006) The geometry of the forisome-sieve element-sieve plate complex in the phloem of *Vicia faba* L. leaflets. *Journal of Experimental Botany*, **57**, 3091–3098.

Petrescu, A.S., Mondor, E.B. & Roitberg, B.D. (2001) Subversion of alarm communication: Do plants habituate aphids to their own alarm signals? *Canadian Journal of Zoology*, **79**, 737–740.

Pettersson, J., Karunaratne, S., Ahmed, E. & Kumar, V. (1998) The cowpea aphid , *Aphis craccivora*, host plant odours and pheromones. *Entomologia Experimentalis et Applicata*. **88**, 177–184.

Phanchaisri, B., Chandet, R., Yu, L.D., Vilaithong, T., Jamjod, S. & Anuntalabhochai, S. (2007) Low-energy ion beam-induced mutation in Thai jasmine rice (*Oryza sativa* L.) cv. KDML 105. *Surface and Coatings Technology*, **201**, 8024–8028.

Powell, G., Tosh, C.R. & Hardie, J. (2006) Host plant selection by aphids: Behavioral, evolutionary, and applied perspectives. *Annual Review of Entomology*, **51**, 309–330.

Prado, E. & Tjallingii, W.F. (1997) Effects of previous plant infestation on sieve element acceptance by two aphids. *Entomologia Experimentalis et Applicata*, **82**, 189–200.

Prakash, A., Rao, J. & Nandagopal, V. (2008) Future of botanical pesticides in rice, wheat, pulses and vegetables pest management. *Journal of Biopesticides*, **1**, 154–169.

Prasanna, B., Huesing, J.E., Eddy, R. & Peschke, V.M. (2018) Fall armyworm in Africa: A Guide for Integrated Pest Management. USAID, pp. 45–62.

Putra, R., Powell, J.R., Hartley, S.E. & Johnson, S.N. (2020a) Is it time to include legumes in plant silicon research? *Functional Ecology*, **34**, 1–16.

R Core Team. (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Radcliffe, E.B. & Chapman, R. (1966) Varietal resistance to insect attack in various cruciferous crops. *Journal of Economic Entomology*, **59**, 120–125.

Rahbé, Y., Sauvion, N., Febvay, G., Peumans, W. & Gatehouse, A. (1995) Toxicity of lectins and processing of ingested proteins in the pea aphid *Acyrthosiphon pisum*. *Entomologia Experimentalis et Applicata*, **76**, 143–155.

Rahman, A., Wallis, C.M. & Uddin, W. (2015) Silicon-induced systemic defense responses in perennial ryegrass against infection by *Magnaporthe oryzae*. *Phytopathology*, **105**, 748–757.

Rains, D., Epstein, E., Zasoski, R. & Aslam, M. (2006) Active silicon uptake by wheat. *Plant and Soil*, **280**, 223–228.

Ramanujam, B., Poornesha, B., Dileep, R.C. & Japur, K. (2017) Field evaluation of entomofungal pathogens against cowpea aphid ,*Aphis craccivora* Koch , and their effect on two coccinellid predators, **63**, 101–104.

Ramírez-Jiménez, A.K., Reynoso-Camacho, R., Tejero, M.E., León-Galván, F. & Loarca-Piña, G. (2015) Potential role of bioactive compounds of *Phaseolus vulgaris* L. on lipid-lowering mechanisms. *Food Research International*, **76**, 92–104.

Rana, J.S., Khokhar, K.S. & Singh, H. (2001) Influence of Morphological Traits of Plants on the Infestation by Mustard Aphid, *Lipaphis erysimi* (Kalt.) in Rapeseed and Mustard Cultivars. *Indian Journal of Plant Protection*, **29**, 159–160.

Ranger, A.C.M., Singh, A.P., Frantz, J.M. & Locke, J.C. (2019) Influence of Silicon on Resistance of *Zinnia elegans* to *Myzus persicae* (Hemiptera: Aphididae). *Environmental* entomology, **38**, 129–136.

Ratnadass, A., Fernandes, P., Avelino, J. & Habib, R. (2012) Plant species diversity for sustainable management of crop pests and diseases in agroecosystems: A review. *Agronomy for Sustainable Development*, **32**, 273-303.

Raven, J. (2003) Cycling silicon – the role of accumulation in plants. *New Phytologist*, **158**, 419–430.

Ren, J., Zhou, F., Chen, X., Hu, Q., Yang, A., Zhou, J., Shao, J. & Qian, Y. (2014) Effect of physical characteristics of leaf on resistance of cucumber to aphis. *Chinese Journal of Eco-Agriculture*, **22**, 52–57.

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., Johnson, S.N. & Reynolds, O.L. (2016) Silicon: Potential to Promote Direct and Indirect Effects on Plant Defense Against Arthropod Pests in Agriculture. *Frontiers in Plant Science*, **7**, 1–13.

Richmond, K.E. & Sussman, M. (2003) Got silicon? The non-essential beneficial plant nutrient. *Current Opinion in Plant Biology*, **6**, 268–272.

Riddick, E.W. & Simmons, A.M. (2014) Do plant trichomes cause more harm than good to predatory insects? *Pest Management Science*, **70**, 1655–1665.

Rodríguez De Luque, J.J. & Creamer, B. (2014) Major constraints and trends for common bean production and commercialization; establishing priorities for future research. *Agronomía Colombiana*, **32**, 423–431.

Rodriguez, P.A. & Bos, J.I.B. (2013) Toward understanding the role of aphid effectors in plant infestation. *Molecular Plant-Microbe Interactions*, **26**, 25–30.

Ronner, E., Descheemaeker, K., Almekinders, C.J.M., Ebanyat, P. & Giller, K.E. (2018) Farmers' use and adaptation of improved climbing bean production practices in the highlands of Uganda. *Agriculture, Ecosystems and Environment*, **261**, 186–200.

Roubos, C.R., Rodriguez-Saona, C. & Isaacs, R. (2014) Mitigating the effects of insecticides on arthropod biological control at field and landscape scales. *Biological Control*, **75**, 28–38.

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

Ruengphayak, S., Ruanjaichon, V., Saensuk, C., Phromphan, S., Tragoonrung, S., Kongkachuichai, R. & Vanavichit, A. (2015) Forward screening for seedling tolerance to Fe toxicity reveals a polymorphic mutation in ferric chelate reductase in rice. *Rice*, **8**, 1-10.

Saeed, N.A. & Razaq, M. (2014) Effect of sowing dates within a season on incidence and abundance of insect pests of canola crops. *Pakistan Journal of Zoology*, **46**, 1193–1203.

Samireddypalle, A., Boukar, O., Grings, E., Fatokun, C.A., Kodukula, P., Devulapalli, R., Okike, I. & Blümmel, M. (2017) Cowpea and groundnut haulms fodder trading and its lessons for multidimensional cowpea improvement for mixed crop livestock systems in west Africa. *Frontiers in Plant Science*, **8**, 1–9.

Sandström, J. & Pettersson, J. (1994) Amino acid composition of phloem sap and the relation to intraspecific variation in pea aphid (*Acyrthosiphon pisum*) performance. *Journal of Insect Physiology*, **40**, 947–955.

Saranya, S., Ushakumari, R., Jacob, S. & Philip, B.M. (2010) Efficacy of different entomopathogenic fungi against cowpea aphid, *Aphis craccivora* (Koch). *Journal of Biopesticides*, **3**, 138–142.

Sarria, E., Cid, M., Garzo, E. & Fereres, A. (2009) Workbook for automatic parameter calculation of EPG data. *Computers and Electronics in Agriculture*, **67**, 35–42.

Sarwar, M. & Sattar, M. (2013) Varietals variability of winter rapes (*Brassica napus* L.) for their susceptibility to green aphid, *Myzus persicae* (Sulzer) (Homoptera: Aphididae). *Pakistan Journal of Zoology*, **45**, 883–888.

Sasamoto, K. (1958) Studies on the relation between silica content of the rice plant and insect pests on the injury of silicated rice plant caused by the rice-stem-borer and its feeding behaviour. *Journal of Applied Entomology and Zoology*, **2**, 88–92.

Saska, P., Skuhrovec, J., Tylová, E., Platková, H., Tuan, S.J., Hsu, Y.T. & Vítámvás, P. (2020) Leaf structural traits rather than drought resistance determine aphid performance on spring wheat. *Journal of Pest Science*, **94**, 423-434.

Schaub, S., Huber, R. & Finger, R. (2020) Tracking societal concerns on pesticides - A Google Trends analysis. *Environmental Research Letters*, **15**, 084049.

Schoonhoven, A. V., Cardona, C. & Valor, J. (1983) Resistance to the Bean Weevil and the Mexican Bean Weevil (Coleoptera: Bruchidae) in Noncultivated Common Bean

Accessions. *Journal of Economic Entomology*, **76**, 1255–1259.

Seah, S., Telleen, A.C. & Williamson, V.M. (2007) Introgressed and endogenous Mi-1 gene clusters in tomato differ by complex rearrangements in flanking sequences and show sequence exchange and diversifying selection among homologues. *Theoretical and Applied Genetics*, **114**, 1289–1302.

Sharma, H.C. (1998) Bionomics, host plant resistance, and management of the legume pod borer, *Maruca vitrata* - A review. *Crop Protection*, **17**, 373–386.Sharma, S.K., Punam, J. & Chadha, S. (2014) Management of Aphid Pests By Using Organic Inputs in Organically Grown. *International Journal of Agricultural Sciences*, **2**, 2320–3730.

Shedeed, S., Khater, A. & Ali, M. (2018) Silicon-enhanced reduction of some heavy elements accumulation in Cowpea Plants (*Vigna unguiculata* L.). *Journal of Innovations in Pharmaceutical and Biological Sciences*, **5**, 1–10.

Shone, M.G.T. (1964) Initial uptake of silica by excised barley roots. *Nature*, **202**, 314–315.

Sichilima, T., Mapemba, L. & Tembo, G. (2016) Drivers of Dry Common Beans Trade in Lusaka , Zambia : A Trader ' s Perspective. *Sustainable Agriculture Research*, **5**, 15-26.

Siisii, T.Z. & Moonga, M.K. (2015) Pest Management Decision Guide: Green and Yellow List, Black aphids in beans. Zambia Agricultural Research Institute (ZARI)-Plantwise [WWW Document]. *CABI*. URL https://www.plantwise.org/KnowledgeBank/pmdg/20167800130 [accessed on 2015].

Sikombe, F., Lungu, O.I., Munyinda, K. & Sakala, M. (2003) Response of bean (*Phaseolus vulgaris*, L.) cultivars to inoculation and nitrogen fertilizer in Zambia. In *Grain Legumes and Green Manures for Soil Fertility in Southern Africa: Taking Stock of Progress*. Soil Fert Net and CIMMYT-Zimbabwe, Harare, pp. 39–42.

Sileshi, G., Maghembe, J.A., Rao, M.R., Ogol, C.K.P.O. & Sithanantham, S. (2000) Insects feeding on Sesbania species in natural stands and agroforestry systems in southern Malawi. *Agroforestry Systems*, **49**, 41–52.

Silva, L.B., Sales, M.P., Oliveira, A.E.A., Machado, O.L.T., Fernandes, K.V.S. & Xavier-Filho, J. (2004) The seed coat of *Phaseolus vulgaris* interferes with the development of the cowpea weevil [*Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae)]. *Anais da Academia Brasileira de Ciencias*, **76**, 57–65.

Simmons, A.T., McGrath, D. & Gurr, G.M. (2005) Trichome characteristics of F1 *Lycopersicon esculentum* × *L. cheesmanii* and *L. esculentum* × *L. pennellii* hybrids and effects on *Myzus persicae*. *Euphytica*, **144**, 313–320.

Simunji, S., Munyinda, K.L., Lungu, O.I., Mweetwa, A.M. & Phiri, E. (2019) Evaluation of Cowpea (*Vigna unguiculata* L. walp) Genotypes for Biological Nitrogen Fixation in Maize-cowpea Crop Rotation. *Sustainable Agriculture Research*, **8**, 82–93.

Singh, D.P., Sharma, S.P., Lal, M., Ranwah, B.R. & Sharma, V. (2013) Induction of genetic variability for polygenic traits through physical and chemical mutagens in cowpea [*Vigna unguiculata* (L.) walp]. *Legume Research-An International Journal*, **36**, 10–14.

Singh, G. & Singh, R. (2017) Distribution and economic importance of Aphis (*Aphis craccivora* Koch), 1854 (Aphidini: Aphidinae: Aphididae: Hemiptera) and its food plants in India. *International Journal of Recent Advances in Multidisciplinary*, **4**, 2274–2286.

Singh, S.R. (1990) *Insect pests of tropical food legumes*. John Wiley & Sons, Chichester. pp 133-135.

Singh, S.R. & Rachie, K.O. (1985) *Cowpea Research, Production and Utilization*. John Wiley & Sons, Chichester.

Sitko, N.J. & Jayne, T.S. (2014) Structural transformation or elite land capture? The growth of “emergent” farmers in Zambia. *Food Policy*, **48**, 194–202.

Smillie, I., Pyke, K. & Murchie, E. (2012) Variation in vein density and mesophyll cell architecture in a rice deletion mutant population. *Journal of Experimental Botany*, **63**, 4563–4570.

Smith, C.M. & Chuang, W. (2014) Plant resistance to aphid feeding: behavioral, physiological, genetic and molecular cues regulate aphid host selection and feeding. *Pest management science*, **70**, 528–540.

Smith, J.L., Moraes, C.M. De & Mescher, M.C. (2009) Jasmonate- and salicylate-mediated plant defense responses to insect herbivores, pathogens and parasitic plants. *Pest Management Science*, **65**, 497–503.

Snapp, S., Rahmanian, M. & Batello, C. (2018) *Pulse Crops for Sustainable Farms in Sub-Saharan Africa*. United Nations, pp. 102.

Snelling, R.O. (1941) Resistance of plants to insect attack. *The Botanical Review*, **7**, 543–586.

Soffan, A. & Aldawood, A.S. (2014) Biology and Demographic Growth Parameters of Cowpea Aphid (*Aphis craccivora*) on Faba Bean (*Vicia faba*) Cultivars. *Journal of Insect Science*, **14**, 1–10.

Song, J.Y., Kim, D.S., Lee, M.C., Lee, K.J., Kim, J.B., Kim, S.H., Ha, B. K., Yun, S.J. & Kang, S. Y. (2012) Physiological characterization of gamma-ray induced salt tolerant rice mutants. *Australian Journal of Crop Science*, **6**, 421–429.

Song, X.P., Verma, K.K., Tian, D.D., Zhang, X.Q., Liang, Y.J. & Huang, X. (2021) Exploration of silicon functions to integrate with biotic stress tolerance and crop improvement. *Biological Research*, **54**, 1–12.

Souleymane, A., Ova Aken, M.E., Fatokun, C.A. & Alabi, O.Y. (2013) Screening for resistance to cowpea aphid (*Aphis craccivora* Koch) in wild and cultivated cowpea (*Vigna unguiculata* L. Walp.) accessions. *International Journal of Science, Environment and Technology*, **2**, 611–621.

Spencer, N.J. & Walkey, D.G.A. (1994) *Bean Common Mosaic Virus and related viruses in Africa*. Kent. pp. 109–111.

Srikanth, J. & Lakkundi, N.H. (1988) Instar period, fecundity and longevity of cowpea aphid, *Aphis craccivora* Koch. on seven leguminous hosts. *Journal of Aphidology*, **2**, 18–21.

Stanley, J.N., Baqir, H.A. & Mclaren, T.I. (2014) Effect on larval growth of adding finely ground silicon-bearing minerals (wollastonite or olivine) to artificial diets for *Helicoverpa spp*. (Lepidoptera: Noctuidae). *Austral Entomology*, **53**, 436–443.

Stewart, S.A., Hodge, S., Ismail, N., Mansfield, J.W., Feys, B.J., Prospéri, J.M., Huguet, T., Ben, C., Gentzbittel, L., Powell, G. (2009) The RAP1 gene confers effective, race-specific resistance to the pea aphid in *Medicago truncatula* independent of the hypersensitive reaction. *Molecular Plant-Microbe Interactions*, **22**, 1645–1655.

Stout, M.J. (2013) Reevaluating the conceptual framework for applied research on host-plant resistance. *Insect Science*, **20**, 263–272.

Stout, M.J., Workman, J. & Duffey, S.S. (1994) Differential induction of tomato foliar proteins by arthropod herbivores. *Journal of Chemical Ecology*, **20**, 2575–2594.

Sullivan, D.J. (2004) Aphids. In *Encyclopedia of Entomology*. Springer, Dordrecht. pp. 70–83.

Sun, H., Duan, Y., Jing, N.M., Liu, J., Guo, J., Feng, J. & Gong, H. (2020) Tomato roots have a functional silicon influx transporter but not a functional silicon efflux transporter. *Plant Cell Environment*, **43**, 732–744.

Taiwo, M.A., Kareem, K.T., Nsa, I.Y. & D'A Hughes, J. (2007) Cowpea viruses: Effect of single and mixed infections on symptomatology and virus concentration. *Virology Journal*, **4**, 1–5.

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

Tatchell, G.M. (1985) Aphid-control advice to farmers and the use of aphid-monitoring data. *Crop Protection*, **4**, 39–50.

Tembo, L., Namebo, M., Chanda, R., Kafwa, K. & Munyindaq, K. (2019) Genotypic Variation for Response to Phosphorus Fertilization in Common Bean Mutants. *Canadian Journal of Agriculture and Crops*, **4**, 11–16.

Tembo, L., Pungulani, L., Sohati, P., Mataa, J. & Kalaluka, M. (2017) Resistance to *Callosobruchus maculatus* Developed Via Gamma Radiation in Cowpea, *Journal of Agriculture and Crops*, **3**, 65–71.

Thieme, T. & Heimbach, U. (1996) Development and reproductive potential of cereal aphids (Homoptera: Aphididae) on winter wheat cultivars. *Bulletin OILB SROP*, **19**, 1–8.

Tjallingii, W.F. (1978) Electronic recording of penetration behaviour by aphids. *Entomologia experimentalis et applicata*, **24**, 721–730.

Tjallingii, W.F. (2006) Salivary secretions by aphids interacting with proteins of phloem wound responses. *Journal of Experimental Botany*, **57**, 739–745.

Tomanović, Ž., Athanassiou, L., Kavallieratos, N.G. & Athanassiou, C.G. (2003) A review of the West Palaearctic aphidiines (Hymenoptera: Braconidae: Aphidiinae) parasitic on *Uroleucon spp*., with the description of a new species. *Annales de la Societe Entomologique de France*, **39**, 343–353.

Tu, Y., Jiang, A., Gan, L., Hossain, M., Zhang, J., Peng, B., Xiong, Yuguo, S., Zhaojian, C.D., Xu, Weifeng, Z. & Jianhua, H.Y. (2014) Genome duplication improves rice root resistance to salt stress. *Rice*, **7**, 1–13.

Tulmann Neto, A., Ando, A., Figueira, A., Latado, R.R., Santos, P.C., Correa, L.S., Peres, L.E.P., Hauagge, R., Pulcinelli, C.E., Ishiy, T. & Ferreira, F. (2011) Genetic improvement of crops by mutation techniques in Brazil. *Plant Mutation Reports*, **2**, 24–37.

Vandenborre, G., Smagghe, G. & Damme, E.J.M. Van. (2011) Plant lectins as defense proteins against phytophagous insects. *Phytochemistry*, **72**, 1538–1550.

Verma, K.K., Song, X., Tian, D., Guo, D., Chen, Z., Zhong, C., Nikpay, A., Singh, M.R., Vishnu, D., Singh, R.K., Minkina, T. & Li, Y. (2021) Influence of Silicon on Biocontrol Strategies to Manage Biotic Stress for Crop Protection , Performance , and Improvement. *Plants*, **10**, 1–22.

Viana, V.E., Pegoraro, C., Busanello, C. & Costa de Oliveira, A. (2019) Mutagenesis in Rice: The Basis for Breeding a New Super Plant. *Frontiers in Plant Science,* **10**, 1–28.

Voss-Fels, K.P., Stahl, A. & Hickey, L.T. (2019) Q&A: Modern crop breeding for future food security. *BMC Biology*, **17**, 1–7.

Wainaina, J.M., Kubatko, L., Harvey, J., Ateka, E., Makori, T., Karanja, D., Boykin, L.M. & Kehoe, M.A. (2019) Evolutionary insights of Bean common mosaic necrosis virus and Cowpea aphid- borne mosaic virus. *PeerJ*, **7**, e6297.

Wang, S.Y., Qi, Y.F., Desneux, N., Shi, X.Y., Biondi, A. & Gao, X.W. (2017) Sublethal and transgenerational effects of short-term and chronic exposures to the neonicotinoid nitenpyram on the cotton aphid *Aphis gossypii*. *Journal of Pest Science*, **90**, 389–396.

Wang, X., Gao, Y., Chen, Z., Li, J., Huang, J., Cao, J., Cui, M. & Ban, L. (2019) (E)-β-farnesene synthase gene affects aphid behavior in transgenic *Medicago sativa*. *Pest Management Science*, **75**, 622–631.

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 Signaling and Behavior*, **7**, 1306–1320.

Ward, S.A., Leather, S.R., Pickup, J. & Harrington, R. (1998) Mortality during dispersal and the cost of host specificity in parasites: How many aphids find hosts? *Journal of Animal Ecology*, **67**, 763–773.

Webster, B., Bruce, T., Dufour, S., Birkemeyer, C., Birkett, M., Hardie, J. & Pickett, J. (2008) Identification of volatile compounds used in host location by the black bean aphid, *Aphis fabae*. *Journal of Chemical Ecology*, **34**, 1153–1161.

Webster, B., Bruce, T., Pickett, J. & Hardie, J. (2010) Volatiles functioning as host cues in a blend become nonhost cues when presented alone to the black bean aphid. *Animal Behaviour*, **79**, 451–457.

Webster, J.A., Inayatullah, C., Hamissou, M. & Mirkes, K.A. (1994) Leaf pubescence effects in wheat on yellow sugarcane aphids and greenbugs (Homoptera: Aphididae). *Journal of Economic Entomology*, **87**, 231–240.

Weibull, J. (1994) Glutamic acid content of phloem sap is not a good predictor of plant resistance to *Rhopalosiphum padi*. *Phytochemistry*, **33**, 601–602.

Westwood, J.H. & Stevens, M. (2010) Resistance to Aphid Vectors of Virus Disease. Elsevier Inc., Cambridge, pp. 179–201.

White, C. & Eigenbrode, S.D. (2000) Effects of surface wax variation in *Pisum sativum* on herbivorous and entomophagous insects in the field. *Environmental Entomology*, **29**, 773–780.

Wilkinson, T.L. & Douglas, A.E. (2003) Phloem amino acids and the host plant range of the polyphagous aphid, *Aphis fabae*. *Entomologia Experimentalis et Applicata*, **106**, 103–113.

Will, T., Furch, A.C.U. & Zimmermann, M.R. (2013) How phloem-feeding insects face the challenge of phloem-located defenses. *Frontiers in Plant Science*, **4**, 1–12.

Worrall, E.A., Wamonje, F.O., Mukeshimana, G., Harvey, J.J.W., Carr, J.P. & Mitter, N. (2015) Bean Common Mosaic Virus and Bean Common Mosaic Necrosis Virus: Relationships, Biology, and Prospects for Control. In *Advances in Virus Research*. pp. 1–46.

Wroblewski, T., Piskurewicz, U., Tomczak, A., Ochoa, O. & Michelmore, R.W. (2007) Silencing of the major family of NBS-LRR-encoding genes in lettuce results in the loss of multiple resistance specificities. *Plant Journal*, **51**, 803–818.

Wyatt, I.J. & White, P.F. (1977) Simple estimation of intrinsic increase rates for aphids and tetranychid mites. *Journal of Applied Ecology*, **14**, 757–766.

Xing, Z., Liu, Y., Cai, W., Huang, X., Wu, S. & Lei, Z. (2017) Efficiency of trichome-based plant defense in *Phaseolus vulgaris* depends on insect behavior, plant ontogeny, and structure. *Frontiers in Plant Science*, **8**, 1–8.

Xu, J., Shi, S., Wang, L., Tang, Z., Lv, T., Zhu, X., Ding, X., Wang, Y.Z., Fang, J. & Wu, Z. (2017) OsHAC4 is critical for arsenate tolerance and regulates arsenic accumulation in rice. *New Phytologist*, **215**, 1090–1101.

Yan, G. chao, Nikolic, M., YE, M. jun, Xiao, Z. & Liang, Y. (2018) Silicon acquisition and accumulation in plant and its significance for agriculture. *Journal of Integrative Agriculture*, **17**, 2138–2150.

Yang, J., Song, J. & Byoung, R. (2022) Drenched Silicon Suppresses Disease and Insect Pests in Coffee Plant Grown in Controlled Environment by Improving Physiology and Upregulating Defense Genes. *International Journal of Molecular Sciences*, **23**, 3543.

Yates, A.D. & Michel, A. (2018) Mechanisms of aphid adaptation to host plant resistance. *Current Opinion in Insect Science,* **26**, 41–49.

Ye, M., Song, Y., Long, J., Wang, R., Baerson, S.R., Pan, Z. & Zhu-salzman, K. (2013) Priming of jasmonate-mediated antiherbivore defense responses in rice by silicon. *Proceedings of the National Academy of Sciences*, **110**, 3631-3639.

Yuan, L., Bao, Z., Zhang, H., Zhang, Y. & Liang, X. (2017) Habitat monitoring to evaluate crop disease and pest distributions based on multi-source satellite remote sensing imagery. *Optik*, **145**, 66–73.

Zadda, K., Ragjendran, R. & Vijayaraghavan, C. (2007) Induced systemic resistance to major insect pests of brinjal through organic farming. *Crop Research (Hisar)*, **34**, 125–129.

Zaidi, S.S.E.A., Vanderschuren, H., Qaim, M., Mahfouz, M.M., Kohli, A., Mansoor, S. & Tester, M. (2019) New plant breeding technologies for food security. *Science*, **363**, 1390–1391.

Zehnder, G., Gurr, G.M., Kühne, S., Wade, M.R., Wratten, S.D. & Wyss, E. (2007) Arthropod Pest Management in Organic Crops. *Annual Review of Entomology*, **52**, 57–80.

Zhaozhi, L., Likai, F., Guizhen, G., Ling-Ling, G., Han, P., Sharma, S. & Zalucki, M.P. (2017) Differences in the high-temperature tolerance of *Aphis craccivora* (Hemiptera: Aphididae) on cotton and soybean: implications for ecological niche switching among hosts. *Applied Entomology and Zoology*, **52**, 9–18.

Zhu-Salzman, K. & Zeng, R. (2015) Insect Response to Plant Defensive Protease Inhibitors. *Annual Review of Entomology*, **60**, 233–252.

Zimba, K.J., Sohati, P.H., Munyinda, K., Kamfwa, K., Roberts, J.M. & Pope, T.W. (2022a) Evaluation of resistance to black bean aphid (*Aphis fabae*) in selected varieties and mutant genotypes of common bean (*Phaseolus vulgaris*). *Annals of Applied Biology*, **181**, 298–308.

Zimba, K.J., Sohati, P.H., Munyinda, K., Roberts, J.M. & Pope, T.W. (2022b) Induced mutagenesis: An underutilised component in the integrated management of aphid pests in sub-Saharan Africa. *Crop Protection*, **159**, 106030.

Zimba, K.J., Sohati, P.H., Munyinda, K., Roberts, J.M. & Pope, T.W. (2022c) Gamma irradiation as a tool to produce cowpea (*Vigna unguiculata* (L.) Walp.) genotypes resistant to aphid pests. *Arthropod-Plant Interactions*, **16**, 665–675.

Züst, T. & Agrawal, A.A. (2016) Mechanisms and evolution of plant resistance to aphids. *Nature Plants*, **2**, 1–9.

8 APPENDICES

8.1 Section 3.1

8.1.1 Analysis of Variance (ANOVA) for assessment of nymph development (Days) on common bean varieties

```
Analysis of Variance Table
Response: Days
          Df Sum Sq Mean Sq F value    Pr(>F)
Line       5 129.68 25.9367  12.584 4.081e-08 ***
Residuals 54 111.30  2.0611
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

8.1.2 Mean separation (Holm-Sidak test) for assessment of nymph development (Days) on common bean varieties

```
P value adjustment: sidak method for 15 tests
 Line          emmean    SE df lower.CL upper.CL .group
 ADP 684          9.4 0.454 54     8.16     10.6  a
 ADP 466          9.6 0.454 54     8.36     10.8  ab
 ADP 1            9.7 0.454 54     8.46     10.9  ab
 Kabulangeti     11.2 0.454 54     9.96     12.4  ab
 ADP 765         11.4 0.454 54    10.16     12.6   b
 AO 1012-29-3A   13.6 0.454 54    12.36     14.8    c

Confidence level used: 0.95
Conf-level adjustment: sidak method for 6 estimates
P value adjustment: sidak method for 15 tests
significance level used: alpha = 0.05
```

8.1.3 ANOVA for assessment of nymph development (Days) on mutation-derived common bean lines

```
Analysis of Variance Table
Response: Days
          Df Sum Sq Mean Sq F value   Pr(>F)
Line       4  43.48 10.8700  4.2646 0.005196 **
Residuals 45 114.70  2.5489
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

8.1.4 Mean separation (Holm-Sidak test) for assessment of nymph development (Days) on mutation-derived common bean lines

```
P value adjustment: sidak method for 10 tests
 Line  emmean    SE df lower.CL upper.CL .group
 CA 3    11.3 0.505 45     9.95     12.7  a
 CA 15   11.5 0.505 45    10.15     12.9  a
 CA 24   12.4 0.505 45    11.05     13.8  ab
 CA      13.2 0.505 45    11.85     14.6  ab
 CA 38   13.7 0.505 45    12.35     15.1   b

Confidence level used: 0.95
Conf-level adjustment: sidak method for 5 estimates
P value adjustment: sidak method for 10 tests
significance level used: alpha = 0.05
```

8.1.5 ANOVA for determination of hooked-trichome density (TD) on common bean varieties

```
Analysis of Variance Table
Response: TD
          Df Sum Sq Mean Sq F value   Pr(>F)
Gen        5 2600.8  520.17  19.985 8.89e-07 ***
Residuals 18  468.5   26.03
---
```

```
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

8.1.6 Mean separation (Holm-Sidak test) for determination of hooked-trichome density on common bean varieties

```
P value adjustment: sidak method for 15 tests
 Gen           emmean   SE df lower.CL upper.CL .group
 AO-1012-29-3A    9.0 2.55 18     1.47     16.5  a
 Kabulangeti     13.5 2.55 18     5.97     21.0  a
 ADP 1           17.5 2.55 18     9.97     25.0  a
 ADP 684         20.8 2.55 18    13.22     28.3  ab
 ADP 765         30.5 2.55 18    22.97     38.0   bc
 ADP 466         39.8 2.55 18    32.22     47.3    c

Confidence level used: 0.95
Conf-level adjustment: sidak method for 6 estimates
P value adjustment: sidak method for 15 tests
significance level used: alpha = 0.05
```

8.1.7 ANOVA for determination of hooked-trichome density (TC) on mutation derived common bean lines

```
Analysis of Variance Table
Response: TC
          Df Sum Sq Mean Sq F value  Pr(>F)
Gen        4 202.80  50.700  3.6962 0.02754 *
Residuals 15 205.75  13.717
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

8.1.8 Mean separation (Holm-Sidak test) for determination of hooked-trichome density on mutation-derived common bean lines

```
P value adjustment: sidak method for 10 tests
 Gen   emmean   SE df lower.CL upper.CL .group
 CA 24   12.5 1.85 15     7.06     17.9  a
 CA 38   13.8 1.85 15     8.31     19.2  ab
 CA 15   14.5 1.85 15     9.06     19.9  ab
 CA 3    17.0 1.85 15    11.56     22.4  ab
 CA      21.5 1.85 15    16.06     26.9   b

Confidence level used: 0.95
Conf-level adjustment: sidak method for 5 estimates
P value adjustment: sidak method for 10 tests
significance level used: alpha = 0.05
```

8.1.9 ANOVA for determination of glandular-trichome density (TD) on common bean varieties

```
Analysis of Variance Table

Response: TD
          Df Sum Sq Mean Sq F value    Pr(>F)
Gen        5 36.875  7.3750  7.9254 0.0004258 ***
Residuals 18 16.750  0.9306
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

8.1.10 Mean separation (Holm-Sidak test) for determination of glandular-trichome density on common bean varieties

```
P value adjustment: sidak method for 15 tests
 Gen           emmean    SE df lower.CL upper.CL .group
 AO-1012-29-3A   0.25 0.482 18  -1.1743     1.67  a
```

```
 ADP 1           1.50 0.482 18   0.0757     2.92  ab
 ADP 466         2.25 0.482 18   0.8257     3.67  ab
 ADP 765         2.75 0.482 18   1.3257     4.17   b
 ADP 684         3.75 0.482 18   2.3257     5.17   b
 Kabulangeti     3.75 0.482 18   2.3257     5.17   b

Confidence level used: 0.95
Conf-level adjustment: sidak method for 6 estimates
P value adjustment: sidak method for 15 tests
significance level used: alpha = 0.05
```

8.1.11 ANOVA for determination of glandular-trichome density (TC) on mutation derived common bean lines

```
Analysis of Variance Table
Response: TC
          Df Sum Sq Mean Sq F value  Pr(>F)
Gen        4  70.20 17.5500  2.8383 0.06179 .
Residuals 15  92.75  6.1833
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

8.1.12 ANOVA for Settling preference of alate aphids on bean genotypes after 24 hours

```
Analysis of Variance Table
Response: Count
          Df  Sum Sq Mean Sq F value Pr(>F)
Line      10  220.36  22.036  1.1231 0.3551
Residuals 84 1648.22  19.622
```

8.1.13 ANOVA for Settling preference of alate aphids on bean genotypes after 48 hours

```
Analysis of Variance Table
```

```
Response: Count
          Df  Sum Sq Mean Sq F value Pr(>F)
Gen       10  254.48  25.448  1.2188 0.2903
Residuals 88 1837.36  20.879
```

8.1.14 ANOVA for Settling preference of alate aphids on bean genotypes after 72 hours

```
Analysis of Variance Table
Response: Count
          Df  Sum Sq Mean Sq F value Pr(>F)
Gen       10  204.16  20.416  1.1821 0.3138
Residuals 88 1519.84  17.271
```

8.1.15 ANOVA for assessment of nymph survival (Surv.) on common bean varieties

```
Analysis of Variance Table
Response: Surv
          Df  Sum Sq Mean Sq F value Pr(>F)
Line       5  2168.3  433.67  1.4167 0.2331
Residuals 54 16530.0  306.11
```

8.1.16 ANOVA for assessment of nymph survival (Surv.) on mutation-derived common bean lines

```
Analysis of Variance Table
Response: Surv
          Df Sum Sq Mean Sq F value    Pr(>F)
Line       4   7612 1903.00  7.0949 0.0001617 ***
Residuals 45  12070  268.22
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

8.1.17 Mean separation (Holm-Sidak test) for assessment of nymph survival (Surv.) on mutation-derived common bean lines

```
P value adjustment: sidak method for 10 tests
 Line   emmean   SE df lower.CL upper.CL .group
 CA         33 5.18 45     19.1     46.9  a
 CA 3       42 5.18 45     28.1     55.9  a
 CA 24      49 5.18 45     35.1     62.9  ab
 CA 15      53 5.18 45     39.1     66.9  ab
 CA 38      70 5.18 45     56.1     83.9   b

Confidence level used: 0.95
Conf-level adjustment: sidak method for 5 estimates
P value adjustment: sidak method for 10 tests
significance level used: alpha = 0.05
```

8.1.18 ANOVA for monitoring of aphid feeding behaviour (tim.) on common bean varieties

```
Analysis of Variance Table
Response: tim
          Df  Sum Sq Mean Sq F value   Pr(>F)
Gen        5  6213.5 1242.70  4.4386 0.001851 **
Residuals 54 15118.9  279.98
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

8.1.19 Mean separation (Holm-Sidak test) for monitoring of aphid feeding behaviour (tim.) on common bean varieties

```
 P value adjustment: sidak method for 15 tests
 Gen           emmean   SE df lower.CL upper.CL .group
 AO 1012-29-3A   4.90 5.58 54  -10.332     20.1  a
 Kabulangeti     7.07 6.32 54  -10.200     24.3  a
```

```
 ADP 1           7.65 5.05 54   -6.123     21.4  a
 ADP 765        10.08 4.64 54   -2.589     22.8  a
 ADP 466        13.28 5.05 54   -0.496     27.1  a
 ADP 684        36.46 5.58 54   21.224     51.7   b

Confidence level used: 0.95
Conf-level adjustment: sidak method for 6 estimates
P value adjustment: sidak method for 15 tests
significance level used: alpha = 0.05
```

8.2 Section 3.2

8.2.1 ANOVA of cowpea aphid colony growth (Num.) on mutation derived Bubebe genotypes

```
Kruskal-Wallis rank sum test
data:  Num by Line
Kruskal-Wallis chi-squared = 19.687, df = 6, p-value = 0.003148
```

8.2.2 ANOVA of cowpea aphid colony growth (Num.) on mutation derived Lutembwe genotypes

```
Kruskal-Wallis rank sum test
data:  Num by Line
Kruskal-Wallis chi-squared = 18.164, df = 3, p-value = 0.000407
```

8.2.3 Mean separation (Holm-Sidak test) of cowpea aphid colony growth (Num.) on mutation derived Lutembwe genotypes

```
P value adjustment: sidak method for 6 tests
 Line emmean   SE df lower.CL upper.CL .group
 LT11   32.1 9.75 28     6.18     58.1  a
 LT3    46.4 9.75 28    20.43     72.3  a
 LT4    49.0 9.75 28    23.05     74.9  a
```

```
 LT    151.0 9.75 28   125.05    176.9   b

Confidence level used: 0.95
Conf-level adjustment: sidak method for 4 estimates
P value adjustment: sidak method for 6 tests
significance level used: alpha = 0.05
```

8.2.4 ANOVA of duration of the first aphid probe on mutation derived Lutembwe genotypes

```
Analysis of Variance Table
Response: prb
          Df Sum Sq Mean Sq F value  Pr(>F)
Line       3  18721  6240.5  3.1188 0.03244 *
Residuals 61 122055  2000.9
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

8.2.5 ANOVA of pathway phase (duration until first phloem puncture) on mutation derived Lutembwe genotypes

```
Kruskal-Wallis rank sum test

data:  tim by Gen
Kruskal-Wallis chi-squared = 22.178, df = 3, p-value = 5.989e-05
```

8.2.6 Mean separation of pathway phase (duration until first phloem puncture) on mutation derived Lutembwe genotypes

```
P value adjustment: sidak method for 6 tests
 Gen   emmean   SE df lower.CL upper.CL .group
 LTP    2.33 9.82 38   -23.35     28.0  a
 LT3   22.56 7.70 38     2.42     42.7  a
 LT11  32.19 8.38 38    10.29     54.1  ab
 LT4   56.57 8.78 38    33.60     79.5   b
```

```
Confidence level used: 0.95
Conf-level adjustment: sidak method for 4 estimates
P value adjustment: sidak method for 6 tests
     significance level used: alpha = 0.05
```

8.2.7 Mean separation (Holm-Sidak test) of cowpea aphid colony growth (Num.) on mutation derived Lutembwe genotypes, at UNZA-Agricultural Technology Demonstration Centre

```
P value adjustment: sidak method for 6 tests
 Gen  emmean   SE df lower.CL upper.CL .group
 LT4    13.2 10.1 92   -12.43     38.8  a
 LT3    17.0 10.1 92    -8.64     42.6  a
 LT11   29.6 10.1 92     3.99     55.2  a
 LTP    78.7 10.1 92    53.11    104.3   b

Confidence level used: 0.95
Conf-level adjustment: sidak method for 4 estimates
P value adjustment: sidak method for 6 tests
significance level used: alpha = 0.05
```

8.2.8 Mean separation (Holm-Sidak test) of cowpea aphid colony growth (Num.) on mutation derived Lutembwe genotypes, at UNZA-Liempe farm

```
P value adjustment: sidak method for 6 tests
 Gen  emmean   SE df lower.CL upper.CL .group
 LT3     109 17.2 92     65.1      153  a
 LT11    114 17.2 92     70.6      158  a
 LT4     115 17.2 92     70.8      158  a
 LTP     211 17.2 92    167.0      255   b

Confidence level used: 0.95
Conf-level adjustment: sidak method for 4 estimates
P value adjustment: sidak method for 6 tests
```

```
     significance level used: alpha = 0.05
```

8.2.9 Mean separation (Holm-Sidak test) of cowpea aphid colony growth (Num.) on mutation derived Lutembwe genotypes, at UNZA-Field Research Station

```
P value adjustment: sidak method for 6 tests
 Gen  emmean   SE df lower.CL upper.CL .group
 LT11   22.0 7.64 92     2.64     41.4  a    
 LT3    23.4 7.64 92     3.97     42.8  a    
 LT4    32.4 7.64 92    13.01     51.8  a    
 LTP    61.6 7.64 92    42.18     81.0   b   

Confidence level used: 0.95 
Conf-level adjustment: sidak method for 4 estimates 
P value adjustment: sidak method for 6 tests 
significance level used: alpha = 0.05
```

8.3 Section 3.3

8.3.1 Welch Two Sample t-test of silicon accumulation capacity of LT-11-3-3-12 genotype

```
Welch Two Sample t-test
data:  ppm by Gen
t = -6.1833, df = 2.2113, p-value = 0.01962
alternative hypothesis: true difference in means is not equal to
0
95 percent confidence interval:
 -0.020666422 -0.004600245
sample estimates:
mean in group L11C mean in group L11T 
        0.02830000         0.04093333
```

8.3.2 Welch Two Sample t-test of silicon accumulation capacity of Lutembwe genotype

```
Welch Two Sample t-test
data:  ppm by Gen
t = -3.9087, df = 2.3346, p-value = 0.04618
alternative hypothesis: true difference in means is not equal to
0
95 percent confidence interval:
 -0.0334951352 -0.0006381981
sample estimates:
mean in group LPC mean in group LPT
       0.03220000        0.04926667
```

8.3.3 Welch Two Sample t-test of laboratory assessment of aphid colony growth on Lutembwe genotype

```
Welch Two Sample t-test
data:  Num by Gen
t = 2.3025, df = 17.561, p-value = 0.03378
alternative hypothesis: true difference in means is not equal to
0
95 percent confidence interval:
   4.519403 100.680597
sample estimates:
mean in group LPC mean in group LPT
            106.3              53.7
```

8.3.4 Welch Two Sample t-test of nymph mean relative growth rate on Lutembwe genotype

```
Welch Two Sample t-test
data:  GR by Line
t = 3.0091, df = 14.926, p-value = 0.008848
```

```
alternative hypothesis: true difference in means is not equal to
0
95 percent confidence interval:
 0.01528125 0.08961675
sample estimates:
mean in group LPC mean in group LPT
         0.193816          0.141367
```

8.3.5 Wilcoxon rank sum exact test of aphid phloem ingestion duration (t) on Si- and Si+ Lutembwe plants

```
Wilcoxon rank sum exact test
data:  t by Tret
W = 15, p-value = 0.03571
alternative hypothesis: true location shift is not equal to 0
```

8.3.6 Welch Two Sample t-test of aphid pathway duration on Si- and Si+ plants of LT 4-2-4-1 genotype

```
Welch Two Sample t-test
data:  Pth by Trt
t = -3.206, df = 16.804, p-value = 0.005241
alternative hypothesis: true difference in means is not equal to
0
95 percent confidence interval:
 -374.09982  -76.98244
sample estimates:
mean in group Si- mean in group Si+
         374.1410      599.6822
```

8.3.7 Wilcoxon rank sum exact test of 1st stylet probe duration on Si+ and Si- plants of LT 4-2-4-1 genotype

```
Wilcoxon rank sum test with continuity correction
```

```
data:  DFP by Trt
W = 13.5, p-value = 0.01133
alternative hypothesis: true location shift is not equal to 0
```

8.3.8 Welch Two Sample t-test of Aphid colony growth on Si+ and Si- plants of Lutembwe genotype in field plot A

```
Welch Two Sample t-test
data:  Num by Treat
t = 2.5161, df = 18.028, p-value = 0.02156
alternative hypothesis: true difference in means is not equal to
0
95 percent confidence interval:
  11.14421 123.85579
sample estimates:
mean in group LTC mean in group LTT
         109.5625           42.0625
```

8.3.9 Welch Two Sample t-test of Aphid colony growth on Si+ and Si- plants of Lutembwe genotype in field plot B

```
Welch Two Sample t-test
data:  Num by Treat
t = 2.362, df = 18.708, p-value = 0.02918
alternative hypothesis: true difference in means is not equal to
0
95 percent confidence interval:
   6.623345 110.662369
sample estimates:
mean in group LTC mean in group LTT
        110.85714          52.21429
```

8.4 Journal publications

Zimba, K. J., Sohati, P. H., Munyinda, K., Roberts, J. M., & Pope, T. W. (2022). Induced mutagenesis: An underutilised component in the integrated management of aphid pests in sub-Saharan Africa. *Crop Protection*, *159*, 106030. https://doi.org/10.1016/j.cropro.2022.106030

Zimba, K. J., Sohati, P. H., Munyinda, K., Kamfwa, K., Roberts, J. M., & Pope, T. W. (2022). Evaluation of resistance to black bean aphid (*Aphis fabae*) in selected varieties and mutant genotypes of common bean (*Phaseolus vulgaris*). *Annals of Applied Biology*, *181*(3), 298-308. https://doi.org/10.1111/aab.12776

Zimba, K. J., Sohati, P. H., Munyinda, K., Roberts, J. M., & Pope, T. W. (2022). Gamma irradiation as a tool to produce cowpea (*Vigna unguiculate* (L.) Walp.) genotypes resistant to aphid pests. *Arthropod-Plant Interactions*, *16*(6), 665-675. https://doi.org/10.1007/s11829-022-09919-8

Zimba, K. J., Sohati, P. H., Munyinda, K., Roberts, J. M., & Pope, T. W. Can silicon complement mutation-derived resistance to cowpea aphid? (Submitted to the Journal of Pest Science).