The possible effect of insecticide drift from citrus orchards, and acute toxicity of insecticides on the biocontrol agents of *Pontederia crassipes* (Mart.) Solms-Laub (Pontederiaceae) established along citrus orchards in the Lowveld region of Mpumalanga Province, South Africa

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

MASTER OF SCIENCE

By

Mefika Michael Mabuza

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RHODES UNIVERSITY
Where leaders learn

DECLARATION

I Mefika Michael Mabuza proclaim that this research is my original work. The information and materials that has been used from other sources have been acknowledged fully. This dissertation has not been submitted for any degree or for examination at this or any other institution for a formal degree. The reported results were not presented in this or any other form elsewhere in the world.

Supervisor: Professor Martin Hill

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ABSRACT

This study investigated the possible effect of insecticide drift on naturalized biological control agents of *Pontederia crassipes* (Mart.) Solms-Laub (Pontederiaceae), in the Lowveld region of Mpumalanga Province of South Africa. Occurrence and abundance of biocontrol agents were recorded at three sites on the Crocodile River and at three dams adjacent to citrus orchards. Leaves of *P. crassipes* and water samples were collected for insecticide residues and also nutrient status of the water and plants. *Eccritotarsus catarinensis* Carvalho (Hemiptera: Miridae), *Neochetina* spp. (combined) **(**Coleoptera: Curculionidae), and *Orthogalumna terebrantis* Wallwork (Sarcoptiformes: Galumnidae) were recorded with notable variation in abundance between the river and dams across regions. Insecticide residues were not detected on all leaves sampled across study regions, however, nutrients were detected with nitrate ranging between oligotrophic and mesotrophic. Phosphorus was also detected, but, neither of the nutrients correlated with the occurrence and abundance of naturalized biological control agents of *P. crassipes*.

Bioassays were conducted to measure the effect of commonly used insecticides (*viz*. Methomyl and Chlorpyrifos) on the survival and feeding damage of biological control agents of *P. crassipes*. Survival of individual insects was recorded between 0.5 and 120 hours for *Megamelus scutellaris* and *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae) adults for treatments where insecticides were topically applied onto the insects or leaves were dipped into the pesticides. Concentrations below field rates, recommended and above field rates of Methomyl and Chlorpyrifos on either exposure techniques significantly reduced survival and feeding of biocontrol agents. Methomyl was more toxic compared to Chlorpyrifos and it significantly reduced the survival of *M. scutellaris* and *N. eichhorniae*.

In conclusion, in this study, population abundance of biocontrol agents of *P. crassipes* at the Lowveld region of Mpumalanga was not influenced by pesticide drift, but, insecticides commonly used in the citrus orchards has the potential to negatively impact naturalized biological control of *P. crassipes* as demonstrated by the bioassays.

Keywords: acute toxicity, biocontrol establishment, feeding damage, freshwater bodies, nutrients, Water hyacinth.

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CHAPTER 1: GENERAL INTRODUCTION

1.1. Background to the study

The biological invasion of invasive alien plants is a global concern, as it threatens the functioning of indigenous ecosystems by competing with native plants for natural resources, modifying the community structure, and causing loss of biodiversity (Pimentel et al., 2000), and the impacts are greatest in ecologically diverse regions (Latimer et al., 2004). Invasions are driven by factors such as the propagule pressure, traits of the invasive plant, and vulnerability of the environment to invasion (Lonsdale, 1999). Native communities of plants in exotic regions are more vulnerable to invasion by alien plants when there is sufficient amount of available resources (Davis et al., 2000). This theory is based on the concept that an introduced plant into a non-native region must compete for natural resources and that invasive alien plants will proliferate at high rates if they do not encounter competition for available resources (*viz*. nutrients, water) (Davis et al., 2000). Management options that have been adopted to prevent proliferation and impact of both terrestrial and aquatic invasive alien plants around the world include manual, mechanical, chemical, and biological control (Coetzee et al., 2017; Hill & Coetzee, 2017). Although these interventions can be successful, most of them are expensive and offer short-term relief. Biological control is the most preferred management option because it is self-sustainable and provides long term control of invasive alien plants (Coetzee et al., 2011; Mukwevho et al., 2017a).

Biological control of invasive alien plants is implemented in three ways (*viz*. augmentative, conservation and classical) (Culliney, 2005). Augmentative biological control refers to the periodical release of natural enemies either in high numbers to supress population of the target plant at a short term period or in smaller quantities to reproduce, increase in numbers and inflict significant damage on the plant in a long term period (Eilenberg et al., 2001). Conservation biological control involves modifying the environment to protect the populations of the established agents (Culliney, 2005). Classical biological control involves the introduction of natural enemies of non-native origin to suppress populations of the host plant (Cuda et al., 2008; Schwarzländer et al., 2018). Classical biological control requires agent importation, screening for host-specificity, mass-rearing and release with the intention to lessen the intensities of the target weed to acceptable levels (Coetzee & Hill, 2008; Tipping et al., 2014). During the surveys to search for biological control agents from the native range of invasive alien plants, climate and genotype matching may be used to select potential natural enemies which are likely to feed on the host plant, reproduce and sustain high populations (Goolsby et al., 2006; Paterson et al., 2014; Paterson et al., 2009). The two factors are useful in selecting the target regions in the native range on which to conduct the surveys and field host range along with the damage inflicted by the biological control agents on the host plant to rule out agents which are unlikely to feed on the target weed (Paterson et al., 2014). Choosing the most promising biological control agents for further research in quarantine limits the risk of conducting host specificity trials on unsuitable agents, thus, less resources, time and effort invested by researchers on host specificity studies (Paterson et al., 2014).

Introduction of live organisms to supress populations of invasive alien plants around the world have been carried out since the beginning of the 20th century and a wide range of biological control agents with different feeding guilds have been released (Klein, 2011; Winston et al., 2014). Amongst families of invasive alien plants with the most release of biological control agents includes Asteraceae, Cactaceae, Fabaceae, Pontederiaceae and Verbenaceae (Winston et al., 2014). In South Africa, biological control of invasive alien plants started in 1913 with the release of the cochineal insect, *Dactylopius ceylonicus* Green (Hemiptera: Dactylopiidae), to manage *Opuntia monacantha* Haworth (Cactaceae) (Moran et al., 2013). Ever since the release of *D. ceylonicus,* 146 more host specific agents have been released to keep populations of 57 invasive alien weeds below the acceptable levels (Klein, 2011). The release of biological control agents to suppress invasiveness of aquatic weeds in South Africa was first initiated in 1970s, ever since 15 natural enemies (13 insects species, one pathogen and one mite) have been introduced (Coetzee et al., 2021).

Amongst the five worst water weeds in South Africa include *Salvinia molesta* D.S. Mitchell (Salviniaceae), *Pistia stratiotes* Linnaeus (Araceae), *Myriophyllum aquaticum* (Vellozo Conceição) Verdcourt, *Azolla filiculoides* Lamarck (Azollaceae) and *Pontederia crassipes* (Mart.) Solms-Laub (Pontederiaceae) (the topic of this thesis) (Hill & Coetzee, 2017). The first four invasive weeds are successfully kept below the damaging population levels by biological control agents, meanwhile, *P. crassipes* remains the most widespread and notorious weed (Coetzee et al., 2021; Richardson & Wilgen, 2004).

Pontederia crassipes which is commonly known as water hyacinth is one of the most ecologically and socio-economically damaging water weeds of the tropical, subtropical and temperate regions of the world (Coetzee et al., 2009; Hill & Coetzee, 2017; Pellegrini et al., 2018), and is ranked amongst the top ten worst invasive alien plants around the globe (Patel, 2012; Téllez et al., 2008). *Pontederia crassipes* is native to the Amazon Basin of South America and has invaded a range of water bodies such as rivers, lakes, ponds, wetlands, dams and man-made impoundments of the introduced and naturalized ranges (Cilliers et al., 2003; Coetzee et al., 2017; Hill, 2003).

In attempts to address the invasiveness, spread and impact of *P. crassipes*, different management techniques have been adopted against the weed in South Africa (Hill & Coetzee, 2008; Hill & Coetzee, 2017). These techniques include the use of herbicide sprays, physical and mechanical removal, and biological control (Coetzee & Hill, 2008; Culliney, 2005). Physical and mechanical controls are labour intensive and only feasible at local scales (Hill & Coetzee, 2008) and while chemical or herbicidal control presents immediate relief, it requires follow-up treatments, it is costly to apply and there is the potential of resistance development by the target plant through the frequent use of herbicides (Arias et al., 2005; Culliney, 2005; Hill & Coetzee, 2017). Classical biological control however, has been adopted as it is sustainable, environmentally safe, and cost-effective (Coetzee & Hill, 2008; Hill & Olckers, 2001).

To date, nine biological control agents have been released against *P. crassipes* in South Africa, and amongst the best known and most widely used are the two weevils, *Neochetina bruchi* Hustache and *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae), and a moth *Niphograpta albiguttalis* Warren (Lepidoptera: Pyralidae) (Coetzee et al., 2011; Hill & Coetzee, 2017; Julien, 2000). These biological control agents have successfully suppressed the population of *P. crassipes* in Papua New Guinea, Benin, Malawi, Lake Victoria and South Africa (Coetzee & Hill, 2008; Hill & Olckers, 2001; Phiri et al., 2001; Wilson et al., 2007; Winston et al., 2014).

The overall performance of biological control agents against *P. crassipes* has been variable in some parts of South Africa in comparison to other tropical and subtropical regions of the world (Coetzee et al., 2011; Hill & Olckers, 2001). Among the factors that have been suggested to limit the impact of biological control agents against *P. crassipes* are cool temperatures, flooding, nutrient loads, host plant availability, and injudicious use of herbicides (Coetzee et al., 2007a; Hill & Olckers, 2001; Hopper et al., 2021; Marlin et al., 2013; May & Coetzee, 2013). Despite the long history of weed biological control globally, there have only been a limited number of studies that have assessed the effect of insecticide drift on the establishment and performance of weed biological control agents. For example, Hoffmann & Moran (1995) reported the indirect effect of synthetic insecticides on the performance of *Trichapion lativentre* Beguin Billecocq (Coleoptera: Apionidae) on *Sesbania punicea* (Cav) Benth. (Fabaceae) along citrus orchards in the Western Cape Province, South Africa. Due to the limited performance of biological control agents of *P. crassipes* on the inland regions of Mpumalanga Province, especially at sites where there is high agricultural production, this study assessed the effect of insecticide drift on the establishment of biological control agents of *P. crassipes.*

1.2. Botany, morphology and ecology of *Pontederia crassipes*

Pontederia crassipes is a perennial, herbaceous freshwater weed that invades water bodies such as rivers, impoundments, lakes, ponds and wetlands throughout the world (Coetzee et al., 2017; Winston et al., 2014). After a recent botanical nomenclature revision, the Pontederiaceae was divided into two genera, the *Heteranthera* and *Pontederia* (Pellegrini et al., 2018). The *Pontederia* includes, *P. crassipes*, *Pontederia azurea* (Sw.) Kunth, *Pontederia diversifolia* Vahl, *Pontederia heterosperma* Alexander, and *Pontederia natans* P. Beauv (Pellegrini et al., 2018). While the *Heteranthera* includes, *Heteranthera dubia, Heteranthera rotundifolia, Heteranthera limosa, Heteranthera oblongifolia, Heteranthera gardneri, Heteranthera zosterifolia* and *Heteranthera seubertiana* (Pellegrini et al., 2018).

Pontederia crassipes (Figure 1.1) has a disc-like to oval incurved shape, shiny dark green leaves which are supported by the spongy petioles above the water surface (Gettys, 2014; Penfound & Earle, 1948). Leaves are displayed in a whorl arrangement with older leaves found at the outer edge of the rosette (Penfound & Earle, 1948). The petioles contain air and function as floats, and petiole morphology is primarily dependent on the population density of the plant (Gettys, 2014). In overpopulated conditions, the petioles are tall and slender, up to 2m in height, compared to the swollen petioles found in plants in sparse populations (Center & Spencer, 1981). The slender or swollen shape of petioles influences the amount of air contained and ultimately the ability of the plant to float (Center & Spencer, 1981). Rhizomes of *P. crassipes* and the fibrous and black coloured roots are submerged (Xie & Yu, 2003). *Pontederia crassipes* produces a spike of inflorescence borne on a single erect stalk (Gettys, 2014). The violet flowers develop on the spike in clusters of 8-15 and each flower consists of six petals with a central yellow spot on the uppermost petal (Coetzee et al., 2017; Gettys, 2014).

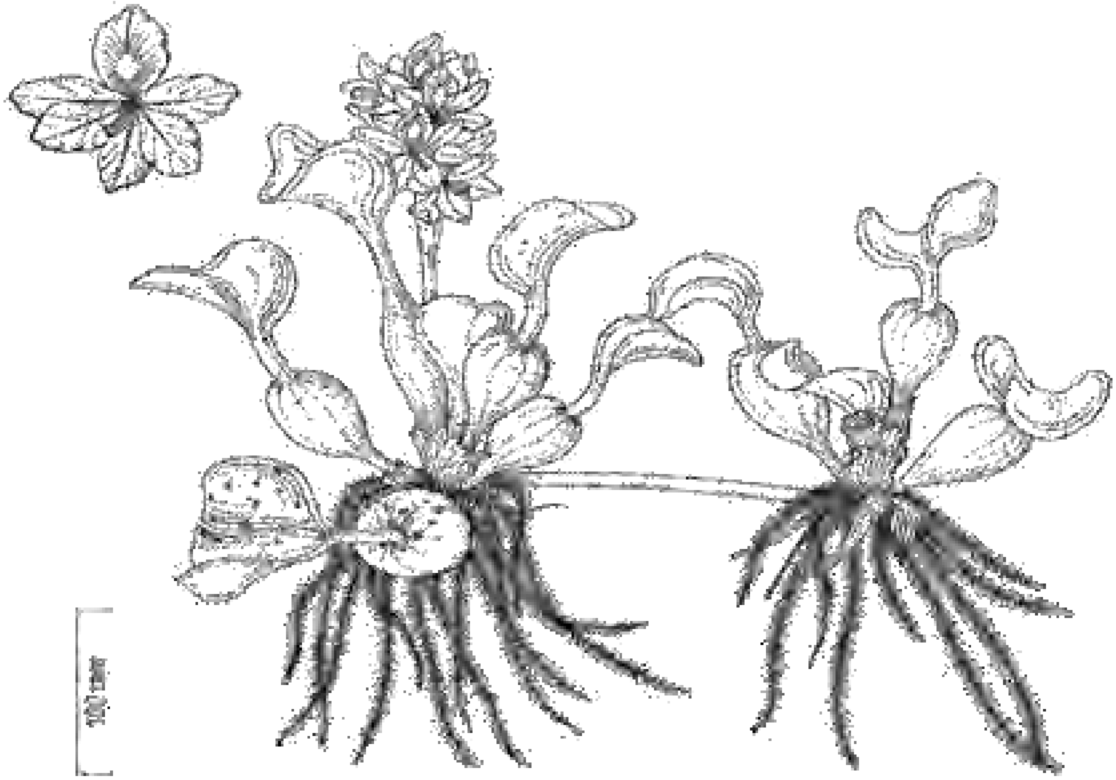

Figure 1.1: A mature *Pontederia crassipes* illustrating bulbous petioles, inflorescence on erect stalk and fibrous roots with fine hairs. Mother plant is attached to a stolon with a daughter plant at end (Coetzee et al., 2011).

Pontederia crassipes reproduces sexually through the production of seeds and vegetatively through stolon's (Patel, 2012; Téllez et al., 2008). A stolon extends horizontally from the base of the stem to produce clones from axillary buds (Gettys, 2014). For sexual reproduction, a single inflorescence produces a cluster of approximately 3000 seeds which are enclosed in a capsule (Barrett, 1980). After the flowering cycle, seeds are released from capsule and either accumulate in floating mat or sink (Coetzee et al., 2017). Seeds on the sediment remain viable for 5- 20 years and germinate successfully when conditions are favorable for growth (Pérez et al., 2011; Malik, 2007).

1.3. Geographic distribution and climatic requirements

Pontederia crassipes is widespread and destructive on every continent of the world except in Antarctica (Coetzee et al., 2017). As a consequence of ornamental trade, *P. crassipes* has spread to more than 50 countries around the globe (Hussner et al., 2017; Zhang et al., 2010). The distribution and naturalization occur abundantly in tropical and subtropical freshwater bodies of the world (Coetzee et al., 2017). In South Africa, invasion by *P. crassipes* was firstly reported in KwaZulu-Natal in 1908 and the weed has since spread throughout the country from the low-lying regions to the high plateau (Figure 1.2) (Cilliers, 1991). The weed in South Africa is listed as category 1b by the Conservation of Agricultural Resources Act (No. 43 of 1983) and National Environmental Management: Biodiversity Act (No. 10 of 2004) (NEMBA), where it is prohibited to plant, transport or sell (Hill & Coetzee, 2017; Jones, 2001).

Pontederia crassipes can withstand a diversity of environmental conditions which leads to its wide distribution around the world (Zhang et al., 2010). The natural drivers that foster invasive traits of *P. crassipes* include temperature, pH, salinity, nutrients and solar radiation (Bick et al., 2020; Téllez et al., 2008; Wilson et al., 2005). *Pontederia crassipes* grows well at a temperature of 28°C to 30°C and relative humidity of 21% to 30% (Mujere, 2015). Leaves of *P. crassipes* exposed to - 3°C for longer than 12 hours are destroyed and eventually, the entire plant dies at -5 °C (Mujere, 2015). During the onset of the growing season, *P. crassipes* recovers from freezing temperatures and in nutrient enriched water bodies, the weed doubles biomass in less than a week (Gettys, 2014). *Pontederia crassipes* tissues under eutrophic water bodies takes up and efficiently utilize nutrients, especially Nitrogen and Phosphorus (El-Gendy et al., 2005). Furthermore, *P. crassipes* tolerate a pH of 6 to 8, but growth prefers neutral pH (Téllez et al., 2008).

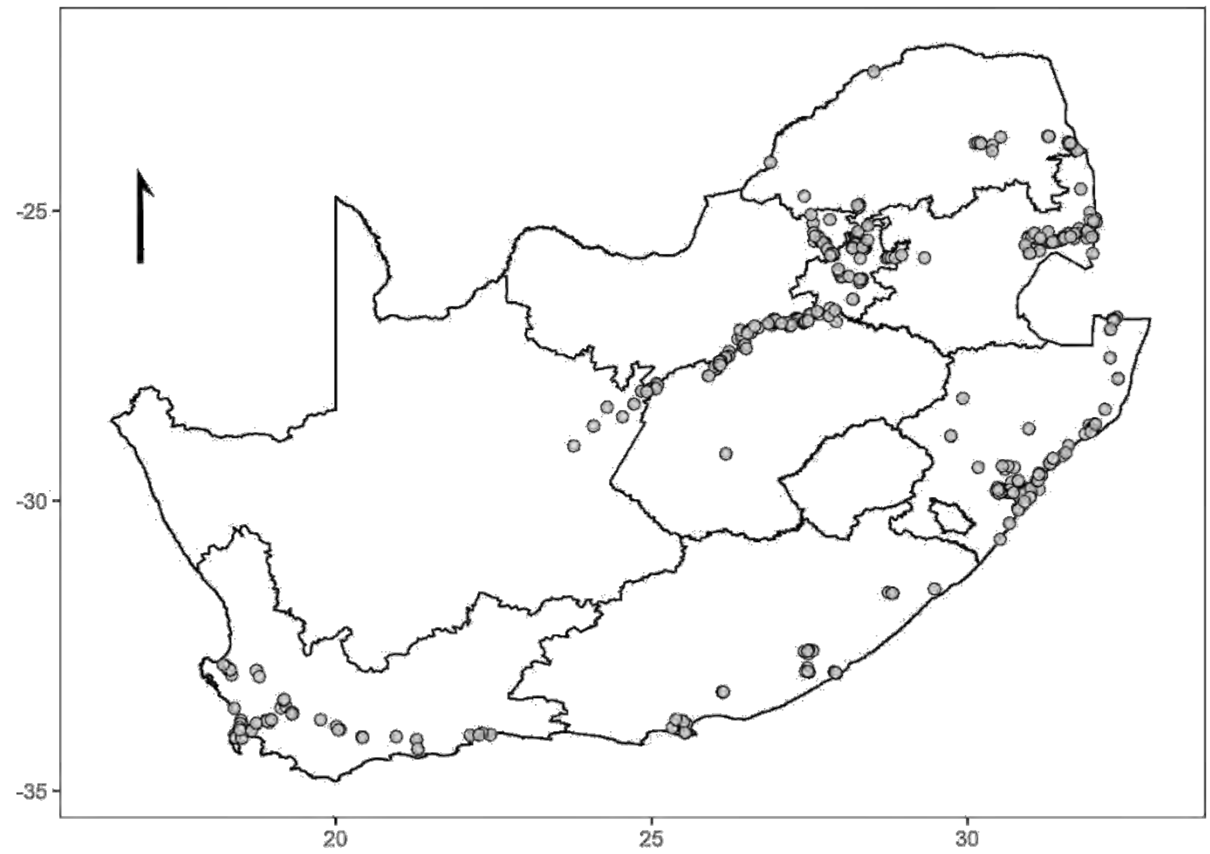

Figure 1.2: The geographic distribution of *Pontederia crassipes* in South Africa. Heavy invasions of *P. crassipes* occur on the coastal and the high lying inland regions of the country.

1.4. Management of *Pontederia crassipes*

Integrated management of *P. crassipes* involves the utilization of different techniques with the intention to suppress the population densities and to limit the spread of the plant in the exotic ranges (Villamagna & Murphy, 2010). Efforts to manage *P. crassipes* include physical and mechanical, chemical and biological control practices, and these tactics may either be applied solely or combined (Byrne et al., 2010; Schwarzländer et al., 2018). Despite the greatest effort to repress populations of *P. crassipes,* the plant is still persistent in the naturalized range (Coetzee et al., 2017).

1.4.1. Physical and mechanical control

Manual and mechanical control entails the use of manpower and machinery to eradicate populations of *P. crassipes* (Caffrey & Monahan, 2006; Patel, 2012). Manual control is usually the first form of invasive alien plant management (Hill, 2003; Hussner et al., 2017), and does not require the utilization of specialized techniques (Villamagna & Murphy, 2010). Efforts to harvest *P. crassipes* has been deemed to be successful at a small scale of about 1ha, and although it is labour intensive and expensive, it presents temporal relief (Caffrey & Monahan, 2006; Edwards & Comas, 2009). Mechanical harvesters can work, but are limited by the depth of the water bodies, hence, chopping and harvesting the weed in shallow systems is often difficult and impractical (Coetzee et al., 2017). Cables and booms can be used and are extended across the system to control the movement of *P. crassipes*, and prevent the weed from spreading to water intakes of hydropower stations (Cilliers et al., 2003). Furthermore, barriers of cables are also used to split large mats into small units intended for different control techniques of the integrated management approach (Jones, 2001).

1.4.2. Chemical or herbicidal control

Chemical control refers to the application of synthetic herbicides to control populations of *P. crassipes* (Hill & Coetzee, 2017; Uka, 2008). *Pontederia crassipes* is highly susceptible to herbicides such as Diquat, Paraquat, 2,4-D, Amitrole and Glyphosate (Cilliers et al., 2003; Datta & Mahapatra, 2015; Yigermal et al., 2020). Herbicides with either Glyphosate or 2,4-D active ingredients are among the commonly utilized to suppress the weed (Datta & Mahapatra, 2015; Katembo et al., 2013; Osmond & Petroeschevsky, 2013). Glyphosate is the most commonly used active ingredient and is still utilized to maintain the populations of *P. crassipes* at levels below the threshold at some sites in South Africa (Hill & Coetzee, 2017).

The use of herbicides requires skilled personnel and intense follow-up treatments to prevent regrowth from the seed bank (Coetzee et al., 2017; Hill & Coetzee, 2017). Registered herbicides are applied to the foliage of *P. crassipes* with the aid of a knapsack, boom, handgun mounted on a boat, or aerially using aircraft (Ueckermann, 2001). Herbicides are quickly taken up by the plant tissues, translocated within the vascular system and destroy the emergent parts of *P. crassipes* (Datta & Mahapatra, 2015; Uka, 2008). Although herbicides are fast-acting, their extensive use is costly and offer only temporal relief (Hill & Coetzee, 2017). *Pontederia crassipes* recover quickly from plants that have been missed during spray operations, and from the seed bank (Malik, 2007; Mujere, 2015). Furthermore, surfactants contained in registered herbicides of *P. crassipes* kill herbivorous insects that feed on the treated plants (Hill et al., 2012). As a result, more emphasis has been on the use of sub-lethal dosages or to avoid spraying of the biological control reserves (Byrne et al., 2010; Jadhav et al., 2008; Katembo et al., 2013).

1.4.3. Biological control

Weed biological control involves the utilization of live organisms such as insects, pathogens and mites to suppress the target weed population, in this example *P. crassipes* (Coetzee & Hill, 2008; Coetzee et al., 2009). Biological control as a management strategy has been adopted throughout the globe and a diverse suite of phytophagous agents with narrow host-ranges have been intentionally introduced against *P. crassipes* (Coetzee et al., 2011; Klein, 2011; Winston et al., 2014). The efficiency of the biological control agents varies between countries and regions, and amongst the primary drivers is the different micro-environmental factors (Hill & Olckers, 2001; Wilson et al., 2007; Winston et al., 2014).

In South Africa, the classical biological control programme for *P. crassipes* was first initiated in 1973 and at least nine agents have been released to date (Cilliers, 1991; Hill & Coetzee, 2017; Klein, 2011). Among the released agents are two weevils *Neochetina bruchi* Hustache and *Neochetina eichhorniae* Warner (both Coleoptera: Curculionidae), a moth *Niphograpta albiguttalis* Warren (Lepidoptera: Pyralidae), two mirids *Eccritotarsus catarinensis* Carvalho and *Eccritotarsus eichhorniae* Henry (both Hemiptera: Miridae) (establishment unconfirmed), a mite *Orthogalumna terebrantis* Wallwork (Sarcoptiformes: Galumnidae), a pathogenic fungus *Cercospora piaropi* Tharp (Mycosphaerellales: Mycosphaerellaceae), a planthopper *Megamelus scutellaris* Berg (Hemiptera: Delphacidae) and a grasshopper *Cornops aquaticum* Bruner (Orthoptera: Acrididae) (establishment unconfirmed) (Henry, 2017; Hill, 2003; Hill & Coetzee, 2017; Klein, 2011). Furthermore, screened agents that were not developed fully include *Bellura densa* Walker (Lepidoptera: Noctuidae), *Xubida infusella* Walker (Lepidoptera: Pyralidae), *Thrypticus* spp. (Diptera: Dolichopodidae), *Dyscinetus dubius Olivier* (Scarabaeidae: Dynastinae), and *Taosa longula* Remes Lenicov (Hemiptera: Dictyopharidae) (Center & Hill, 2002; Coetzee et al., 2011; Klein, 2011).

South Africa has the highest number of agents released against *P. crassipes* compared to other countries where biological control programme has been initiated (Hill & Olckers, 2001; Wilson et al., 2007; Winston et al., 2014). Irrespective of the release efforts of the biological control agents, the establishment and performance of these agents is variable (Coetzee et al., 2011). Abiotic factors, host plant quality (compatibility), release techniques and parasitism are among factors that limit the efficacy of the biological control agents (Bownes et al., 2013; Hill & Olckers, 2001; Katembo et al., 2013; Kraus et al., 2019).

1.4.4. Establishment and impact of biocontrol agents on *P. crassipes* in South Africa.

As a result of ecological and economic benefits, greater emphasis has been on the extensive use of biological control to suppress populations of *P. crassipes* (Coetzee et al., 2020; Coetzee et al., 2014; Midgley et al., 2006; Van Wilgen et al., 2004). The success of the biological control agents is determined by the ability of the agents to establish, spread and inflict significant damage to the weed (Mukwevho et al., 2017a; Schwarzländer et al., 2018). Among the nine agents released on water hyacinth in South Africa, the two weevils are the most widespread and abundant with recognizable impact on *P. crassipes* (Table 1.1). *Neochetina eichhorniae* was the first agent to establish on *P. crassipes* in South Africa (Cilliers, 1991), followed by the *N. bruchi* (Coetzee et al., 2011; Coetzee & Hill, 2008). However, the establishment of other released agents such as *C. aquaticum, E. catarinensis, M. scutellaris* and *N. albiguttalis* has been sporadic due to environmental constraints (Byrne et al., 2010; Coetzee et al., 2011; Coetzee et al., 2007b; May & Coetzee, 2013; Miller et al., 2021; Paterson et al., 2016, 2019). Therefore, the distribution of the agents and the impact on *P. crassipes* is restricted to the warmer regions of South Africa (Coetzee et al., 2011; Coetzee et al., 2007b).

Table 1.1: Biocontrol agents released to reduce the proliferation of *P. crassipes* in South Africa (Klein, 2011; Coetzee et al. 2011; Winston et al. 2014**)**

Order: Family/ biocontrol agent	**Origin**	**Main release**	**Agent status**	**Feeding mode**	**Damage inflicted**
Coleoptera: Curculionidae					
Neochetina bruchi Hustache	Argentina	1990	Widely established and abundant throughout invaded range	Stem borer	Extensive damage on leaves and petioles
Neochetina eichhorniae Warner	Argentina	1974	Widely established and abundant throughout invaded range	Stem borer	Extensive damage on leaves and petioles
Hemiptera: Delphacidae					
Megamelus scutellaris Berg	Argentina via USA	2013	Established, limited by extreme high and cold temperatures	Leaf sucker	Unknown
Hemiptera: Miridae					
Eccritotarsus catarinensis Carvalho	Brazil	1996	Widely established, present in low numbers in temperate climate	Leaf sucker	Considerable, remove leaf chlorophyll
Eccritotarsus eichhorniae Henry	Peru	2007	Unconfirmed	Leaf sucker	-
Lepidoptera: Pyralidae					
Niphograpta albiguttalis Warren	Argentina via USA	1990	Established, present in low numbers in the temperate climate	Petiole borer	Considerable, reduce leaf surface area for photosynthesis

Table 1.1 (Continued): Biocontrol agents released to reduce the proliferation of *P. crassipes* in South Africa (Klein, 2011; Coetzee et al. 2011; Winston et al. 2014)

Order: Family/ biocontrol agent	Origin	Main release	Agent status	Feeding mode	Damage inflicted
Mycosphaerellales: Mycosphaerellaceae					
Cercospora piaropi Tharp	USA	1992	Established in low numbers	Leaf pathogen	Considerable, causes foliar lesions
Orthoptera: Acrididae					
Cornops aquaticum Bruner	Brazil	2011	Establishment unconfirmed	Leaf feeder	-
Sarcoptiformes: Galumnidae					
Orthogalumna terebrantis Wallwork	Argentina	1989	Established, occur in low numbers in temperate regions	Leaf miner	Considerable, reduces leaf surface area for photosynthesis

1.5. Factors limiting the establishment of biological control agents.

The ability of biological control agents to establish successfully depends on the suitability of abiotic factors (climate, anthropogenic activities) and biotic factors (host-plant quality, predation or parasitism) (Cuda et al., 2008; Harms et al., 2020). The abiotic and biotic factors influence the population dynamics, fecundity, feeding behaviour, distribution and survival of biological control agents (Byrne et al., 2010; Khaliq et al., 2014).

1.5.1. Climate

Climatic incompatibility is often cited as the common constraint affecting the establishment and fecundity of biological control agents (Chen et al., 2019; Coetzee & Hill, 2008; Cuda et al., 2008; Régnière et al., 2012). Climatic factors include temperature, light, rainfall and humidity (Hill & Olckers, 2001; Khaliq et al., 2014). The climate in South Africa is not similar to the warm tropical climate of the biological control agents' native South American range (Cowie et al., 2016; Griffith et al., 2019; Hill & Olckers, 2001; May & Coetzee, 2013; Maseko, 2020). Although *P. crassipes* has managed to overcome the climatic variability in South Africa by recovering in spring from frost in the higher-altitude sites, biological control agents do not always respond in the same way (Coetzee et al., 2007b; Hopper et al., 2017; Singh & Olckers, 2017). Biological control agents enter the onset of the growing season in low numbers (Byrne et al., 2010; Reddy et al., 2019), and as a result, some time is required for the agents to re-populate the invaded site to effect considerable damage on *P. crassipes*, usually peaking near the end of the growing season (Byrne et al., 2010; Hill & Olckers, 2001; Hopper et al., 2017).

1.5.2. Eutrophication

The nutrient status of the host plant influences the fecundity of biological control agents (Awmack & Leather, 2002; Hong et al., 2019; Inbar et al., 2001; Salgado & Saastamoinen, 2019). Many water bodies where *P. crassipes* is invasive are enriched with nutrients, thereby influencing the impact of biological control agents (Coetzee & Hill, 2012; Heard & Winterton, 2000; Ismail et al., 2017; Marlin et al., 2013). The highly enriched plants support the reproduction and development of biological control agents but the weed displays a high recovery rate to compensate for the damage inflicted (Bownes, 2008; Bownes et al., 2013a; Burke et al., 2014; Canavan et al., 2014; Center & Dray, 2010; Coetzee et al., 2007a; Mukarugwiro et al., 2018; Ripley et al., 2008).

1.5.3. Host-plant factors

Whilst most failures of biological control in different geographic ranges is linked to commonly cited factors (*viz*. climate, eutrophication, predation and parasitism), phenological and genetical changes in the target host causes incompatibility between introduced agents and plants (Harms et al., 2020). There is evidence that relatively high growth rate and reproduction of introduced plants in the adventive ranges is due to limited resources invested in defense mechanisms (Blossey & Notzold, 1995; Keane & Crawley, 2002). This phenomenon is known as the evolution of increased competitive ability (EICA) by non-native plants (Blossey & Notzold, 1995). In practice, introduced specialized biological control agents may successfully feed and establish on the invaded ranges of the host plant, but fail to limit spread and invasion intensities over time (Hill & Coetzee, 2020). Limited success of a sap-feeding mirid *Falconia intermedia* Distant (Hemiptera: Miridae) on *Lantana camara* has been associated with induced biotic resistance where a decline in average densities of the agent was as a consequence of increased leaf laminae toughness, leaf hairs and defence chemical compounds (Heshula & Hill, 2011, 2014; Ngxande-Koza et al., 2017).

Introduced plants into exotic ranges undergo physical and chemical evolutionary changes as a defensive or compensatory response to herbivory (Grodowitz et al., 2010; Manrique et al., 2008; Moody et al., 2016; Mukwevho et al., 2017a; Nabity et al., 2009). It is widely accepted that the level of response by the host plant is influenced by habitat type, the quantity of resource available (bottom-up regulators) and the plant growth rates (Marazzi et al., 2004). Defensive secondary metabolites influence palatability and insect female oviposition preference, thus the impact of biological control agents on invasive weeds is limited (Marazzi et al., 2004; War et al., 2012). The compensatory responses are elicited to nullify the impact inflicted by herbivory (Schwachtje & Baldwin, 2008).

1.5.4. Parasitism and predation

Although biological control agents are checked in quarantine and released without natural enemies, they remain vulnerable to predators and parasitoids in their new geographic ranges (Cuda et al., 2008; Tipping et al., 2020). For example, *M. scutellaris* has rapidly recruited the indigenous parasitoid wasp *Echthrodelphax migratorius* Benoit (Hymenoptera: Dryinida) in South Africa (Kraus et al., 2019). An egg parasitizing mymarid wasp, *Kalopolynema ema* Schauff and Grissell (Hymenoptera: Mymaridae) has been also recorded on *M. scutellaris* in the United States of America (USA) (Minteer et al., 2016). Although the long-term effect of these parasitoids on the performance of *M. scutellaris* has not been reported, it is likely that they may be delaying the agent from attaining high numbers to inflict significant damage on *P. crassipes* in the field (Coetzee et al., 2021).

1.5.5. Pesticidal effects

The application of pesticides in agroecosystems is conducted within stringent regulations and is permitted only after risk assessments on the potential effects on non-target habitats, which involves studies on the impacts on beneficial arthropods (Maltby, 1999; Maltby & Hills, 2008). These assessments are primarily based on laboratory studies using the worst case events (*viz*. ensure optimum exposure, determining the most sensitive developmental stages and limited potential to avoid treated sites and recover post treatment) (Desneux et al., 2004; Desneux et al., 2007). The potential risk effects of insecticides on non-target arthropods is determined by susceptibility of the species, type of pesticide, concentration and duration of exposure (Kjær et al., 2021; Prouty et al., 2021). Despite the measures (*viz*. hedgerows or buffer) employed to prevent the loss of insecticides from agroecosystems, the toxic compounds continue to threaten the survival and performance of beneficial arthropods in habitats adjacent to arable lands (Lazzaro et al., 2008; Maltby & Hills, 2008; Otto et al., 2009). The knowledge on the chemical, physical and physiological processes, including effects of exposure to spatial and temporal distribution of residues in the field is important in understanding the potential effects of insecticides on non-target arthropods (Antwi & Reddy, 2015). The capacity of non-target ecosystems to recover from insecticide effects is determined by factors such as the life-history traits of the affected organism, insecticide persistence and the distance from recolonization sites (Fairchild et al., 1992).

Insecticides from agroecosystems reach non-target adjacent ecosystems indirectly through run-offs and directly via spray drifts (Schulz et al., 2001). The latter involves the transport of tiny chemical droplets by wind currents from agroecosystems to adjacent natural habitats which serve as recolonization sites and home to beneficial arthropods (Dabrowski & Schulz, 2003; Schulz, 2004). Toxic compounds in non-target ecosystems kill beneficial arthropods and reduce the heterogeneity, population density, and ecosystem services (Antwi & Reddy, 2015; Brittain et al., 2010; Crossland et al., 1982; Feber et al., 2007). On the toxicological effects of insecticides, more studies have focused on pollinators, in particular honeybees (Brittain et al., 2010; Mužinić & Želježić, 2018). The worldwide decline in honeybees has been attributed to the non-target effects of neonicotinoids, their systemic translocation within a plant, pollen and flowers interfere with feeding behavior, mobility and social communication (Botías et al., 2015, 2016; Chagnon et al., 2015; Fairbrother et al., 2014; Tison et al., 2016).

On the extensive studies conducted on toxicological effects of insecticides on beneficial insects inhabiting non-target ecosystems, aquatic arthropods have proven to be more sensitive to pesticides than terrestrial species (Siegfried, 1993). Ephemeroptera, Odonata, Plecoptera, Hemiptera, Coleoptera, and Trichoptera are amongst the highly sensitive groups of arthropods to spray drift contaminants (Crossland et al., 1982). Although emergent aquatic weeds have shown success in reducing the direct deposition of spray drift contaminants on the surface of rivers, the accumulation of the toxic chemicals on their leaves could reduce the population of insects inhabiting the plants (Dabrowski et al., 2005). In the context of biological control of invasive alien plants invading rivers adjacent to agroecosystems, interception and absorption of spray drift droplets by the plants expose biological control agents to toxic compounds and limit their success in managing the growth of the host plant (Brock et al., 1992; Hoffmann & Moran, 1995; Schulz et al., 2003).

In efforts to assess the efficacy of biological control agents, insecticide exclusion studies have been employed where parts of the target host plants are treated with a synthetic chemical widely used in agriculture and others left untreated. Although insecticides selected for this purpose have proven less or no phytotoxic effects on the growth of the treated plants, rapid growth rates along with biomass accumulation and high reproductive performance have been reported on chemically protected plants compared to herbivory on untreated plants (Jones et al., 2018; Mukwevho et al., 2017b; Tipping et al., 2008; Tipping & Center, 2002). Moreover, greater heterogeneity and abundance of biological control agents have been recorded on unsprayed plants compared to insecticide protected plants (Katembo et al., 2019, 2020). As a result, insecticide drift from intensified agricultural production has the potential to interfere with the establishment and impact of biological control agents of invasive alien plants (Hoffmann & Moran, 1995; Otto et al., 2013). The current study, therefore, investigated the potential effects of insecticide drift on the establishment and abundance of naturalized biological control agents of *P. crassipes.*

- The aim of this study was to evaluate the effects of insecticides drifted from citrus orchards on the performance of biological control agents of *P. crassipes.*

The specific objectives of the thesis were:

- To measure establishment and population densities of biological control agents of *P. crassipes* on the Crocodile River and dams adjacent to citrus orchards at the Lowveld region of Mpumalanga Province.
- To measure percentage survival of *M. scutellaris* and *N. eichhorniae* adults exposed to selected insecticides commonly used in citrus orchards at the Lowveld region of Mpumalanga Province.

CHAPTER TWO

Eutrophication is more important than pesticide drift on the success of biocontrol agents of *Pontederia crassipes* (Pontederiaceae) in the Lowveld regions of Mpumalanga Province.

2.1. INTRODUCTION

Establishment and impact of biological control agents on *P. crassipes* is variable with sometimes negligible impacts recorded in the inland regions of South Africa, especially in rivers (Hill & Coetzee, 2017; Hill & Olckers, 2001; Klein, 2011; Moran et al., 2021). The establishment success and impact of naturalized biological control agents of *P. crassipes* are determined by abiotic and biotic factors (Harms et al., 2020; Hill & Olckers, 2001; Hopper et al., 2021). Amongst common factors limiting the establishment of biocontrol agents is climate, whilst eutrophication and pesticide drift can influence the level of control achieved (Byrne et al., 2004; Coetzee et al., 2007a; Coetzee & Hill, 2012; Hoffmann & Moran, 1995; Miller et al., 2021). To address this, augmentative release efforts have resulted in higher establishment success in the Lowveld region of Mpumalanga, however, the impact remains less than anticipated (Byrne et al., 2010; Hill et al., 2021).

Eutrophication in aquatic ecosystems influences the population abundance and impact of biological control agents of *P. crassipes* (Bownes et al., 2013a; Byrne et al., 2010; Coetzee & Hill, 2012; Mukarugwiro et al., 2018). Nitrogen and phosphorus amongst other nutrients influence the nutritional value of *P. crassipes*, and thereby the ability of the agents to feed and the ability of the plant to withstand herbivory (Heard & Winterton, 2000; Reddy et al., 1990). Nitrogen has the most significant impact on the survival, development and fecundity of biological control agents (Awmack & Leather, 2002; Bownes et al., 2013a; Center & Dray, 2010; Mukarugwiro et al., 2018), while phosphorus influences the uptake and level of nitrogen stored in the tissues of *P. crassipes* (Reddy et al., 1990). The availability of these nutrients in the tissues of *P. crassipes* varies spatially and temporally, and the establishment success of biological control agents increases with tissue nutrient concentration (Bownes et al., 2013b; Byrne et al., 2010; Center & Dray, 2010; Heard & Winterton, 2000; Marlin et al., 2013). Despite the success in establishment, their impact is negated by the high rate at which the plants grow in enriched water to compensate for their damage (Bownes et al., 2013a; Byrne et al., 2010; Marlin et al., 2013).

There has been intensive use of pesticides to manage insects, pathogens and weeds (including invasive alien weeds) in agroecosystems (Hill et al., 2012; Hoffmann & Moran, 1995). Irrespective of their efficiency against target pests and weeds, pesticides threaten beneficial organisms such as biological control agents infesting plants adjacent to agroecosystems (Hoffmann & Moran, 1995; Langhof et al., 2005; Otto et al., 2013). Pesticides from agricultural ecosystems reach nearby aquatic ecosystems through runoff and spray drift in air currents (Dabrowski et al., 2005; Maltby & Hills, 2008; Schulz et al., 2001), and these pesticides may kill naturalized biocontrol agents on contact or through systemic means for insects feeding on contaminated hosts (Hoffmann & Moran, 1995; Langhof et al., 2005).

The effects of insecticide drift on the performance of biological control agents has been poorly studied (Hoffmann & Moran, 1995), and there has been limited focus on the impact of pesticide drift on the establishment of biological control agents of *P. crassipes*. Due to the intensive use of insecticides on citrus orchards along aquatic ecosystems in the Lowveld region of Mpumalanga, we speculated that pesticides may be contributing to the limited success of biological control of *P. crassipes* (see Chapter 1). The aim of this chapter was to assess the potential effect of insecticide drift, and eutrophication on the establishment and impact of biological control agents on *P. crassipes*.

2.2. MATERIALS AND METHODS

2.2.1. Study sites

The study was conducted on the Crocodile River and dams selected at Hectospruit, Kanyamazane and Vergenoeg farms. The river and dam at each of the three selected sites were heavily invaded with *P. crassipes* (Henderson, 2015). Each river site was paired to a dam site. The hypothesis was that the small irrigation dams within orchards would be more prone to insecticide drift than the river sites which were further from the orchards (Figure 2.1). To adjust for the effect of river flow, *P. crassipes* plants were only surveyed in well-established mats at each of the three river sites that had not moved for at least a season. All plots/ sites were selected within the subtropical region characterized by short, cool winter and long-warm summer. The average temperature ranges from 6°C to 26°C in winter, and summer temperatures ranges between 16°C and 32°C. Dry winters receive less than 10mm of average rainfall, meanwhile in summer precipitation ranges between 70mm and 95mm. Data was collected during the austral autumn (i.e., May 2022) which coincided with the insecticide application periods in citrus orchards in the Lowveld region. Furthermore, the populations of biological control agents were highest during the data collection period.

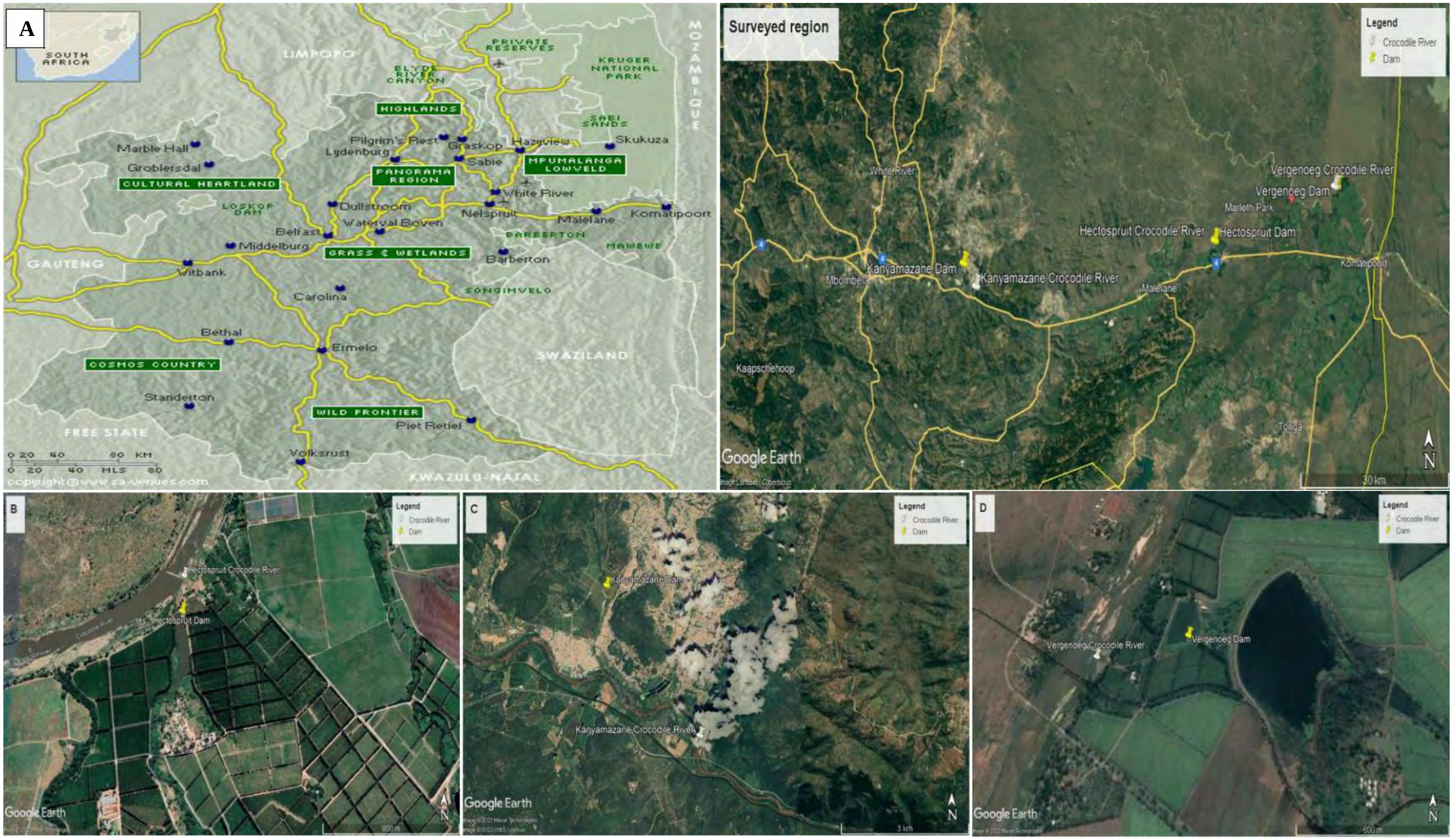

Figure 2.1: The Lowveld region of Mpumalanga in South Africa (a), and the paired (Crocodile River and dam) surveyed sites selected along citrus orchards at Hectospruit (b), Kanyamazane (c), and Vergenoeg (d).

2.2.2. Insecticides residues and nutrient status of water

Leaf and water samples were collected from each site and sent to Labserve Pty Ltd. (Nelspruit) for insecticide residue and nutrient analysis. Two hundred leaves (i.e., approx. 500g) were randomly harvested from each site, maintained in a cooler box, and submitted within 24 hours for analysis. Leaves were screened for the residues of widely used pesticides such as Methomex® 200, Pyrinex® 250 CS, Delegate™ 250 WG, Tivoli 240 SC, and Walker 240 SC (Table 2.1). Furthermore, the common macro-nutrients (*viz*. Nitrogen, Phosphorus and Potassium) were screened and their levels were determined from water samples collected.

Table 2.1: commonly applied insecticides to manage citrus insect pests in the Lowveld region of Mpumalanga**.**

Trade Name	**Active ingredient**	**Timing (weeks)**	**Concentration/ 100L**
Methomex® 200 SL	Methomyl	43-45	20g
Pyrinex® 250 CS	Chlorpyrifos	5-8	200ml
Delegate™ 250 WG	Spinetoram	38-40	10g
Tivoli 240 SC	Spirotetramat	48-52/ 1-5	10ml
Walker 240 SC	Methoxyfenozide	4 weeks prior to harvest	60ml

2.2.3. Establishment of biocontrol agents on *P. crassipes*

To measure the establishment of biological control agents at each study site, a quadrat ($0.25m^2$) was randomly thrown into the dense mat of *P. crassipes*. Sampling was replicated three times with each sampling unit selected at least 10 m distant from one another. At each of the three quadrats, ten haphazardly selected *P. crassipes* plants were thoroughly searched for damage signs or scars associated with *E catarinensis, Neochetina* spp. (the two species were grouped together as it is very difficult to discriminate between their feeding scars), and *O. terebrantis* (Table 2.2). A four-point damage scale was used to estimate the abundance of the established biocontrol agents. The interpretation and description of the scores were: 0 = healthy plant (no feeding scars), 1 = exploratory feeding (0-30 scars), 2 = moderate feeding (31-60 scars) and 3 = severe/ intensified feeding (above 61 scars). To calculate the establishment intensity of biocontrol agents at each site, the total number of plants damaged was divided by the total number of plants sampled per quadrat (10) and multiplied by 100.

Table 2.2: Distinctive damage signs of biocontrol agents naturalized on *P. crassipes* at the Lowveld region of Mpumalanga.

Biocontrol agent	**Damage signs**
Eccritotarsus catarinensis	Visible as chlorotic marks on the leaf lamina surface (i.e., yellow leaves) (Hill et al., 1999).
Neochetina spp.	The weevil's scrap the petioles and leaf lamina surface of *P. crassipes* causing rectangular scars ranging from 0.5 mm^2, 20 to 25 mm^2 (Deloach & Cordo, 1976).
Orthogalumna terebrantis	Feed on the leaf lamina and create galleries of up to ± 4mm (Deloach & Cordo, 1976).

2.2.4. Relationship between agent establishment and proximity to orchards

The distance between the sampled sites (*viz*. river and dams) and the nearest orchard was measured with the aid of a Google earth Pro. Version 7.3.3. 7786. Google earth ruler tool was used to measure the distance (i.e. in metres) between the selected site and the border of a citrus orchard.

2.2.5. Statistical analysis

The collected data was first tested for normality using Shapiro-Wilk test. Data met the assumptions of normality and parametric analytic procedures were used. Percentage number of plants infested (damaged) by *E. catarinensis* ($W = 0.79663$, $P = 0.05$) and *O. terebrantis* ($W = 0.63989$, $P = 0.001$) at Hectospruit were not normally distributed. Percentage number of plants infested by *Neochetina* spp. ($W = 0.49609$, $P = 0.00002$) and *O. terebrantis* ($W = 0.71448$, $P = 0.008$) at Kanyamazane were not normally distributed. Only *P. crassipes* plants infested by *E. catarinensis* ($W = 0.81940$, $P = 0.08$) on the sites visited at Kanyamazane were normally distributed. At Vergenoeg the percentage number of plants infested by *Neochetina* spp. ($W = 0.70209$, $P = 0.006$), and *O. terebrantis* ($W = 0.66679$, $P = 0.002$) were not normally distributed. Only plants infested by *E. catarinensis* ($W = 0.83824$, $P = 0.13$) on the sites visited at Vergenoeg were normally distributed.

Number of *E. catarinensis* ($W = 0.85589$, $P = 0.17$) and *O. terebrantis* ($W = 0.92157$, $P = 0.52$) on sites visited at Hectospruit were normally distributed. Number of *Neochetina* spp. ($W = 0.49609$, $P = 0.00002$) at Hectospruit was not normally distributed. The number of *E catarinensis* ($W = 0.77516$, $P = 0.03$) and *Neochetina* spp. ($W = 0.79689$, $P = 0.05$) on the visited sites at Kanyamazane were not normally distributed. The number of *O. terebrantis* ($W = 0.82092$, $P = 0.08$) on sampled plants per quadrat were normally distributed at Kanyamazane. Number of *E. catarinensis* ($W = 0.89021$, $P = 0.32$), *Neochetina* spp. ($W = 0.86626$, $P = 0.21$), and *O. terebrantis* ($W = 0.82481$, $P = 0.09$) on the visited sites at Vergenoeg were normally distributed.

To measure statistical differences in the establishment levels and abundance of biological control agents between water bodies (i.e., along river and dams), one-way analysis of variance (ANOVA) was used for data that met the assumptions of normality. The analysis was followed by the Least Significant Difference (LSD) to measure significant differences between plots at 95% Confidence interval. Mann-Whitney U test was conducted to measure statistical differences on the establishment levels and abundance of the biocontrol agents between the aquatic ecosystems for data that was not normally distributed. Spearman's rank correlation was used to determine the relationship between nutrients, distance and establishment levels or abundance of biological control agents. The statistical analysis was performed using Statistica 13.5.0 version.

2.3. RESULTS

2.3.1. Insecticides on *P. crassipes*

Surprisingly there were no toxic compounds or droplets of insecticides detected on the *P. crassipes* leaf samples from either the dam or the river sites (Table 2.3). Thus the role of insecticide drift onto water hyacinth leaves, and thereby the impact on the biological control agents, seems to be negligible at the time that this study was completed.

Table 2.3: Insecticides screened on *P. crassipes* leaf samples

Trade name	Active ingredients	Concentration (mg/l)
Methomex® 200 SL	Methomyl	0
Pyrinex® 250 CS	Chlorpyrifos	0
Delegate™ 250 WG	Spinetoram	0
Tivoli 240 SC	Spirotetramat	0
Walker 240 SC	Methoxyfenozide	0

2.3.2. Establishment of biocontrol agents on *P. crassipes*

Of the nine biological control agents released on *P. crassipes* in South Africa, *E. catarinensis*, *Neochetina* spp. (considered together), and *O. terebrantis* were established on sampled plants on the Crocodile River and dams. *Eccritotarsus catarinensis*, *Neochetina* spp., and *O. terebrantis* were present on *P. crassipes* on both water body types and at the three sites of each surveyed region.

Although there was no statistical difference (U = 1.500000, $P < 0.19$) in the percentage number of plants that had *E. catarinensis* between the aquatic ecosystems at Hectospruit, more plants were infested by the agent on the dam near to citrus orchards (Figure 2.2). There was significantly ($F_{1, 4}$ = 21.235, P= 0.009) fewer plants that had *E. catarinensis* on the dam compared to the Crocodile River at Kanyamazane. Although there was no statistical difference ($F_{1, 4}$ = 2.4194, P= 0.19) in the mean percentage number of plants infested by *E. catarinensis* between the visited water bodies at Vergenoeg, fewer plants had the agent on dam near to citrus orchards.

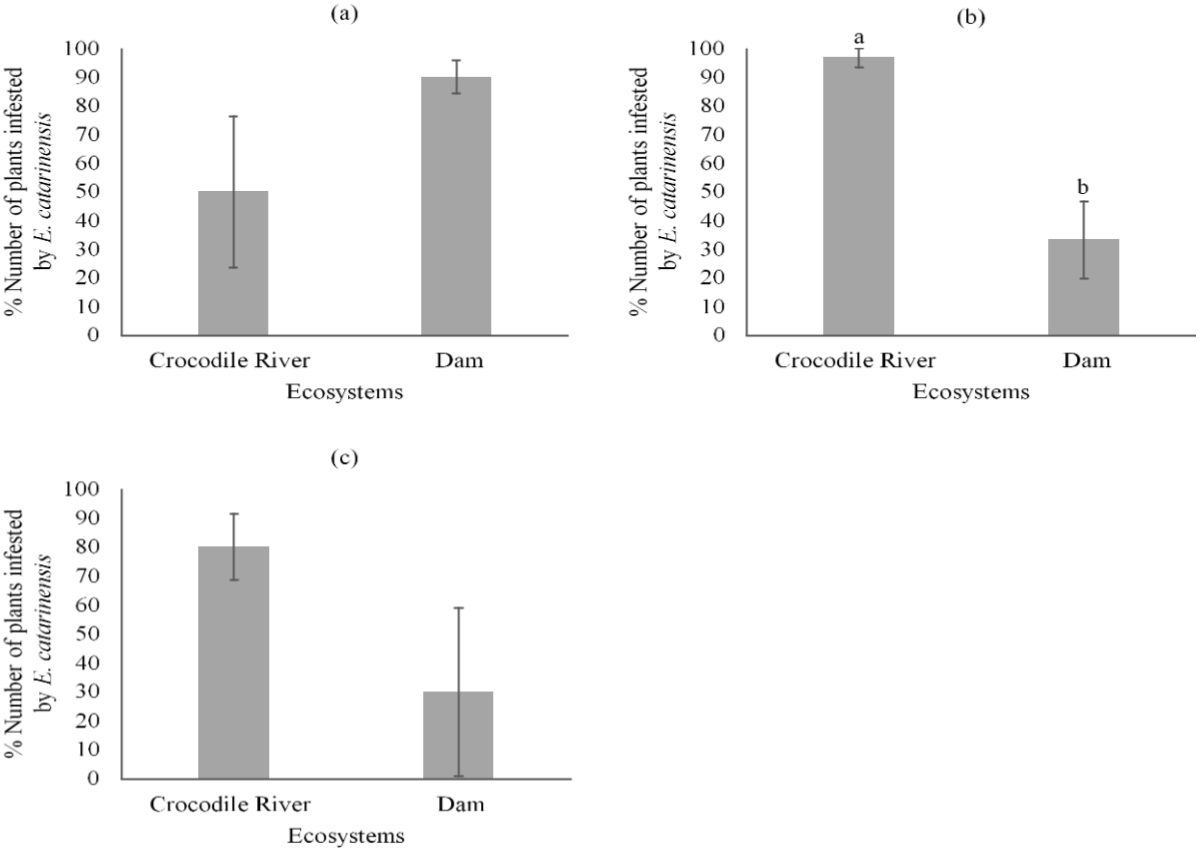

Figure 2.2: Mean (±SE) percentage number of plants that had *E. catarinensis* on *P. crassipes* reserves on the Crocodile River and dams adjacent to citrus orchards at Hectospruit (a), Kanyamazane (b), and Vergenoeg (c). Means with the same letter are not statistically different ($P < 0.05$).

All plants assessed on either freshwater bodies at Hectospruit were infested by *Neochetina* spp. There was no statistical difference in the percentage number of plants infested by *Neochetina* spp. between the Crocodile River and dam adjacent to citrus orchards at Kanyamazane (U = 3.000000, $P < 0.51$) (Figure 2.3). There was also no statistical difference in the percentage number of plants infested by *Neochetina* spp. between Crocodile River and dam at Vergenoeg (U = 4.000000, $P < 0.83$).

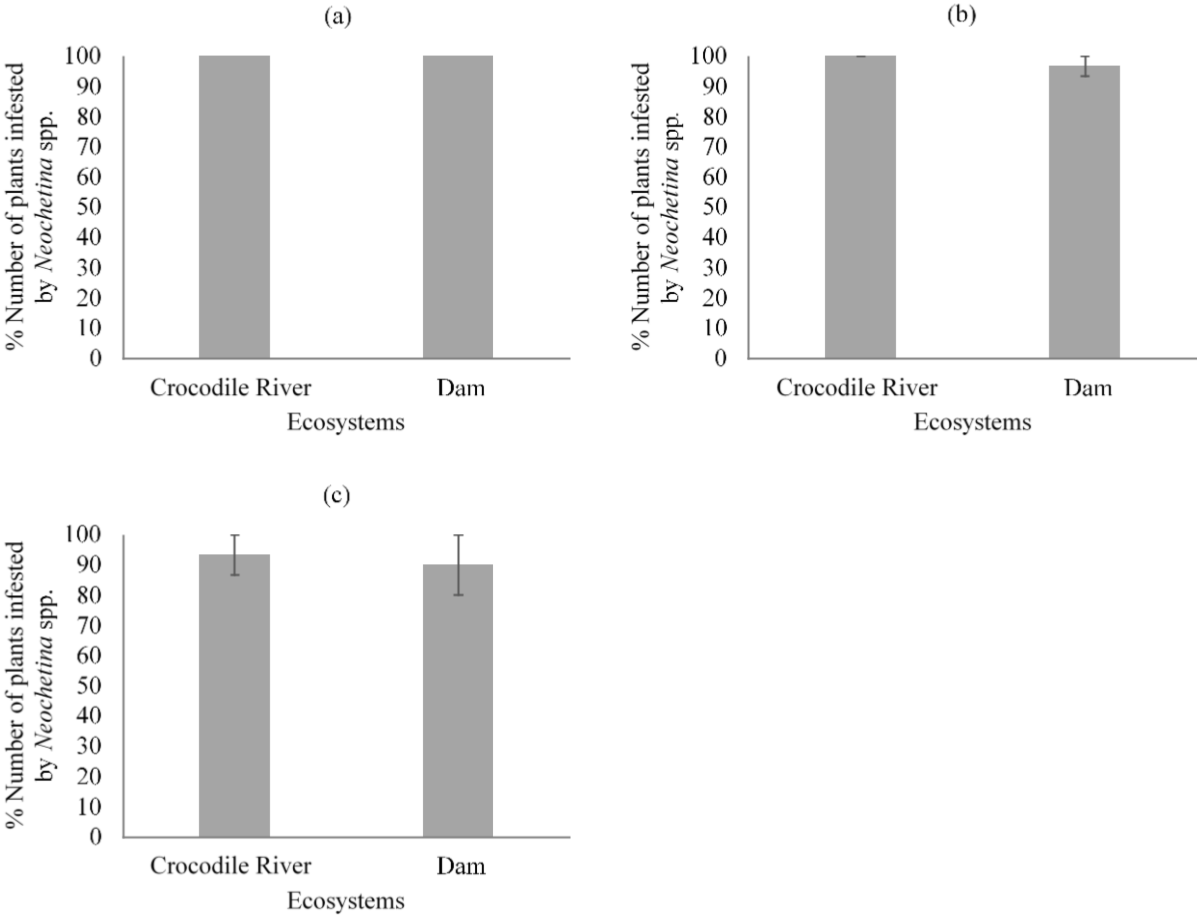

Figure 2.3: Mean (±SE) percentage number of plants that had *Neochetina* spp. on *P. crassipes* reserves on the Crocodile River and dams adjacent to citrus orchards at Hectospruit (a), Kanyamazane (b), and Vergenoeg (c).

There was no statistical difference in the percentage number of plants that had *O. terebrantis* (*U* = 4.500000, *P* < 1.00) between *P. crassipes* plants sampled on the Crocodile River and dam adjacent to citrus orchards at Hectospruit (Figure 2.4). However, there was a statistical difference in the percentage number of plants that had *O. terebrantis* between plants sampled on the Crocodile River and dam at Kanyamazane (*U* = 0.00, *P* < 0.049). Although the number of plants that had *O. terebrantis* did not differ statistically (*U* = 2.000000, *P* < 0.28) between the aquatic ecosystems at Vergenoeg, more plants were infested by the agent on the dam.

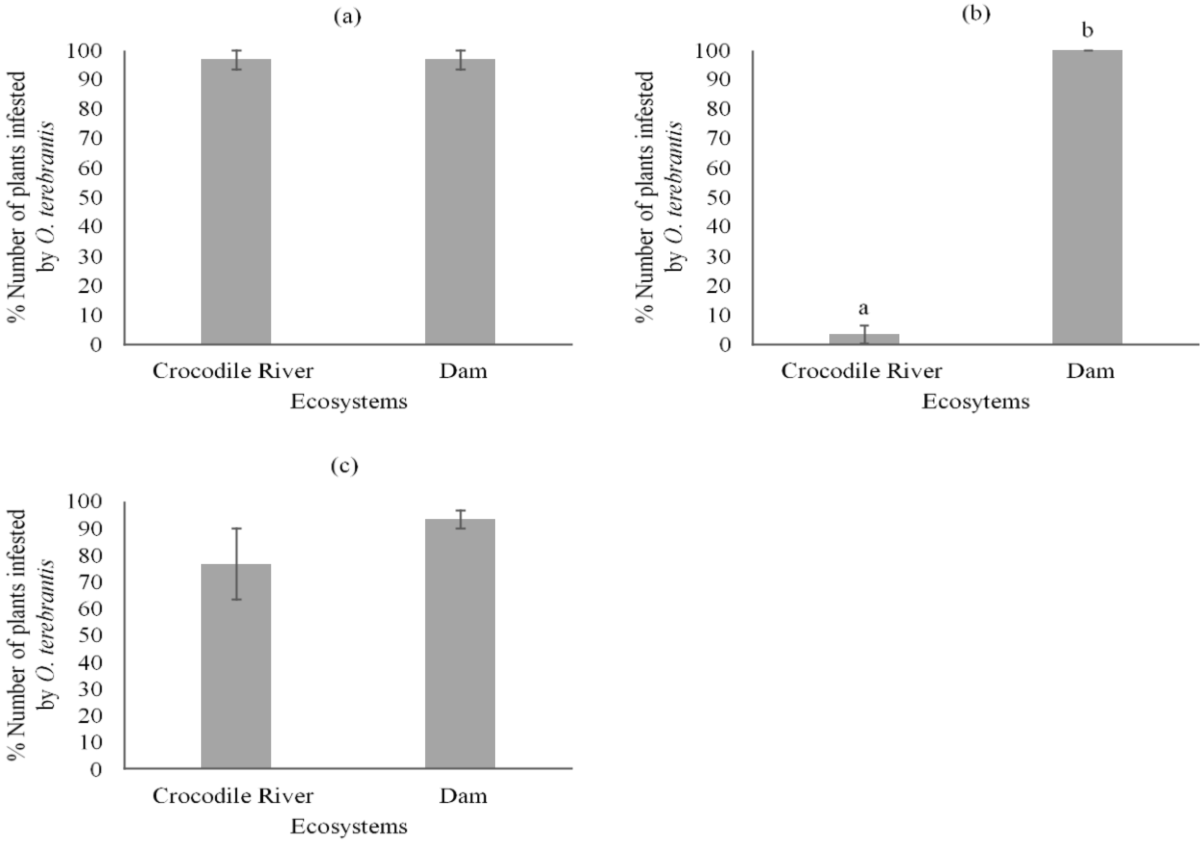

Figure 2.4: Mean (±SE) percentage number of plants that had *O. terebrantis* on the Crocodile River and dam adjacent to citrus orchards at Hectospruit (a), Kanyamazane (b), and Vergenoeg (c). Means with the same letter are not statistically different (*P*< 0.05).

2.3.3. Abundance of biocontrol agents on *P. crassipes*

The number of each of the naturalized biological control agents recorded on *P. crassipes* differed between sites visited along citrus orchards on the Crocodile River and dams (Table 2.4). But, there was no statistical difference in the number of *E. catarinensis* ($F_{1,4} = 2.4143$, $P = 0.19$) between the Crocodile River and dam at Hectospruit. Similarly, there was no statistical difference in the number of *Neochetina* spp. ($U = 3.000000$, $P < 0.51$), and *O. terebrantis* ($F_{1,4} = 4.6875$, $P = 0.09$) between the Crocodile River and dam at Hectospruit.

There was a significantly higher number of *E. catarinensis* ($U = 0.00$, $P < 0.04$) on the Crocodile River compared to the dam at Kanyamazane. Although there was no statistical difference in the number of *Neochetina* spp. ($U = 3.000000$, $P < 0.51$) between the water bodies at Kanyamazane, the numbers were slightly high on the dam. There was significantly high number of *O. terebrantis* ($F_{1,4} = 60{,}017$, $P = 0.0015$) on the dam compared to the Crocodile River at Kanyamazane.

There was no statistical difference in the number of *E. catarinensis* ($F_{1,4} = 0.71915$, $P = 0.44$) recorded on *P. crassipes* between the sites on the Crocodile River and dam at the Vergenoeg. There was also no statistical difference in the number of *Neochetina* spp. ($F_{1,4} = 0.0000$, $P = 1.00$) between the visited sites on the Crocodile River and dam at Vergenoeg. There was significantly high number of *O. terebrantis* ($F_{1,4} = 11.529$, $P = 0.027$) on the dam compared to the number recorded on the Crocodile River at Vergenoeg.

Table 2.4: Mean (±SE) number of biocontrol agents on *P. crassipes* plants sampled on the Crocodile River and dams adjacent to citrus orchards at three selected sites

	Number of individuals per quadrat		
Site	***E. catarinensis***	***Neochetina* spp.**	***O. terebrantis***
Hectospruit			
Crocodile River	5.00 (± 2.65) a	12.0 (± 2.00) a	20.0 (± 2.00) a
Dam	9.33 (± 0.88) a	10.0 (± 0.00) a	15.0 (± 1.15) a
Kanyamazane			
Crocodile River	10.0 (± 0.00) a	10.0 (± 0.00) a	0.33 (± 0.33) a
Dam	3.33 (± 1.33) b	11.3 (± 1.45) a	20.0 (± 2.52) b
Vergenoeg			
Crocodile River	9.00 (± 2.08) a	9.33 (± 0.67) a	8.00 (± 1.00) a
Dam	4.67 (± 4.67) a	9.33 (± 1.20) a	22.0 (± 4.00) b

Means followed by different letters indicate statistical difference by post-hoc using least significant difference, and Mann-Whitney U test ($P < 0.05$).

2.3.4. Water nutrients

Amongst the nutrients that influence growth of *P. crassipes* and population dynamics of biological control agents, nitrate was slightly higher on the Crocodile River than dams at Hectospruit, Kanyamazane and Vergenoeg (Table 2.5). The visited sites were all considered eutrophic for phosphorus, and they ranged from oligotrophic to mesotrophic for nitrate.

Table 2.5: Nutrients in water sampled on the Crocodile River and dams at Hectospruit, Kanyamazane, and Vergenoeg.

		Sites	
Farm	**Nutrients (mg/l)**	**River**	**Dam**
Hectospruit	Nitrate as N	0.40	0.34
	Nitrite as N	<0.02	<0.02
	Potassium (K)	1.3	1.6
	Phosphorous (P)	<0.10	<0.10
Kanyamazane	Nitrate as N	0.76	0.36
	Nitrite as N	<0.02	<0.02
	Potassium (K)	1.1	1.4
	Phosphorous (P)	<0.10	<0.10
Vergenoeg	Nitrate as N	1.12	0.49
	Nitrite as N	<0.02	<0.02
	Potassium (K)	0.91	1.5
	Phosphorous (P)	<0.10	<0.10

2.3.5. Relationship between nutrients and establishment of biocontrol agents on *P. crassipes*

There was no significant relationship between nitrate and *P. crassipes* infested by *E. catarinensis* (r_s = -0.45, *P*= 0.45), *Neochetina* spp. (r_s = -0.56, *P*= 0.32), and *O. terebrantis* (r_s = -0.67, *P*= 0.22) at the visited sites on the Crocodile River and dams.

2.3.6. Relationship between nutrients and abundance of biocontrol agents on *P. crassipes*

There was no significant relationship between nitrate and number of *E. catarinensis* (r_s = -0.45, *P*= 0.45), *Neochetina* spp. (r_s = -0.56, *P*= 0.32), and *O. terebrantis* (r_s = -0.10, *P*= 0.87) on *P. crassipes* reserves at the visited sites.

2.3.7. Relationship between distance from citrus orchards and establishment of biocontrol agents on *P. crassipes*

The sites on the Crocodile River were 78.63m (Hectospruit), 291.98m (Kanyamazane), and 79.99m (Vergenoeg) from the edge of the sprayed citrus orchards respectively. The dams were 10.50m (Hectospruit), 76.31m (Kanyamazane), and 29.91m (Vergenoeg) away from citrus orchards.

There was no significant relationship between distance from citrus orchards and *P. crassipes* plants infested by *E. catarinensis* (r_s = 0.43, *P*= 0.39), *Neochetina* spp. (r_s = 0.15, *P*= 0.77), and *O. terebrantis* (r_s = -0.69, *P*= 0.12) on *P. crassipes* reserves at the visited sites.

2.3.8. Relationship between distance from citrus orchards and abundance of biocontrol agents on *P. crassipes*

There was no significant relationship between distance from citrus orchards and number of *E. catarinensis* (r_s = 0.20, *P*= 0.70), *Neochetina* spp. (r_s = -0.18, *P*= 0.74), and *O. terebrantis* (r_s = -0.08, *P*= 0.86) on *P. crassipes* reserves at the visited sites.

2.4. DISCUSSION

This study demonstrated that infestation levels on the weed were high, while the population sizes of the naturalized biocontrol agents at the surveyed sites was low in comparison to other parts of the country (Coetzee et al., 2021). Surprisingly insecticide residues were not recorded on any sampled leaves of the plants on the dam and river sites, suggesting that at the time this study was undertaken, spray drift was not important. The findings are not consistent with other studies (Dabrowski et al., 2005; Langhof et al., 2003, 2005) that recorded insecticides drift residues on the surface of non-target plants adjacent to agroecosystems, and showed their detrimental effects on the performance of biocontrol agents of invasive weeds (Hoffmann & Moran, 1995). It is possible that surveyed sites were visited sometime when insecticide application was low or when the droplets residues had either degraded or washed off by rainfall. Hoffmann & Moran, (1995) only reported a decline in the population of a weevil, *T. lativentre* on *S. punicea* from early spring to mid-summer during which there was high application of insecticides from citrus orchards. Langhof et al. (2005) in a study to assess the potential deposition of insecticide drift residues on non-target plants bordering wheat fields, and their acute toxicity to two beneficial insects showed that rainfall one hour post application of λ-cyhalothrin reduced the impact.

At low or undetectable concentrations insecticides cause chronic toxicity which could still influence feeding behaviour, reproductive potential, and delay development rate of biological control agents (Amarasekare & Shearer, 2013; Desneux et al., 2007; Maltby & Hills, 2008). Hasan et al. (2020) in laboratory bioassays reported slow development rate of immatures, reduced fecundity, extended pre-oviposition period, and male bias sex ratio of *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) (a biocontrol agent of *Parthenium hysterophorus* L. (Asteraceae)) adults which survived exposure to recommended field rates of neonicotinoids and diamides. Hasan & Ansari, (2017) also reported slow development rate of immatures, reduced fecundity and extended pre-oviposition period of *Z. bicolorata* in response to contact exposure to insecticides.

The significantly high infestation levels of *O. terebrantis* on the dams suggests that flooding could have an influence on this species. Floating *P. crassipes* plants are easily carried away with the biocontrol agents during high water flow. Thus, the population growth rate of especially sessile agents, such as the mite is delayed and their impact could be limited (Coetzee et al., 2011). Hopper et al. (2021) in the Sacramento-San Joaquin River Delta in California (USA), reported reduction of population intensity of *P. crassipes* and *Neochetina* spp. with the increase in water flow rate. Although agents in enclosed impoundments which are less prone to floods may build up, the lack of wind and wave action prevent the damaged plants from sinking population of *P. crassipes* and biological control agents (Byrne et al., 2010; Hill & Olckers, 2001).

Infestation levels and population abundance of the biological control agents was not influenced greatly by nutrient levels at the surveyed freshwater bodies. The eutrophic level of phosphorus did not correspond to the hypertrophic nutrient status of many freshwater bodies, and the range from oligotrophic to mesotrophic level of nitrates only corresponded to few of the 14 monitoring sites in the country (Byrne et al., 2010). It appears that the nitrate levels were too low to have a significant positive influence on the population abundance of the biological control agents. Bownes et al. (2013a) at mesotrophic (2.52 N mg/l), and oligotrophic (0.034 N mg/l) concentrations of nitrate recorded low fecundity of females, body weights, and survival of *C. aquaticum* nymphs compared to eutrophic level of nitrate in the tissues of *P. crassipes*. This study also realized a female bias sex ratio (65 females: 35 males) at the eutrophic nutrient level and a male bias ratio (39:61) at oligotrophic nutrient level.

Phosphorus is the reproductive and growth limiting nutrient of *P. crassipes*, and increase in this nutrient to 1.06 mg/l has been reported to increase the biomass of the plant (Reddy et al., 1990). Reddy et al. (1990) also demonstrated that at a concentration below or equal to 0.06 mg/l *P. crassipes* biomass declined. Although the concentration of phosphorus in the current study suggest that the growth of *P. crassipes* may have stopped during the period of sampling, the low population density of the biological control agents as a response to lower foliar nitrogen may not inflict significant damage to reduce reproductive potential and proliferation of the plant.

2.5. CONCLUSION

The outcomes of the current study indicated that population abundance of biological control agents of *P. crassipes* was lower than those of other parts of the country, but insecticide drift from citrus orchards may not be responsible for the slow population increase of the insects. High water levels along with the spatial and temporal variation of nutrient availability in aquatic ecosystems appeared to be the factors influencing the establishment and impact of the biological control agents on *P. crassipes* at the Lowveld regions of Mpumalanga. Understanding how each of the factors influencing the insect-plant complex interactions in invasive weed management systems may assist in decision making on the timing of augmentative releases. Surveys to assess the pattern of establishment and population abundance of biocontrol agents should be also conducted in the spring or summer to possibly determine the variation of potential insecticide drift with seasons. Acute toxicity assessments of commonly applied insecticides on citrus orchards would be necessary to determine the possible influence of spray drift residues on the feeding intensity and population abundance of the biocontrol agents.

CHAPTER THREE

The impact of insecticides used against citrus pests on biocontrol agents of *Pontederia crassipes* (Pontederiaceae) in laboratory bioassays

3.1. INTRODUCTION

Extensive use of broad-spectrum insecticides successfully reduce pest loads in agroecosystems, but injudicious use can have undesired harmful effects (Cordeiro et al., 2010; Maltby & Hills, 2008). Although inappropriate use of insecticides is discouraged and ways to mitigate detrimental effects (*viz*. integrated management, novel pesticide use) have been implemented, their non-target effects in agroecosystems and adjacent habitats remains a global concern (Botías et al., 2015, 2017; Dai et al., 2020; Ricupero et al., 2020). Notable effects of insecticides on the performance of beneficial insects inhabiting plants adjacent to agroecosystems has been reported (Hoffmann & Moran, 1995; Langhof et al., 2003, 2005; Otto et al., 2013). Amongst threatened beneficial insects are pollinators, parasitoids, predators and biological control agents of invasive alien plants (El-heneidy et al., 2016; Hoffmann & Moran, 1995; Muslim et al., 2018; Ricupero et al., 2020). Prolonged development rate and reduced population abundance are amongst the effects of insecticides which limit the performance of biological control agents of invasive alien plants (Hasan et al., 2020; Hasan & Ansari, 2017; Hoffmann & Moran, 1995).

Multitudes of studies have shown harmful effects of commonly used insecticides (*viz*. Methomyl and Chlorpyrifos) on beneficial insects in agroecosystems and adjacent habitats (Botías et al., 2016, 2017; Dai et al., 2020; Frampton & Brink, 2007; Langhof et al., 2003). Beneficial insects in the field are inadvertently exposed to insecticides directly during their application or by coming into contact with the toxic residues on a plant surface, and indirectly by foraging on the contaminated tissues of the host plant (Botías et al., 2016; Calvo-Agudo et al., 2020; Katembo et al., 2020). The magnitude of impacts by the insecticides varies between life stages (Langhof et al., 2005; Hasan et al., 2020; Ono et al., 2017; Wang et al., 2008). Immature life stages are the most affected developmental stage and the impact is demonstrated by the prolonged development rates and reduced body size (Desneux et al., 2004; George et al., 2007; Ono et al., 2017; Prouty et al., 2021; Whitehorn et al., 2018), while exposure at the adult stage influences longevity, mobility, sex ratio, fecundity, feeding, ovipositional behaviour, and survival (Desneux et al., 2007; Hasan et al., 2020).

The success of biological control of invasive alien plants is determined by the ability of biological control agents to establish and their impact on the target weed (Mukwevho et al., 2017a; Schwarzländer et al., 2018). In some instances, biological control agents have successfully established but have had negligible impact in some parts of South Africa (Coetzee et al., 2011; Hill & Coetzee, 2017). This failure to limit growth and reproductive potential of invasive weeds has been reported along agroecosystems where there is intensive use of insecticides (Hoffmann & Moran, 1995). Potential insecticide drift from agroecosystems could reduce the population abundance and impact of naturalized biological control agents of *P. crassipes,* although not shown in this study (chapter two). Prolonged persistence of the spray drift residues on the surface of the host plant may have greater impact on the feeding behaviour and survival of biological control agents (Hasan et al., 2020; Langhof et al., 2003).

Whilst studies have demonstrated the successful exclusion of biological control agents of invasive alien plants by the commonly used insecticides against citrus pests (Katembo et al., 2019, 2020; Mukwevho et al., 2017b; Mukwevho & Mphephu, 2020), and their acute toxicity (Hasan et al., 2020), little is known about the effects of insecticides on the population abundance and feeding behaviour of biocontrol agents of *P. crassipes*. Jones et al. (2018) reported significant reduction in feeding damage of *N. eichhorniae* on *P. crassipes* treated with Actara SC^{TM} (active ingredient: thiamethoxam) when compared to herbivory on untreated plants on the Nseleni River, South Africa. The current study investigated the acute toxicity of neurotoxic insecticides that are used to manage citrus pests on the biological control agents of *P. crassipes* under laboratory conditions.

3.2. MATERIALS AND METHODS

3.2.1. Collection and maintenance of *P. crassipes*

Pontederia crassipes plants were collected from the Crocodile River (25°27′45.45″S 030°57′51.10″E). Newly recruited populations of the plant were propagated and maintained in a pool at the University of Mpumalanga in a tunnel (25°25′36.81″S 30°58′17.75″E). Water was added to keep the roots submerged and N.P.K (2.3.2) fertiliser was applied to ensure that plants were well nourished. Plants were used as rearing material for the insect culture and the day and night temperatures in the tunnel ranged between 20°C and 30°C.

3.2.2. Rearing of insects

Immature and adults of *Megamelus scutellaris,* and *N. eichhorniae* adults were collected from the Crocodile River (25°27′45.45″S 030°57′51.10″E). Additional colonies of the two biological control agents were sourced from SASRI and Rhodes University. Distinctive morphological features such as the chevron stripe on the elytra of *N. bruchi* which is absent on *N. eichhorniae* was used to differentiate between the two weevils and for this experiment only *N. eichhorniae* was used (Deloach and Cordo, 1976). The colour of the markings on the dorsal surface were used to distinguish between immatures and adults of *M. scutellaris* (Sosa et al., 2005).

The agents were released separately on *P. crassipes* plants in large cages (56cm x 36cm x 38cm) to mate and lay eggs for 14 days. Fertiliser was applied and the cages were covered with a fine mesh net to prevent insects from escaping. Plants were moved after 14 days from cages to the main pool for insects to complete their development. Insects were provided with a new set of plants to mate and lay eggs. The number of adults emerging were recorded monthly from December 2020 to June 2021. Adults were collected from the rearing pool at the university and taken to the laboratory two days before the start of the experiment. Insects in the laboratory were then sexed under a microscope based on their distinct morphological characters (*viz*. body size, markings and shape of rostrum). *Neochetina eichhorniae* males and females are approximately 3.2 and 3.7 mm in length (Deloach & Cordo, 1976; Kariuki & Minteer, 2021). The males have a thick and slightly curved rostrum, whereas, female rostrum is strongly curved and slender (Deloach & Cordo, 1976). Adult males of *M. scutellaris* are between 2.5 and 2.9 mm in length, the female body length is 3.1 to 3.7 mm (Sosa et al., 2005).

3.2.3. Insecticides used

For the purpose of this experiment, three concentrations of insecticides were used at rates below recommended, recommended and above recommended field rates (see Table 3.1). To determine the range of concentrations to use, preliminary studies were conducted using *N. bruchi*. A two-fold geometric ratio was used between concentrations of each chemical and the dose regimes that caused 0-100% mortality were identified. The recommended dosages of 20g/ 100L and 200ml/ 100L for Methomex (Methomyl) and Pyrinex (Chlorpyrifos) were used and each was reduced to a litre of deionised water for dilution. The two chemical products were selected due to their wide usage in citrus orchards and their known toxic effects on beneficial insects (Muslim et al., 2018).

Each concentration was prepared separately in a 250ml beaker. Undiluted deionized water was used as a control. Insects were exposed to a range of concentrations of the selected insecticides by topical treatment which closely resemble the contact exposure route in the field. Using a pipette, a 0.02-ml droplet of each solution was applied directly on the dorsal part of each test species. Leaf dipping which closely resemble the chemical residue exposure route was also conducted. The sexed groups of *M. scutellaris* were refrigerated for less than a minute to limit their activeness prior to treatment as they are very active and difficult to manipulate.

Table 3.1: Detailed information of insecticides tested on biocontrol agents of *P. crassipes*

			Tested concentrations (mL/L)		
Trade name	Chemical group	Manufacturer	Above	Recommended	Below
Methomex® 200 SL	Carbamate	ADAMA	0.4	0.2	0.1
Pyrinex® 250 CS	Organophosphate	ADAMA	4	2	1

3.2.4. Experimental layout

For the two exposure techniques, a total of 126 individuals for each of the two biocontrol agents (54 for each insecticide and 18 for the control) were used for the purpose of this study. Forty two plastic containers (18 per insecticide, 9 for each exposure technique and 6 for controls) were used for each insect (Figure 3.1). The biological control agents were placed in one container in groups of six (three males and females). To acclimatize, the biological control agents were then placed in respective containers (10.5cm diameter x 10cm height) a day before the treatments. The treatments were replicated three times. Leaves of different sizes (6 x 4cm to 11 x 6cm (diameter and width)) were provided as food. To maintain moisture, the petioles were cut 5cm from the tip of the leaf lamina and wrapped with moist cotton. Fresh leaves of *P. crassipes* were also dipped for 10s in prepared insecticide solutions for contact insecticide residue exposure. Excess moisture was allowed to evaporate and leaves were placed in plastic containers of respective treatments.

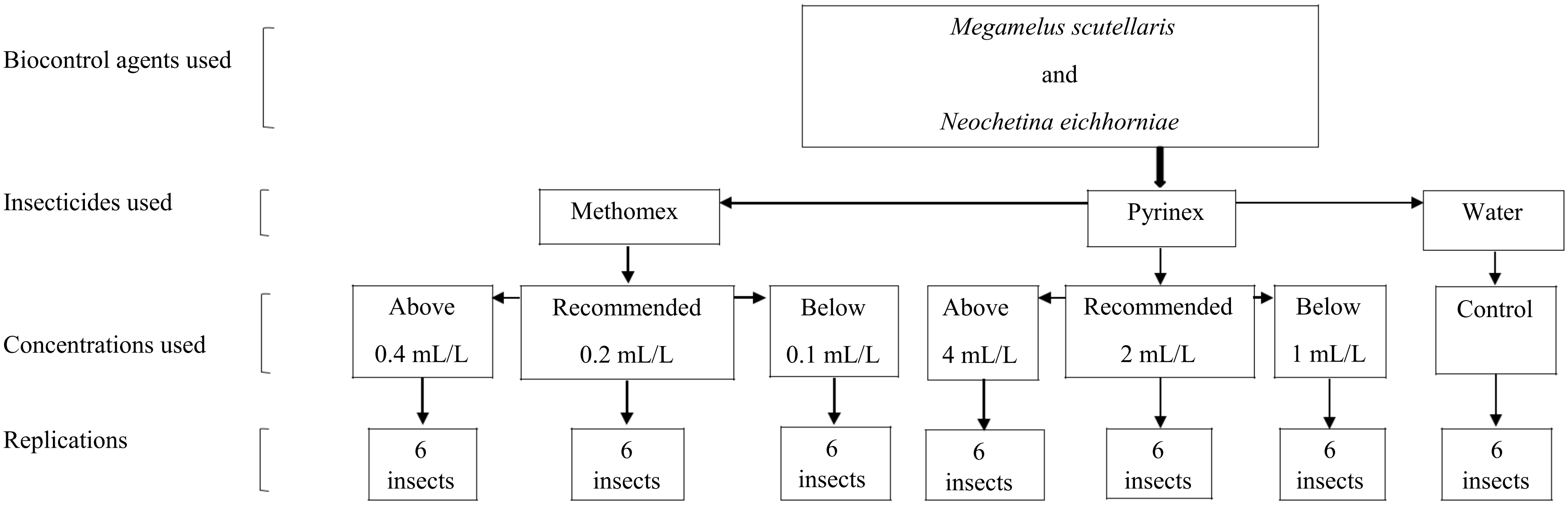

Figure 3.1: The experimental layout for the biological control agents that were exposed to Methomyl and Chlorpyrifos at concentrations above recommended, recommended, and below the recommended field rates. The treatments were replicated three times, and deionized water was applied as a control.

3.2.5. Effect of insecticides on the feeding damage and survival of biocontrol agents

To assess the killing effect of different concentrations of insecticides against *Megamelus scutellaris* and *N. eichhorniae,* topically treated insects and those feeding on dipped leaf of *P. crassipes* were prodded with a fine paintbrush. Individuals which displayed no movement were considered dead, those that moved were recorded as live. Number of adults alive per experimental unit was recorded at 24 h intervals for 120 h. Within the first 24 h number alive per experimental unit were recorded at 0.5 h, 1 h, 2 h, 4 h, 8 h and 16 h. The study was terminated when survival reached 0% for the tested concentrations. The percentage survival of each insect at different concentrations and time intervals was calculated. Percentage survival was determined by dividing the number of live individuals by the total insects used per experimental unit (6) and multiplied by 100. Feeding scars were also counted and recorded on the leaf surface at the end of the study. Since feeding of *M. scutellaris* does not cause visible damage signs, frass black spots on the surface of leaves were counted under a microscope (Sosa et al., 2007).

3.2.6. Statistical analysis

Survival period, percentage survival (i.e., topically treated and those feeding on dipped leaves of *P. crassipes*) of biocontrol agents and feeding damage data was analyzed statistically with Statistica 13.5.0. Data was tested for normality using the Shapiro-Wilk test. Normally distributed data ($P > 0.05$) was subjected to parametric analysis [i.e., one-way analysis of variance (ANOVA)]. Data which failed to meet normal distribution assumption was analysed using the non-parametric Mann-Whitney U test and Kruskal-wallis test. Post-hoc analysis (i.e., least significant difference (LSD) and multiple comparisons of means at $P < 0.05$ for parametric and non-parametric analysis, respectively) was used to measure the statistical significance on the survival period, percentage survival of biocontrol agents and feeding damage induced on *P. crassipes* leaves.

3.3. RESULTS

3.3.1. Impact of topically applied insecticides on the biocontrol agents of *P. crassipes*

Survival period of *M. scutellaris* varied significantly for different concentrations of Methomyl ($H = 12.69190$, $df = 2$, $P < 0.001$; n = 54) and Chlorpyrifos ($H = 14.86650$, $df = 2$, $P < 0.006$; n = 54) (Table 3.2). The agent survived for up to 2.7 and 10.2 hours when treated with the concentrations below the field recommended rates of Methomyl and Chlorpyrifos, respectively. The survival periods declined by up to 1.2 and 2.6 fold for Methomyl at the field recommended and above recommended rate, respectively. Similarly, the survival period significantly declined by 1.3 and 2.5 fold for *M. scutellaris* treated with field recommended and above recommended rates of Chlorpyrifos, respectively. Survival period of *M. scutellaris* was significantly lower for adults treated with Methomyl compared to those treated with Chlorpyrifos at all three concentrations.

Although survival period of *N. eichhorniae* varied significantly for different concentrations of Methomyl ($H = 8.254822$, $df = 2$, $P < 0.01$; n = 54), survival period did not differ significantly for different concentrations of Chlorpyrifos ($H = 0.9927537$, $df = 2$, $P < 0.61$; n = 48). *Neochetina eichhorniae* survived for up to 4.3 and 72.0 hours when treated with concentrations below the field recommended rates of Methomyl and Chlorpyrifos, respectively. The survival period of *N. eichhorniae* declined by up to 1.1 and 3.5 fold for Methomyl at the field recommended and above recommended rate. Similarly, the survival period significantly declined by 1.1 fold for *N. eichhorniae* treated with field recommended and above recommended rates of Chlorpyrifos. Survival period of *N. eichhorniae* was significantly lower for adults treated with Methomyl compared to those treated with Chlorpyrifos at all three concentrations.

Table 3.2: Survival (mean ±SE) period of biological control agents of *P. crassipes* after being topically treated with different concentrations of Methomyl and Chlorpyrifos**.**

Biocontrol agent	Concentrations	Mean (±SE) Survival		Statistics		
		Methomyl	**Chlorpyrifos**	**N**	***U- test value***	***P* value**
M. scutellaris	Below	2.7 (± 0.5) b	10.2 (± 1.3) b	18	28.000	**< 0.001**
	Recommended	2.2 (± 0.4) b	8 (± 1.3) b	18	26.500	**< 0.001**
	Above	1.0 (± 0.1) a	4.1 (± 0.6) a	18	17.000	**< 0.001**
N. eichhorniae	Below	43.1 (± 8.6) b	72.0 (± 11.3) a	18	74.500	< **0.05**
	Recommended	38.9 (± 8.6) ab	64.6 (± 10.5) a	18	85.500	**< 0.04**
	Above	11.0 (± 3.6) a	58.0 (± 35.8) a	18	29.500	**< 0.002**

Statistically significant (<0.05) *P-values* are marked in bold.

At least 72%, 94% and 100% of *M. scutellaris* survived the topically applied concentrations of Methomyl at above field recommended, recommended and below recommended rate after 0.5 h of exposure (Figure 3.2). Survival rate declined by up to 1.1 and 1.3 fold for *M. scutellaris* exposed to concentrations applied at the field recommended and above recommended rate of Methomyl, respectively. All *M. scutellaris* were dead after being exposed to concentrations of Methomyl applied at the rates above the field recommended for 2 h, whereas, it took up to 8 h to kill the populations of *M. scutellaris* exposed to Methomyl applied at field recommended and that below recommended rates. Survival of *M. scutellaris* which was topically treated with Chlorpyrifos was longer with 100% survival rate recorded for all concentrations at 0.5 h. Noticeable reduction in the survival rate of *M. scutellaris* was 94% at 1 h for the concentration above field recommended, hence, survival rate of 44% and 67% was recorded at 4 h for the field recommended and that below recommended, respectively. After 4 h survival declined by up to 1.5 and 2 fold at the field recommended and above recommended rate of Chlorpyrifos, respectively. Survival was 0% after 8 h for the concentration above field recommended, whereas, it took up to 16 h to kill the population of *M. scutellaris* for the recommended and below recommended rates of Chlorpyrifos. Survival of *M. scutellaris* in the controls declined to 94.4% at 24 h following treatment.

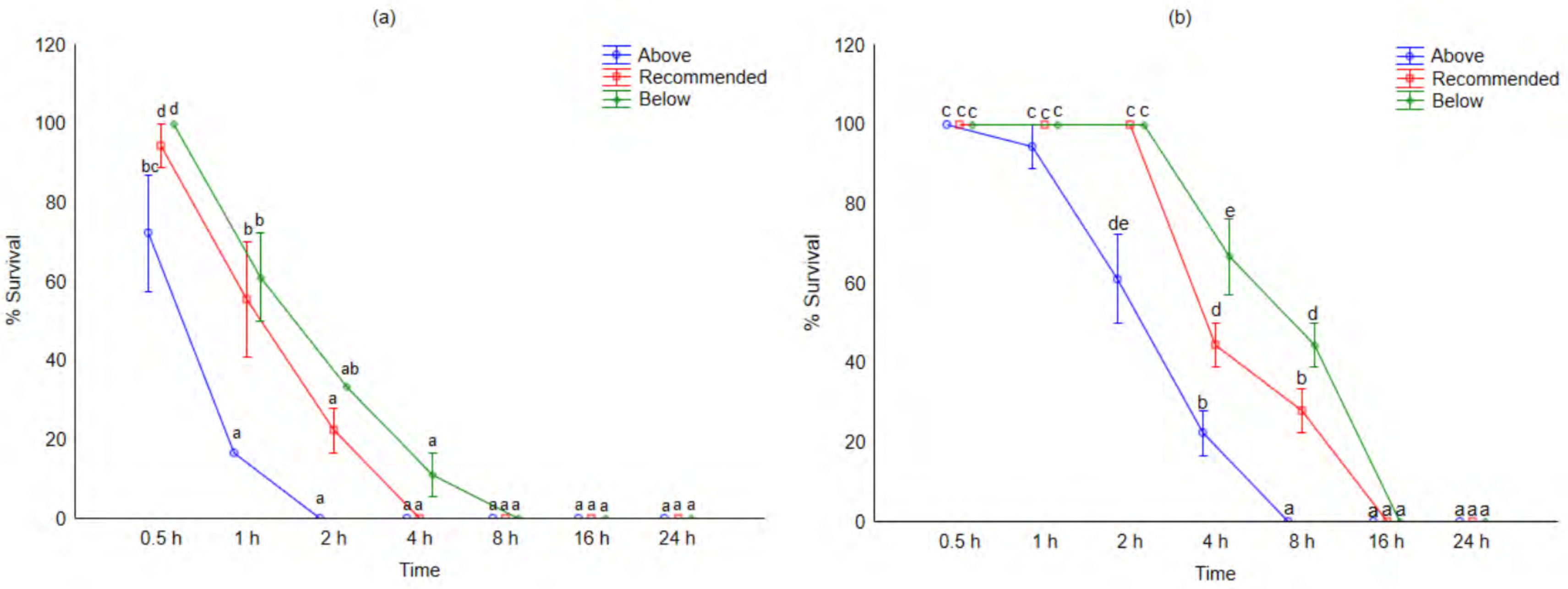

Figure 3.2: Mean (±SE) percentage survival of *M. scutellaris* adults treated topically with Methomyl (a) and Chlorpyrifos (b) at the concentrations below field recommended, recommended, and above recommended rate. Survival rate was evaluated between 0.5 h and 24 h. Means followed by the same letters are not statistically different ($P > 0.05$) between concentrations at each time interval and from 0.5 h to 24 h for each dosage.

At 0.5 h survival of *N. eichhorniae* was 100% for all concentrations of Methomyl (Figure 3.3). A noticeable decline of 67%, 89% and 94% was recorded at 1 h for the concentration above field recommended, recommended and below recommended rate of Methomyl. The survival rate of *N. eichhorniae* declined by up to 1.1 and 1.3 fold at the field recommended and above recommended rate of Methomyl, respectively. Survival was 0% after 48 h for the concentration above field recommended, whilst, it took up to 96 h to induce complete mortality for the field recommended and below recommended rates of Methomyl. Survival of *N. eichhorniae* treated with Chlorpyrifos was longer with 100% survival rate for all concentrations at 2 h. A noticeable decline of 94% was only recorded at 4 h for the concentration above field recommended rate, hence, a decline of 94% and 78% was recorded after 16 h and 24 h for the field recommended and below recommended rate. After 24 h survival declined by up to 1.1 fold at the field recommended and above recommended rate of Chlorpyrifos, respectively. Survival was 0% after 120 h for the concentration above field recommended rate, whilst, it only declined to 11% and 22% at the field recommended and below recommended rates of Chlorpyrifos.

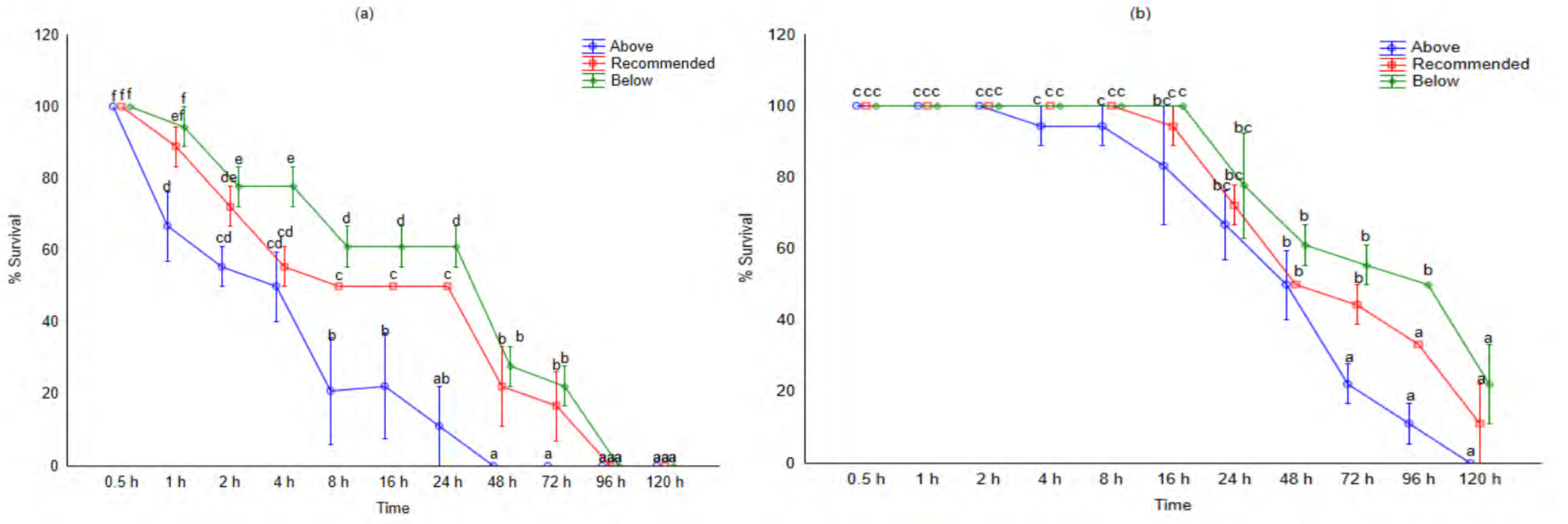

Figure 3.3: Mean (±SE) percentage survival of *N. eichhorniae* treated topically with Methomyl (a) and Chlorpyrifos (b) at the concentrations below field recommended, recommended, and above recommended rate. Survival rate was evaluated between 0.5 h and 120 h. Means followed by the same letters are not statistically different ($P > 0.05$) between concentrations at each time interval from 0.5 h to 120 h for each dosage.

3.3.2. Effect of leaf dip insecticide treatment on biocontrol agents of *P. crassipes*

Although survival period of *M. scutellaris* did not differ significantly when exposed to different concentrations of Methomyl residues on *P. crassipes* leaves ($H = 2.428453$, $df = 2$, $P < 0.29$; n = 54), survival period varied significantly for different concentrations of Chlorpyrifos residues ($H = 7.076004$, $df = 2$, $P < 0.02$; n = 54) (Table 3.3). The agent survived for up to 4 and 9.4 hours when exposed to Methomyl residues at the concentrations below the field recommended rates of Methomyl and Chlorpyrifos. *Megamelus scutellaris* survival period declined by up to 1.2 and 1.3 fold at the field recommended and above recommended rates of Methomyl residues. The survival period declined by 1.3 and 2 fold for *M. scutellaris* exposed to Chlorpyrifos residues at the field recommended and above recommended rate, respectively. Survival period of *M. scutellaris* was significantly lower for adults exposed to Methomyl residues compared to those exposed to residues of Chlorpyrifos at the concentration below field recommended and recommended rate.

Survival period of *N. eichhorniae* did not vary significantly when exposed to different concentrations of Methomyl ($H = 4.246350$, $df = 2$, $P < 0.12$; n = 51) and Chlorpyrifos ($H = 3.067906$, $df = 2$, $P < 0.22$; n = 46) dried residues on *P. crassipes* leaves (Table 3.4). *Neochetina eichhorniae* survived up to 77.2 and 85.7 hours when exposed to dried residues at the concentrations below the field recommended rates for Methomyl and Chlorpyrifos. Survival period of *N. eichhorniae* declined by up to 1.2 and 1.4 fold at the field recommended and above recommended rate of Methomyl dried residues. *Neochetina eichhorniae* survival period declined by 1.1 fold when exposed to the field recommended and above recommended rate of Chlorpyrifos residues. Survival period of *N. eichhorniae* did not differ significantly for adults exposed to Methomyl dried residues when compared to those exposed to residues of Chlorpyrifos at all three concentrations.

Table 3.3: Survival (mean ±SE) period of biological control agents of *P. crassipes* after being exposed to dried residues of Methomyl and Chlorpyrifos on treated leaves**.**

Biocontrol agent	**Concentrations**	**Mean (±SE) Survival**		**Statistics**		
		Methomyl	**Chlorpyrifos**	**N**	***U- test value***	***P* value**
M. scutellaris	Below	4.0 (± 0.6) a	9.4 (± 1.9) b	18	98.000	< **0.04**
	Recommended	3.2 (± 0.6) a	7.4 (± 1.4) ab	18	82.000	< **0.01**
	Above	2.5 (± 0.3) a	3.7 (± 0.6) a	18	120.50	< 0.19
N. eichhorniae	Below	43.1 (± 8.6) b	72.0 (± 11.3) a	18	95.500	< 0.68
	Recommended	38.9 (± 8.6) ab	64.6 (± 10.5) a	18	105.500	< 0.18
	Above	11.0 (± 3.6) a	58.0 (± 35.8) a	18	96.500	< 0.10

Statistically significant (<0.05) *P-values* are marked in bold.

At 0.5 hours survival of *M. scutellaris* was 100% for all concentrations (Figure 3.4). A noticeable decline of 72%, 78% and 89% was only recorded at 1 h for the concentration above field recommended, recommended and below recommended rates. Survival rate of *M. scutellaris* declined by up to 1.1 fold when exposed to Methomyl dried residues at the field recommended and above recommended rate. Survival of *M. scutellaris* 0% after 4 h for the concentration above recommended, whereas, it took up to 8 h to induce complete mortality for the field recommended and below recommended rates of Methomyl dried residues. Survival of *M. scutellaris* treated with Chlorpyrifos residues was longer with 100% survival rate for all concentrations at 1 h. A noticeable decline of 39% and 72% was only recorded at 2 h for Chlorpyrifos dried residues at the concentration above field recommended, recommended, and below recommended rate. Survival declined by up to 1 and 1.9 fold for Chlorpyrifos dried residues at the field recommended and above recommended rate, respectively. Survival was 0% after 8 h for the concentration above field recommended rate, whereas, it took up to 16 h and 24 h for the field recommended and below recommended rates of Chlorpyrifos dried residues to induce complete mortality. Survival of *M. scutellaris* in the controls declined to 94.4% after 24 h following treatment.

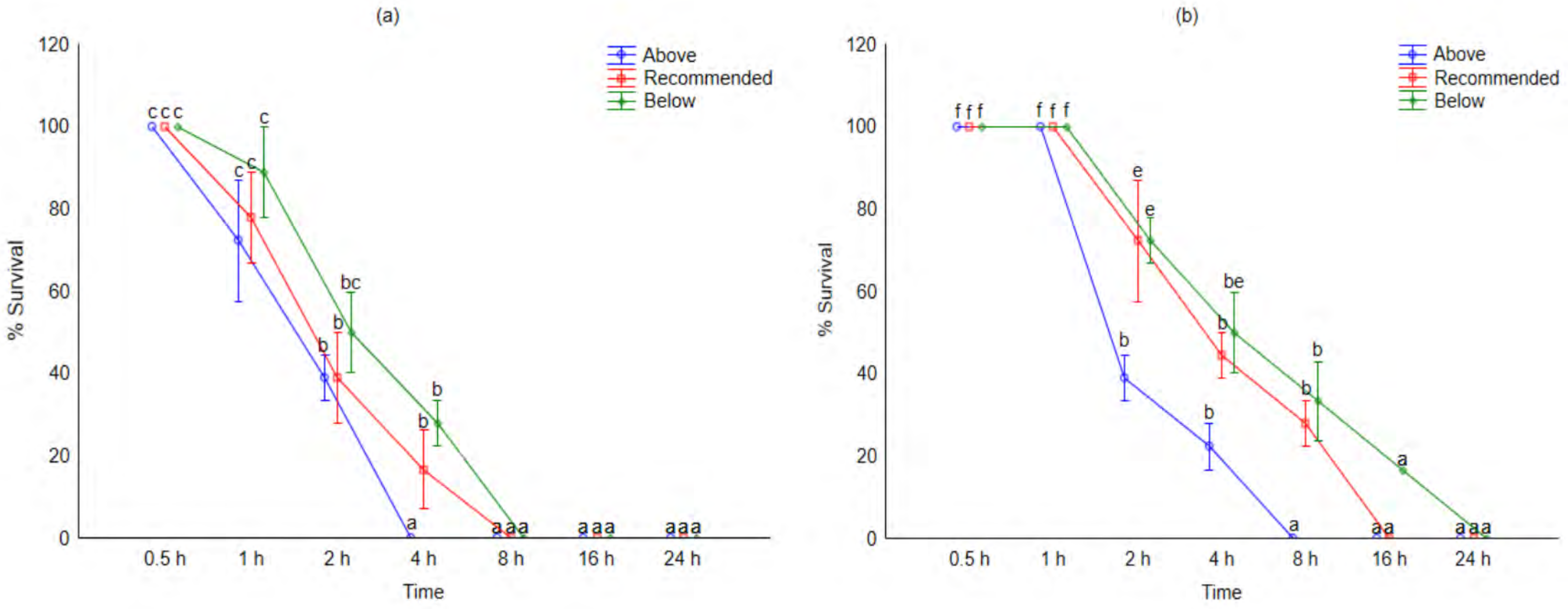

Figure 3.4: Mean (±SE) percentage survival of *M. scutellaris* adults exposed to dried residues of Methomyl (a) and Chlorpyrifos (b) on *P. crassipes* leaves at the concentrations below field recommended, recommended, and above recommended rate. Survival rate was evaluated between 0.5 h and 24 h. Means followed by the same letters are not statistically different ($P > 0.05$) between concentrations at each time interval from 0.5 h to 24 h for each concentration.

At 1 h survival of *N. eichhorniae* exposed to Methomyl dried residues was 100% for all concentrations (Figure 3.5). A noticeable decline of 72%, 89% and 94% was only recorded at 2 h for the concentration above field recommended, recommended and below recommended rate of Methomyl residues. *Neochetina eichhorniae* survival rate declined by up to 1.1 and 1.2 fold when exposed to Methomyl dried residues at the field recommended and above recommended rate. Survival was 0% after 120 h at the concentration above field recommended and recommended rate, whilst, it only declined to 11% for the concentration below field recommended rate of Methomyl dried residues. *Neochetina eichhorniae* survival rate when exposed to dried residues of Chlorpyrifos was longer with 100% survival rate for all concentrations at 4 h. A noticeable decline of 94% was only recorded at 8 h for the concentration above field recommended rate, hence, a decline of 94% and 89% was recorded at 16 h and 24 h for the field recommended and below recommended rate of Chlorpyrifos residues. After 24 h survival declined by up to 1 and 1.1 fold at the field recommended and above recommended rate of Chlorpyrifos residues, respectively. Survival was 22% after 120 h for the concentration below field recommended rate, whilst it only declined to 11% and 6% at the field recommended and above recommended rates of Chlorpyrifos residues.

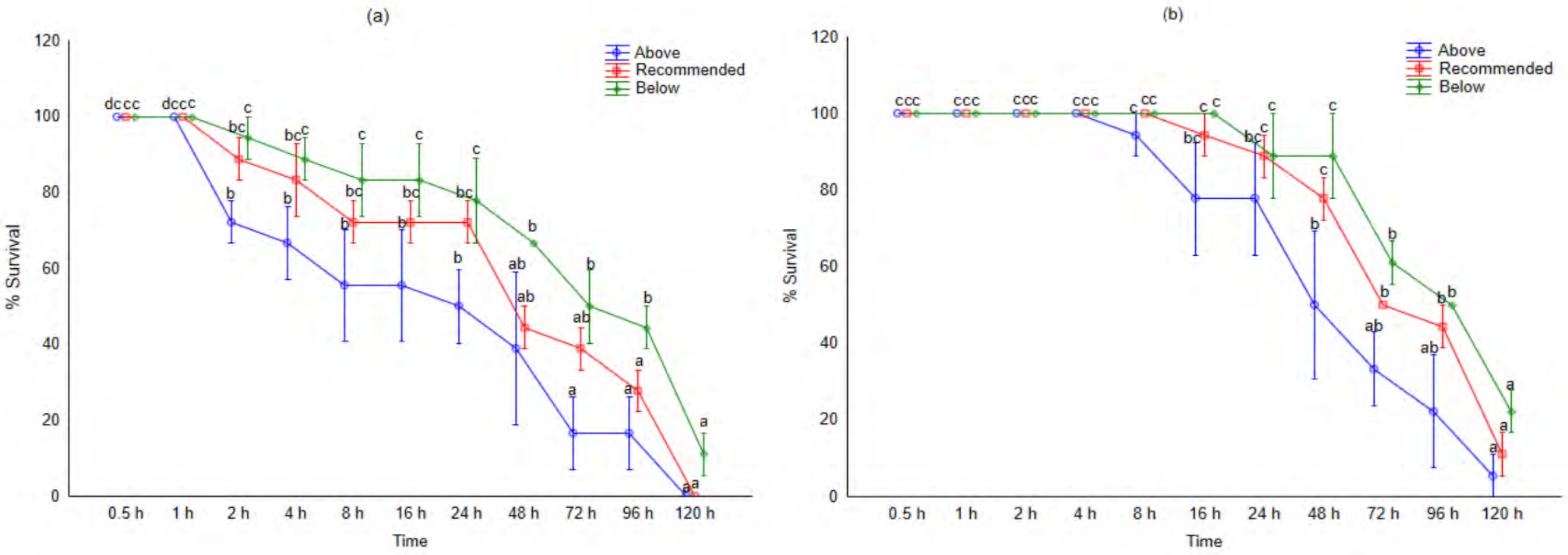

Figure 3.5: Mean (±SE) percentage survival of *N. eichhorniae* adults exposed to dried residues of Methomyl (a) and Chlorpyrifos (b) on *P. crassipes* leaves at the concentrations below field recommended, recommended, and above recommended rate. Survival rate was evaluated between 0.5 h and 120 h. Means followed by the same letters are not statistically different ($P > 0.05$) between concentrations at each time interval and from 0.5 h to 120 h for each concentration.

3.3.3. Feeding damage

Feeding damage of *M. scuttellaris* adults topically treated with Methomyl at the concentration above field recommended rate was significantly reduced ($F_{1,4} = 36.571$, $P = 0.003$) when compared with the control (Figure 3.7). However, feeding damage of *M. scuttellaris* when treated with Methomyl at field recommended rate was not significantly reduced ($F_{1,4} = 4.5714$, $P = 0.09$) when compared with the control. Similarly, feeding by the agent treated with Methomyl at the concentration below field recommended rate was not significantly reduced ($F_{1,4} = 2.5714$, $P = 0.18$) when compared with the control.

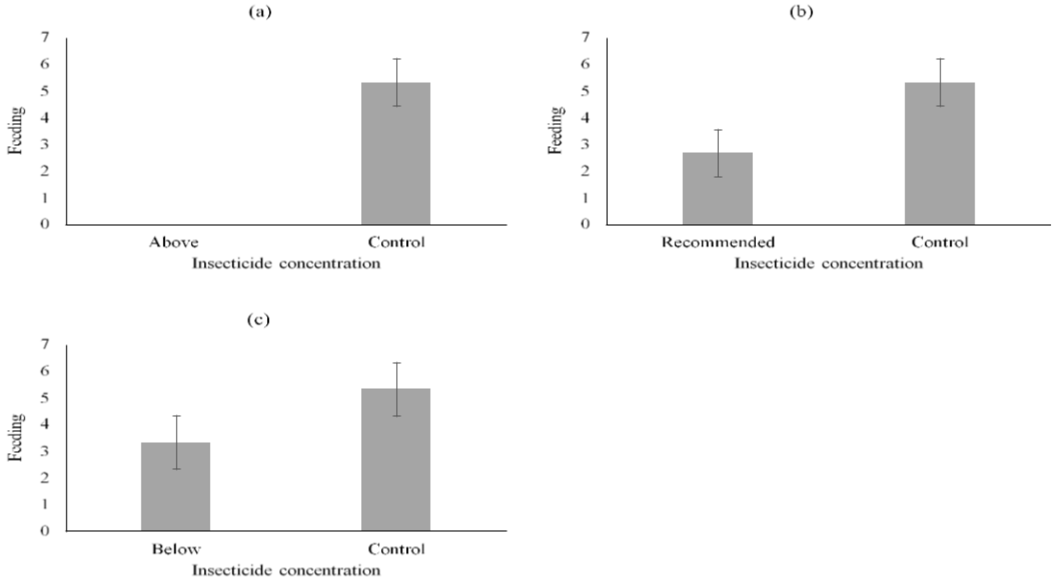

Figure 3.6: Mean (±SE) feeding damge of *M. scutellaris* treated topically with Methomyl at the concentration above recommended (a), recommended (b), and below recommended filed rate (c). Deionized water was used as a control.

Feeding of *M. scuttellaris* treated with Chlorpyrifos concentrations above field recommended rate was significantly reduced ($F_{1,4} = 36.100$, $P = 0.03$) when compared with the control (Figure 3.8). The feeding damage by the agent treated with Chlorpyrifos at field recommended rate was also significantly reduced ($F_{1,4} = 24.596$, $P = 0.007$) when compared with the control. However, feeding damage by *M. scuttellaris* treated with Chlorpyrifos at concentration below field recommended rate was not significantly reduced ($F_{1,4} = 7.2000$, $P = 0.05$) when compared with the control.

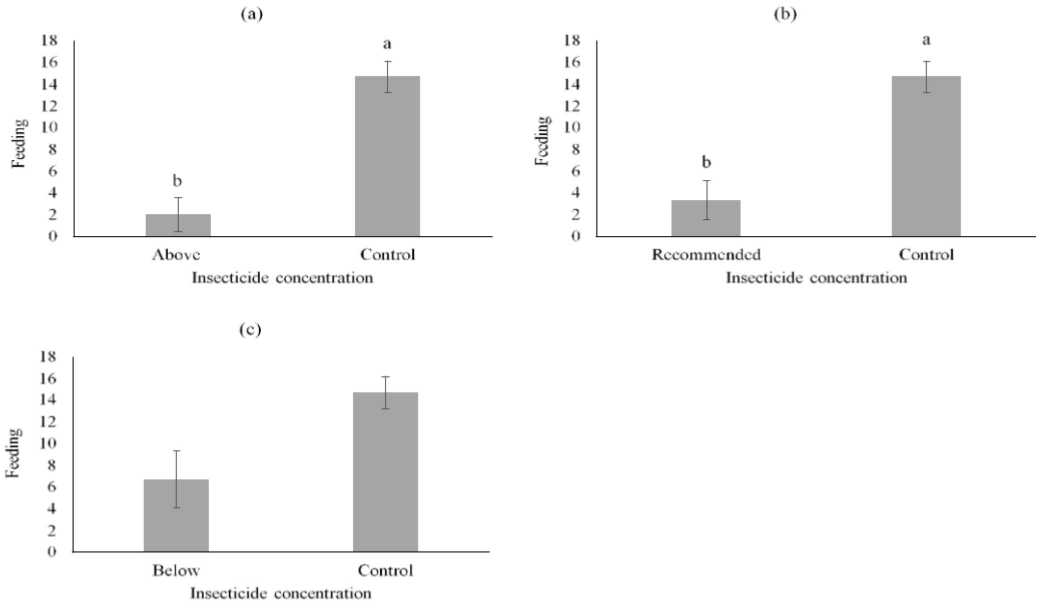

Figure 3.7: Mean (±SE) feeding damage of *M. scutellaris* treated topically with Chlorpyrifos at the concentrations above recommended (a), recommended (b), and below recommended field rate (c). Deionized water was used as a control. Means followed by the same letter are not statistically different ($P > 0.05$).

Feeding damage by *N. eichhorniae* treated with Methomyl concentration above field recommended rate was significantly reduced ($F_{1,4} = 9.8000$, $P = 0.03$) when compared with the control (Figure 3.9). The feeding damage by the weevil treated with Methomyl at field recommended rate was significantly reduced ($F_{1,4} = 18.283$, $P = 0.01$) when compared with the control. Similarly, feeding by the weevil treated with Methomyl concentration below field recommended rate was reduced significantly ($F_{1,4} = 18.000$, $P = 0.01$) when compared with the control.

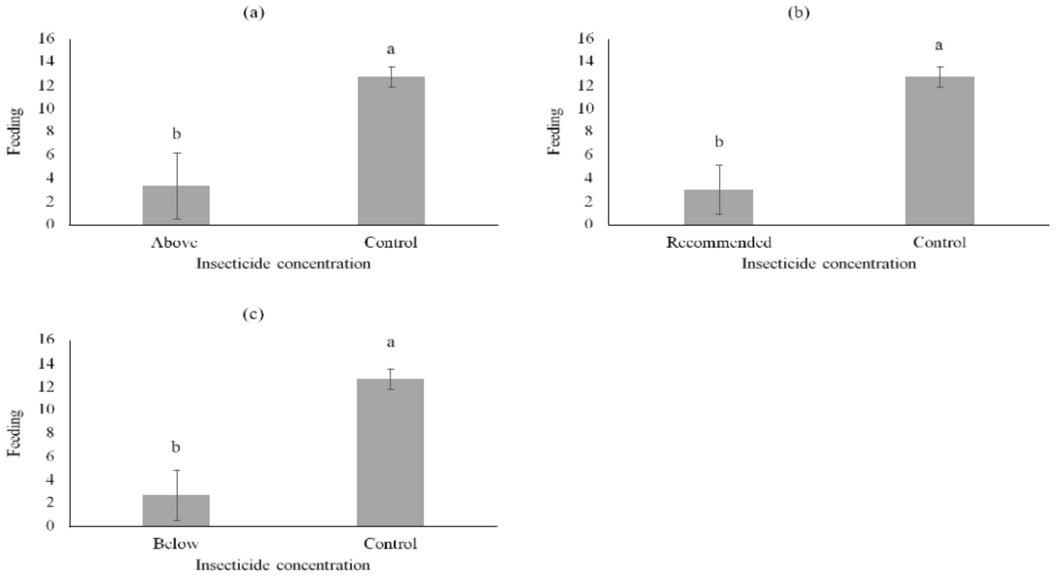

Figure 3.8: Mean (±SE) feeding damage of *N. eichhorniae* adults treated topically with Methomyl (a) at field above recommended (a), recommended (b), and below recommended rates (c). Deionized water was used as a control. Means followed by the same letter are not statistically different (*P* >0.05).

The feeding damage by *N. eichhorniae* treated with Chlorpyrifos concentration above field recommended rate was not significantly reduced ($F_{1,4}$ = 5.0330, *P*= 0.08) when compared with the control (Figure 3.10). Similarly, feeding by the weevil treated with Chlorpyrifos at field recommended rate was not significantly reduced ($F_{1,4}$ = 1.6325, *P*= 0.27) when compared with the control. However, feeding by the weevil treated with Chlorpyrifos at the below field recommended rate was significantly reduced (*U* = 0.00, *P* < 0.04) when compared with the control.

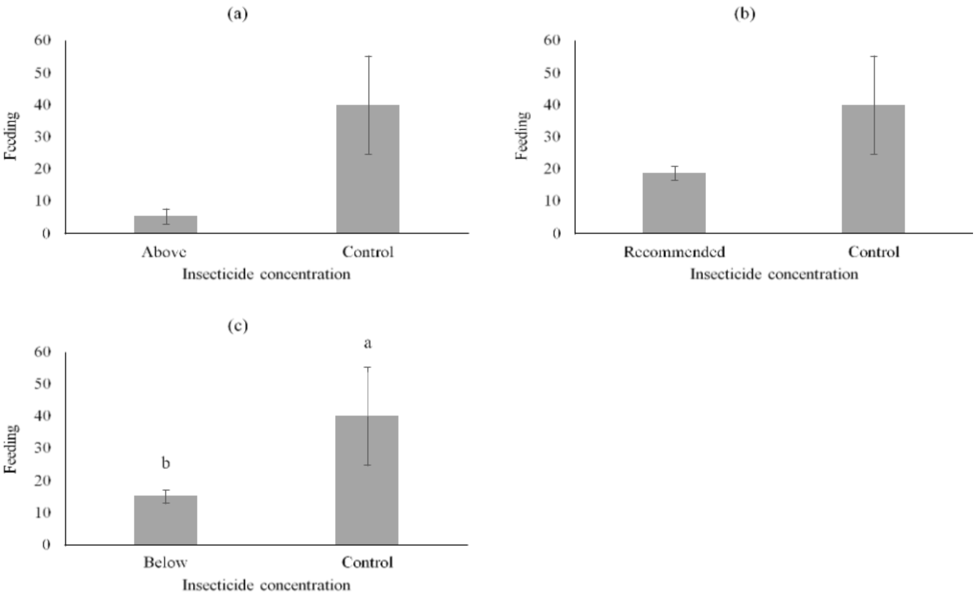

Figure 3.9: Mean (±SE) feeding damage of *N. eichhorniae* topically treated with Chlorpyrifos at the concentration above field recommended (a), recommended (b), and below recommended rate (c). Deionized water was used as a control. Means followed by the same letter are not statistically different ($P > 0.05$).

Feeding by *M. scuttellaris* adults on *P. crassipes* leaves with toxic residues of Methomyl at the concentration above field recommended rate was significantly reduced ($F_{1,4} = 9.4670$, $P = 0.03$) when compared with the control (Figure 3.11). The feeding damage by the agent on leaves treated with toxic residues of Methomyl at field recommended rate was not significantly reduced ($F_{1,4} = 6.2010$, $P = 0.06$) when compared with the control. Similarly, feeding by *M. scuttellaris* on leaves with residues of Methomyl at the below field recommended rate was not significantly reduced ($F_{1,4} = 6.3631$, $P = 0.06$) when compared with the control.

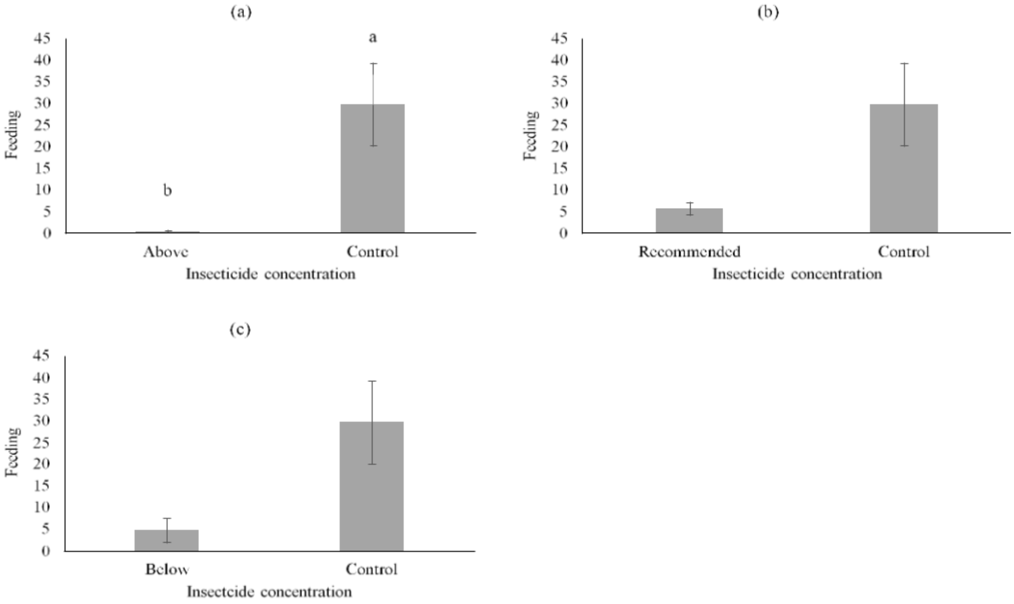

Figure 3.10: Mean (±SE) feeding damage of *M. scutellaris* on *P. crassipes* leaves with Methomyl residues at the concentration above field recommended (a), recommended (b), and below recommended rate (c). Deionized water was used as a control. Means followed by the same letter are not statistically different ($P > 0.05$).

Feeding damage by *M. scuttellaris* on *P. crassipes* leaves with residues of Chlorpyrifos at the concentration above field recommended rate was significantly reduced ($U = 0.00$, $P < 0.04$) when compared with the control (Figure 3.12). The feeding damage by the agent on leaves with residues of Chlorpyrifos at the field recommended rate was significantly reduced ($U = 0.00$, $P < 0.04$) when compared with the control. Feeding by *M. scuttellaris* on leaves with residues of Chlorpyrifos at the concentration below field recommended rate was also significantly reduced ($U = 0.00$, $P < 0.04$) when compared with the control.

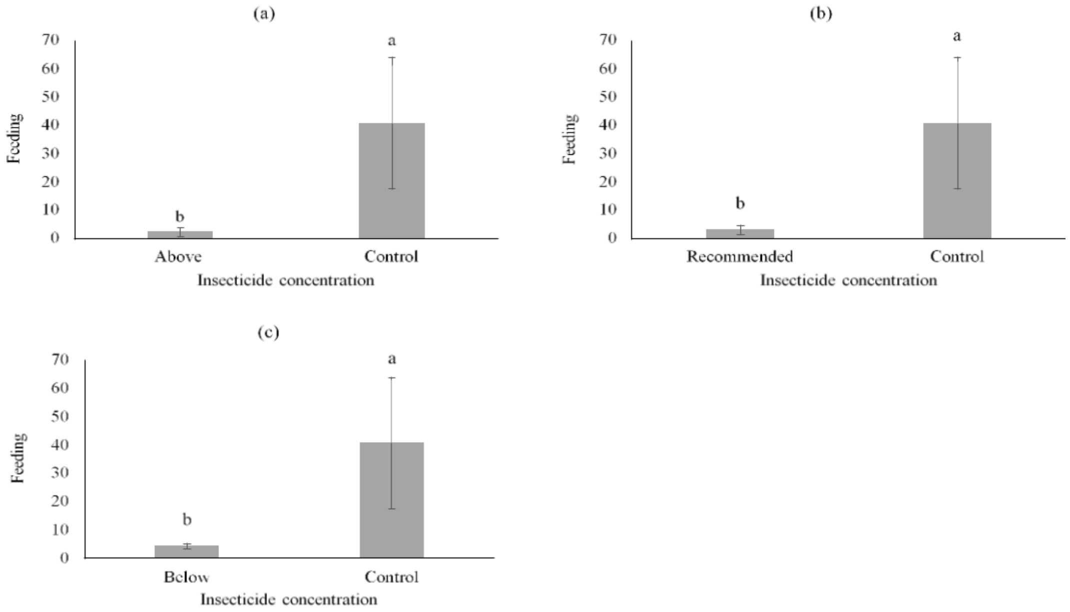

Figure 3.11: Mean (±SE) feeding damage by *M. scutellaris* on *P. crassipes* leaves with Chlorpyrifos residues at the concentration above field recommended, recommended, and below recommended rates. Deionized water was used as a control. Means followed by the same letter are not statistically different (P >0.05).

Feeding damage by *N. eichhorniae* on *P. crassipes* leaves with residues of Methomyl at the concentration above field recommended rate was significantly ($F_{1,4}$ = 24.750, P= 0.007) reduced when compared with the control (Figure 3.13). Similarly, the feeding damage by *N. eichhorniae* on *P. crassipes* leaves with residues of Methomyl at the field recommended rate was siginficantly ($F_{1,4}$ = 40.026, P= 0.003) reduced when compared with the control. Feeding by the weevil on *P. crassipes* leaves with residues of Methomyl at the below field recommended rate was also siginficantly ($F_{1,4}$ = 19.931, P= 0.01) reduced when compared with the control.

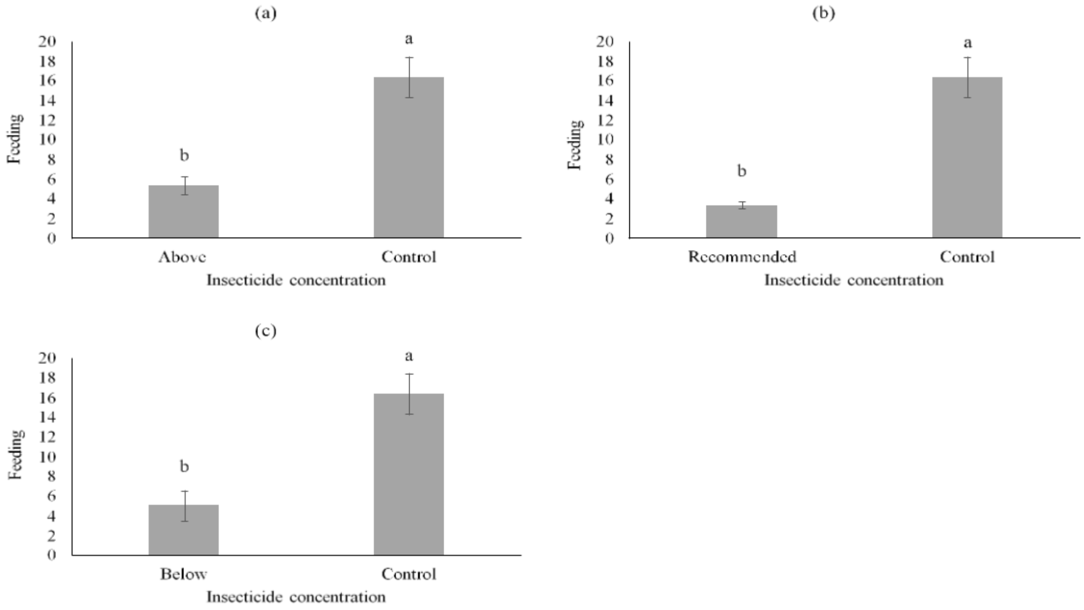

Figure 3.12: Mean (±SE) feeding damage by *N. eichhorniae* adults on *P. crassipes* leaves with residues of Methomyl (a) at the concentration above field recommended (a), recommended (b), and below recommended rates (c). Deionized water was used as a control. Means followed by the same letter are not statistically different ($P > 0.05$).

Feeding damage by *N. eichhorniae* on *P. crassipes* leaves with residues of Chlopryrifos at the concentration above field recommended rate was not significantly ($F_{1,4} = 4.6263$, $P = 0.09$) reduced when compared with the control (Figure 3.14). Similarly, the feeding damage by the weevil on leaves with residues of Chlopryrifos at the field recommended rate was not siginficantly ($F_{1,4} = 5.3272$, $P = 0.08$) reduced when compared with the control. The feeding damage by the weevil on leaves with residues of Chlopryrifos at the concentration below field recommended rate was also not siginficantly ($F_{1,4} = 2.5574$, $P = 0.18$) reduced when compared with the control.

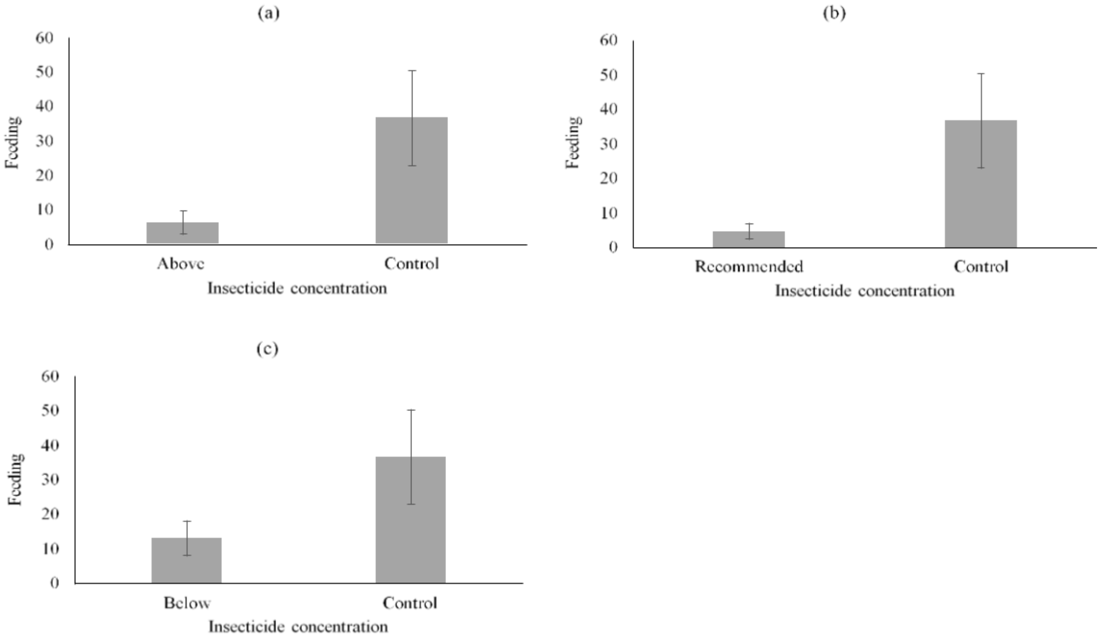

Figure 3.13: Mean (±SE) feeding damage of *N. eichhorniae* adults on *P. crassipes* leaves with Chlorpyrifos residues at the concentrations above field recommended, recommended, and below recommended rates. Deionized water was used as a control. Means followed by the same letter are not statistically different (*P* >0.05).

3.4. DISCUSSION

The present study demonstrated that the tested neurotoxic insecticides through topical and residue contact exposure were highly toxic and significantly reduced the survival of the biological control agents of *P. crassipes*. At all tested concentrations, survival and feeding damage of the biological control agents were significantly reduced by Methomyl and Chlorpyrifos on either exposure methods. These outcomes suggest that potential insecticide drift from citrus orchards to *P. crassipes* reserves may reduce the population abundance of the biological control agents and limit the success of the biological control programme (although this was not shown in chapter two). The findings corroborate with a multitude of previous studies which have reported that Methomyl and Chlorpyrifos are highly toxic to biological control agents (Langhof et al., 2003; Liu et al., 2016; Wang et al., 2008; Wang et al., 2012) and have potential to reduce the establishment and impact of biocontrol agents of invasive alien plants (Hoffmann & Moran, 1995; Mukwevho et al., 2017b; Mukwevho & Mphephu, 2020). Replacing the neurotoxic insecticides in citrus orchards with novel pesticides which are highly selective to pests and less persistent may limit the potential effects on the performance of biological control agents (Gentz et al., 2010; Lowenstein et al., 2019; Smith & Krischik, 2000; Torres & Bueno, 2018).

The reduction in feeding damage of the biological control agents by contact exposure or foraging on the contaminated surface of leaves could allow *P. crassipes* to grow, reproduce and spread at higher rates (Jones et al., 2018; Katembo et al., 2020). Katembo et al. (2019) reported a decline in the number of *L. camara* leaves damaged by *Teleonemia scrupulosa* Stal (Hemiptera: Tingidae) on plants treated with Carbofuran in comparison to untreated plants. Katembo (2018) in a field study in Mpumalanga also recorded a greater number of inflorescences and leaves damaged by suite of naturalized biological control of *L. camara* on untreated plants compared to treated plants.

The average period at which the insecticide concentrations reduced survival of the biological control agents on either contact exposure techniques indicated that Methomyl had the greatest toxicity than Chlorpyrifos. Liu et al. (2016) also recorded the highest toxicity of Methomyl than Chlorpyrifos on adults of two biological control agents of *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae). However, the findings are not in line with other studies (Campos et al., 2011; Desneux et al., 2004; Fontes et al., 2018; Grundy et al., 2000; Muslim et al., 2018;

Wang et al., 2008) where Chlorpyrifos was reported to induce the highest toxicity to biological control agents than Methomyl.

As expected, survival of the biological control agents of *P. crassipes* declined significantly with the increase in concentrations on either exposure techniques. Provided that Methomyl and Chlorpyrifos are applied at concentrations beyond field recommended rates in citrus groves, toxicity to biological control agents could be intensified (Bacci et al., 2008; Katembo et al., 2019). In a previous study, Khan et al. (2009) reported that Methomyl at concentrations above and recommended field rates reduced survival of larval parasitoid *Bracon hebetor* Say (Hymenoptera: Braconidae) to 0% at 36 h and rated the chemical harmful to the biocontrol agent. Although at the concentration below field recommended rate Methomyl reduced survival to 1% after 48 h of treatment, survival was very low for Methomyl to be considered safe or less harmful to *B. hebetor*. Khan et al. (2009) also reported that Chlorpyrifos wiped-out the population of *B. hebetor* after 24 h at the concentration above field rate and at 36 h for the recommended and below field recommended rates. James, (2003) reported that Methomyl reduced survival of a larvae of the ladybeetle, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) to 0% at 48 h following treatment.

Whilst concentrations of tested insecticides induced complete mortality of *M. scutellaris* on either exposure techniques, *N. eichhorniae* showed less sensitivity to insecticides with prolonged decline in survival rates and few survivors at the end of the study period. Sensitivity to insecticides amongst beneficial insects depends on the variation in rate of cuticle penetration and processes of detoxification, thereby lead to differences in physiological toxicity (Croft, 1990). Sensitivity may also depend on ecological and behavioural differences such as nocturnal or diurnal activity, feeding, mating and mobility (Croft, 1990; Desneux et al., 2007). These differences amongst biological control agents lead to variations in ways in which they encounter insecticides or uptake of the chemicals in the field. *Megamelus scutellaris* high sensitivity to either contact exposure routes could be based on penetration rate and time of activity during the day. *Megamelus scutellaris* is diurnal and does not have a hard exoskeleton (Sosa et al., 2005, 2007). Therefore, *M. scutellaris* may have had high penetration rate of Methomyl and Chlorpyrifos and is more likely to come into contact with spray drift droplets during pesticide application in the field. *Neochetina eichhorniae* has a hard cuticle which may have reduced the penetration rate of insecticides, and due to their nocturnal behaviour, they may only come into contact with spray droplet residues on the surface of contaminated leaves when they are feeding (Deloach & Cordo, 1976).

The two classes of insecticides were nearly equally harmful to the biocontrol agents of *P. crassipes* because they share the capacity to disrupt the normal functioning of the enzyme acetylcholinesterase (Fukuto, 1990). The highest acute toxicity of the two classes of insecticides to the biological control agents of *P. crassipes* could be also associated with their low molecular weight (Bacci et al., 2008). Insecticides with low molecular weight have the greater capacity to enter the cuticle of insects, bind to the action site, and induce paralysis and death (Stock & Holloway, 1993).

3.3. CONCLUSION

The current study based on the acute toxicity of Methomyl and Chlorpyrifos showed that the neurotoxic insecticides are highly toxic to the biological control agents of *P. crassipes* on either contact exposure techniques even at the concentrations below field recommended rates. The feeding damage of the biological control agents treated topically or foraging on the contaminated leaves was also significantly reduced. Despite the lack of clear evidence that the low population abundance of the agents in the field could be due to insecticide drift from nearby citrus orchards, the bioassays proved that insecticides could limit their success in reducing the invasion intensity of *P. crassipes*. Therefore, the neurotoxic insecticides should be replaced with safe and novel substances in citrus orchards to prevent potential detrimental effects on the population abundance and feeding behaviour of naturalized biological control agents of *P. crassipes.*

CHAPTER FOUR

GENERAL DISCUSSION AND CONCLUSIONS

4.1. Establishment and abundance of biocontrol agents of *P. crassipes* across regions

Variation in establishment and impact of biological control agents of invasive alien plants between regions and freshwater bodies is common in the management of aquatic weeds (Byrne et al., 2010; Hopper et al., 2017). The spatial variation in population abundance and distribution of biological control agents of floating aquatic plants is commonly influenced by high water levels (floods), herbicide treatment, insecticide drift and nutrient levels (Bownes et al., 2013b; Hoffmann & Moran, 1995; Hopper et al., 2021).

During field surveys, *E. catarinensis, N. eichhorniae, N. bruchi* and *O. terebrantis* were naturalized on the Crocodile River and dams at Hectospruit, Kanyamazane and Vergenoeg. *Eccritotarsus catarinensis* infestation and abundance was low on dams at Kanyamazane and Vergenoeg, but high on the dam compared to the river at Hectospruit. Number of plants infested by the *Neochetina* weevils and *O. terebrantis* were low on dams at Kanyamazane and Vergenoeg, whereas their abundance was only low on the dam at Hectospruit. The lack of measurable insecticide residues on the sampled leaves of *P. crassipes* indicated that the variation in abundance of biological control agents between the visited freshwater bodies in this case may not be influenced by insecticide drift.

4.2. Potential impact of insecticides on biocontrol agents of *P. crassipes*

Insecticides have direct and indirect impacts on establishment and survival of biological control agents (Desneux et al., 2007; Hoffmann & Moran, 1995). Direct exposure to insecticides has demonstrated acute impacts in may studies (James, 2003; Khan et al., 2009; Wang et al., 2008; Wang et al., 2012), whilst, indirect effects through ingestion of insecticide residues from contaminated plant tissues leads to long term sub-lethal impacts on biological control agents (Hasan et al., 2020; Hasan & Ansari, 2017; Sharma et al., 2019; Sharma et al., 2016). Field assessments and laboratory bioassays are conducted to evaluate the long-term (sub-lethal) and short-term (acute) impacts of insecticides on the performance of biological control agents (Desneux et al., 2004; Hasan et al., 2020; Hoffmann & Moran, 1995; Langhof et al., 2003; Otto et al., 2013). The long-term subtle effects of insecticides have been reported to be transgenerational which may slow down the population growth rates and limit the efficacy of biological control agents (Akhtar et al., 2021; Costa et al., 2014; Hasan et al., 2020).

Although the infestation levels of the naturalized biological control agents were notable on the Crocodile River and dams, their population abundance was low. The lack of insecticide residues on the sampled leaves of *P. crassipes* indicated that spray drift from citrus orchards may not be responsible for the low population density of the agents at the time the field surveys were carried out (Chapter two). However, it is possible that insecticide drift occurred before the selected sites were visited and had degraded to undetectable levels by the time of sampling (Hoffmann & Moran, 1995). The laboratory bioassays did clearly demonstrated that commonly applied insecticides (*viz*. Methomyl and Chlorpyrifos) in citrus orchards are highly toxic to the agents of *P. crassipes* and thus may significantly reduce their population abundance through direct exposure to topical treatment and dried residues on leaves (Chapter three). Langhof et al. (2005), in a bioassay study, also recorded mortality of a parasitoid *Aphidius colemani* Viereck (Hymenoptera: Braconidae) and larvae of a predator *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) exposed to λ-cyhalothrin spray drift deposits on leaves from non-target plants adjacent to wheat plantation.

In other studies where spray drift droplet residues were recorded at closer proximity (2-3m) to treated fields, insecticide application was conducted using a tractor mounted sprayer (Langhof et al., 2003, 2005). It could be possible that insecticide residues were not detected at the shortest distance (10.5m) from the edge of citrus orchard on the dam at Hectospruit because a tractor mounted sprayer which tends to keep the insecticide application more localized was used at the time the surveys were conducted in the present study. Therefore, samples of leaves of *P. crassipes* should be collected closer (less than 5m) to citrus orchards or foliage of terrestrial vegetation bordering treated fields may be collected to determine the extent of spray drift towards invaded freshwater bodies. Samplers or traps used in toxicological studies may be also deployed in the field from the edge of the treated area towards invaded aquatic ecosystems to determine potential spray drift deposition on *P. crassipes* reserves (Curchod et al., 2020; Otto et al., 2013).

In a bioassay study, de Morais et al., (2016) reported that Chlorpyrifos was persistent on the surface of Valencia orange leaves and reduced the survival of a parasitoid *Ageniaspis citricola* Longvinovskaya (Hymenoptera: Encyrtidae), a biocontrol agent of citrus leaf miner *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) by 25% when exposed to 7 days old treated leaves. Survival of a parasitoid *Aphidius colemani* Viereck (Hymenoptera; Braconidae) exposed to leaves treated with Methomyl that were collected from the field after two days of treatment was significantly reduced after 48 h of exposure (Langhof et al., 2003). This suggest that the biological control agents of *P. crassipes* which are less likely to come into contact with spray drift droplets during insecticide applications may be exposed to toxic residues on the surface of leaves when they are foraging.

Whilst only direct contact exposure was considered in the present study which reduced the survival and feeding damage of the biological control agents of *P. crassipes* (Chapter three), in the field agents may also encounter insecticides by feeding on the tissues of the plant contaminated by systemic insecticides (Hasan et al., 2020; Katembo et al., 2019). Systemic insecticides absorbed by plants modify chemical and physiological processes in plant cells, thus, negatively influence the feeding behaviour of beneficial insects (Sharma et al., 2017, 2019; Sharma et al., 2016). Adults and immatures of *M. scutellaris* which feed externally by sucking sap on *P. crassipes* are likely to respond in a similar way to contact and systemic insecticides. The larvae of *N. eichhorniae* which feed inside petioles may be only affected by systemic insecticides.

4.3. Effect of nutrients on the performance of biocontrol agents of *P. crassipes*

The reduction in invasiveness of *P. crassipes* by biological control and the response of the plant to damage is influenced by nutrient levels in aquatic ecosystems (Bownes et al., 2013b; Center & Dray, 2010). Performance of biological control agents have been shown to be effective when tissue nutrient levels on *P. crassipes* are intermediate compared to plants with high and low nutrient rates (Coetzee & Hill, 2012). Coetzee et al., (2007a) reported greatest feeding damage of *E. catarinensis* on plants with medium nutrient content, followed by damage on plants treated with low levels and then above nutrient rates. This study also recorded high number of *E. catarinensis* on the medium nutrient treatment compared to high and low nutrient levels.

Generally, impact of biological control agents on enriched plants is intensified, but plants have been shown to recover tissues removed by biological control agents (Bownes et al., 2013b). At high nutrient levels the response of *P. crassipes* to agents feeding damage can be explained by the compensatory continuum hypothesis (CCH) theory which states that under conditions of high resource availability, invasive alien plants are more tolerant to herbivory (Maschinski & Whithan, 1989). Under low nutrient levels plant growth is slow, fecundity of biocontrol agents is low, thus, population size remains low and ineffective in reducing invasiveness of *P.*

crassipes (Bownes et al., 2013a; Mukarugwiro et al., 2018). The response of *P. crassipes* to feeding damage at low tissue nutrient levels may be explained by the growth rate theory which predicts that plants are more likely to recover tissues under low compared to high nutrient levels (Hilbert et al., 1981). This prediction is based on the assumption that plants growing under stressful conditions may not develop to their fullest potential, therefore, they only need small changes in their relative growth rate (RGR) to recover tissues lost during insect feeding, more especially when the damage is low (Hawkes & Sulivan, 2001). Plants on habitats with high nutrient levels are possibly growing near or to their fullest potential such that changes on their relative growth rate may be negligible to recover tissues lost when biocontrol agents are feeding. Meyer & Root, (1993) reported that *Solidago altissimsa* L. only recovered tissues lost when insect were feeding under low nutrient levels compared to high nutrient levels.

Nutrient levels in water at the Crocodile River and dams ranged between oligotrophic and mesotrophic for nitrate and were eutrophic for Phosphorus (chapter two). The nutrient levels were low to have a positive influence on the reproductive performance of the biological control agents of *P. crassipes* (Byrne et al., 2010). Therefore, the low population abundance during the time at which surveys were conducted may be ascribed to lack of sufficient foliar Nitrogen or quality diet for naturalized biological control agents (Bownes et al., 2013a). Despite reports from glasshouse studies that biological control agents lay few eggs and feed voraciously to compensate for shortage of tissue nutrients on low quality plants (Bownes et al., 2013b; Mukarugwiro et al., 2018), low nutrient supply in the field may be short-lived, thus, an increase in nutrient content may allow the plant to recover from damage and outgrow the population of agents. Leaves of *P. crassipes* should be also collected in the field to monitor temporal variation of nutrient levels.

4.4. CONCLUSION

Despite the low population size of the naturalized biological control agents of *P. crassipes* on the Crocodile River and dams adjacent to citrus orchards, the performance of the agents appeared to be influenced by stochastic events (*viz*. poor nutrient and high water levels) than insecticide drift (chapter two) and is inconsistent with the acute toxicity assessments (chapter three). To prevent the potential toxicity of Methomyl and Chlorpyrifos on the biological control agents of *P. crassipes*, highly selective and less persistent insecticides should be used in citrus orchards. Field surveys should be conducted in spring or summer to determine possible seasonal variation in potential spray drift effects on the performance of biological control agents of *P. crassipes*. Samplers or traps should be deployed to determine the extent of spray drift from citrus groves. Furthermore, water and leaf samples should be collected frequently to monitor the variation in nutrient levels in aquatic ecosystems.

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