Susceptibility of *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) to insecticides in South Africa

BC Smit

orcid.org 0000-0002-0621-6430

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Supervisor: Prof MJ du Plessis

Co-supervisor: Prof J van den Berg

Graduation: November 2025

DECLARATION BY THE CANDIDATE

I, Bernhard Conrad Smit, declare that the work presented in this MSc thesis is my own work, that it is not submitted for any degree or examination at any other University and that all the sources I have used or cited have been acknowledged by the complete reference.

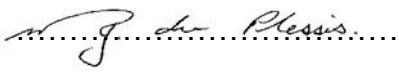
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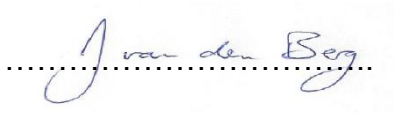
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Prof MJ du Plessis

Unit for Environmental Sciences and Management, North-West University, Private Bag, X6001, Potchefstroom, 2520, South Africa.

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Prof J van den Berg

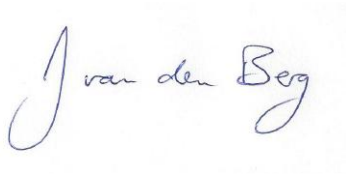
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To whom this may concern,

I, Prof. Johnnie Van den Berg, hereby declare that the thesis titled: “Susceptibility of *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) to insecticides in South Africa” by Bernhard Conrad Smit has been edited for language correctness and spelling. No changes were made to the academic content or structure of this work.



Prof. Johnnie Van den Berg Date: 30 July 2025

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“The whitefly, a small but mighty insect that will drive you crazy” – Bernhard Smit

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General abstract

Trialeurodes vaporariorum (Westwood) (Hemiptera: Aleyrodidae), the greenhouse whitefly, is a highly polyphagous pest that causes severe damage to several economically important crops. Pesticide application is the most common method of control, and many cases of resistance have been reported from countries other than South Africa. No data are therefore available on the susceptibility of *T. vaporariorum* populations to insecticides in South Africa. The aim of this study was to assess the susceptibility of *T. vaporariorum* adults and second-instar nymphs of two populations (WSC-1-24 and BLF-1-25) to six insecticides. Susceptibility was assessed using two IRAC recommended bioassay methods, *viz*. the leaf dip method (IRAC Method No. 15) for adults, and the potted plant immersion method for nymphs (IRAC Method No. 16). Additionally, adult susceptibility to diafenthiuron (Group 12A), flupyradifurone (Group 4D) and imidacloprid (Group 4A), was also evaluated using an adapted IRAC Method No.016. Whiteflies were reared separately by population, and LC_{80} values for each insecticide were estimated and compared to their respective recommended field application rates to assess potential resistance. Adults from both populations were susceptible to most of the abovementioned insecticides, though variation in response was observed across compounds. Both populations exhibited low adult susceptibility to cyantraniliprole, while nymphs were susceptible. Flupyradifurone was effective across both life stages and populations, except for BLF-1-25 nymphs, whose LC_{80} exceeded the recommended rate. Susceptibility to pymetrozine was highly variable, with low efficacy overall. Although control failure is likely for both life stages of individuals of the WSC-1-24 population, BLF-1-25 adults were susceptible. Comparison of the two bioassay methods revealed that the plant immersion method consistently produced higher LC_{80} values than the leaf dip method across all three insecticides, regardless of their mode of action, or action within the plant. Despite these differences, the LC_{80} values estimated for diafenthiuron, flupyradifurone, and imidacloprid using both methods were still below their recommended application rates, indicating that no adult control failure is currently anticipated in South Africa with these products. These findings underscore the importance of using consistent, standardised bioassay protocols in resistance monitoring programs to ensure reliable and comparable data over time. The differences in susceptibility observed between populations likely reflect variations in local insecticide use patterns, emphasising the need for robust insecticide resistance management strategies. Integrated pest management approaches should be prioritised to reduce reliance on chemical control and promote sustainable long-term management of this economically important pest.

Key words: Insecticide, resistance, susceptibility, systemic, *Trialeurodes vaporariorum*

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Chapter 1: Introduction and Literature review

1.1 Distribution of *Trialeurodes vaporariorum*

The greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae), is globally distributed (Fig. 1.1) but its exact origin is still unknown (Gamarra *et al.*, 2020). Vet *et al.* (1980) speculated that this species is possibly native to tropical or sub-tropical America, but Capinera (2020) indicated that *T. vaporariorum* does not thrive in most tropical environments and generally exists in colder climates by virtue of its ability to survive winter seasons.

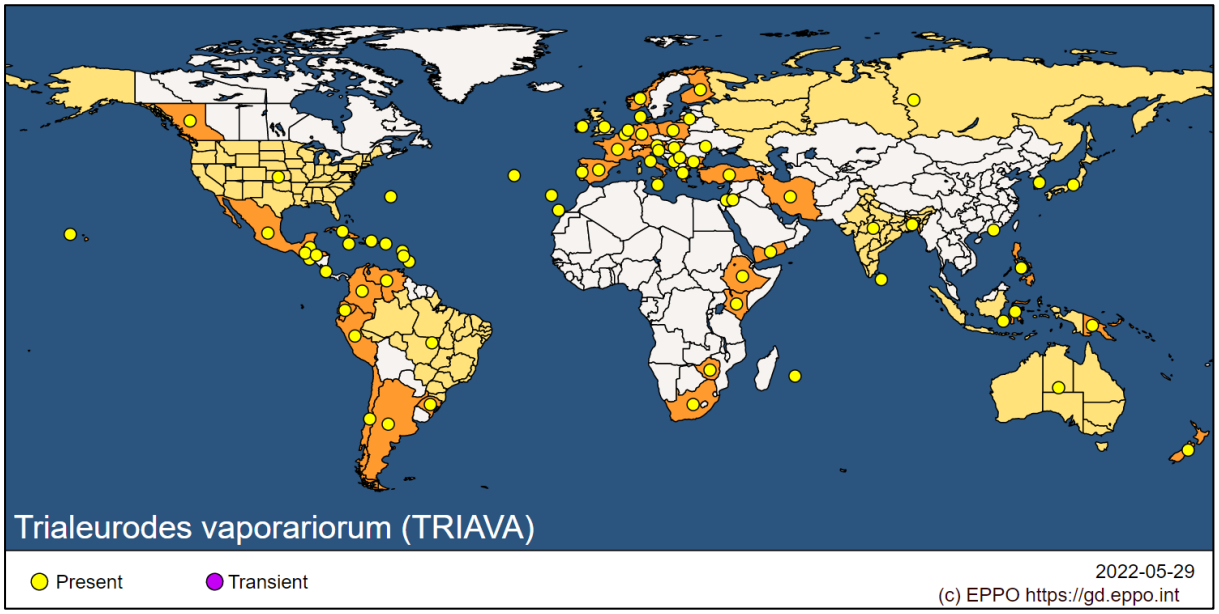


Figure 1.1: Global distribution of *Trialeurodes vaporariorum* (EPPO, 2022).

1.2 Life Cycle

The life cycle of *T. vaporariorum* comprises of six stages, *viz*. eggs, four nymphal stages and the adult stage (Fig. 1.2). The life cycle of *T. vaporariorum* is longer than that of the sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (Sani *et al.*, 2020). The development period from egg to adult ranges from 25–30 days for *T. vaporariorum* compared to 20–30 days for *B. tabaci* at 21º C (Capinera, 2020). Fecundity is also temperature-dependent, and it varies between females. The optimal temperature range for oviposition is between 20 ºC and 25 ºC for *T. vaporariorum* and *B. tabaci*, irrespective of the host plant on which they are reared (Drost *et al.*, 1998; Capinera, 2020).



Figure 1.2: The typical life cycle of *Trialeurodes vaporariorum*: Egg (A); 1st instar nymph (B); 2nd instar nymph (C); 3rd instar nymph (D); 4th instar nymph (E); Pupa (F); Adult female (G); Adult male (H).

1.3 Damage

Trialeurodes vaporariorum adults and nymphs feed by means of piercing-sucking mouthparts and cause direct damage since they are obligate phloem feeders (Byrne & Bellows Jr, 1991; Walker *et al.*, 2010; Perring *et al.*, 2018; Rincon *et al.*, 2019; Li *et al.*, 2021b). Feeding of whiteflies results in stunted plant growth and desiccation, as well as reduced photosynthetic efficacy of the plant, which ultimately results in yield losses (Nabity *et al.*, 2008; Sun *et al.*, 2020; Farina *et al.*, 2022). Indirect damage is caused through the secretion of honeydew during feeding, which supports the growth of secondary fungi (Vieira *et al.*, 2011; Kamikawa *et al.*, 2018; Padilha *et al.*, 2021). Excessive growth of fungi can cause host plant leaves to turn black (Fig. 1.3), resulting in increased solar radiation on the leaf surface causing burns and reduced photosynthesis (Barros *et al.*, 2021).



Figure 1.3: Sooty black mould on peanut leaves, *Arachis hypogaea*. Sooty black mould is the result of the overproduction of honeydew by whiteflies. (Brown, 2011).

Whiteflies are vectors of several plant viruses (Jones, 2003; De Barro *et al.*, 2011; Fiallo-Olivé *et al.*, 2020; Ghosh & Ghanim, 2021). *Trialeurodes vaporariorum* is a known vector of beet pseudo-yellow viruses, tomato infectious chlorosis viruses and lettuce infectious yellow viruses (CABI, 2021). By comparison *B. tabaci*, is a known vector of the more economically important tomato yellow curl leaf virus (TYCLV) and

African cassava mosaic virus (ACMV) (Czosnek *et al.*, 2017; Jacobson *et al.*, 2018; Prasad *et al.*, 2020).

1.4 Economic damage to crops

In the 1970s, *T. vaporariorum* became an economically important pest mainly on greenhouse-grown vegetables and ornamentals (CABI, 2021). Continued inadequate management strategies, mainly through the use of insecticides, have caused high levels of resistance evolution, making whiteflies increasingly difficult to control (Kranthi *et al.*, 2002; Naveen *et al.*, 2017; Saghafipour *et al.*, 2020; Parola-Contreras *et al.*, 2022). It is, therefore, imperative to reduce insecticide use and only apply it when necessary (Gusmão *et al.*, 2006). To determine when applications are needed, economic damage, which is the damage caused by a pest that will validate the cost of integrated pest management strategies (Stern *et al.*, 1959), should be determined. The economic injury level (EIL), and the gain threshold are used to calculate economic damage. The EIL is the minimum number of insect pests required to inflict damage that is equal to the gain threshold (Stern *et al.*, 1959; Higley & Pedigo, 1996; Gusmão *et al.*, 2006). The gain threshold is an expression of the yield loss, which justifies pest management generated from the relationship between the operation costs and crop value (Higley & Pedigo, 1996; Gusmão *et al.*, 2006). Sampling or scouting of plants is essential in this decision-making process and estimation of EILs.

Limited information is available on the economic thresholds for *T. vaporariorum*, but it is well-documented for *B. tabaci.* However, given the economic importance, IPM programs will benefit from the data generated on the EIL's of *T. vaporariorum* on various crops (Pedigo *et al.*, 1986; Pereira *et al.*, 2017; Costa *et al.*, 2019; Rincon *et al.*, 2019). Since *B. tabaci* and *T. vaporariorum* share many of the same host plants (Zhang *et al.*, 2011a). The following examples of economic thresholds for *B. tabaci* on several crops could serve as an indication for the economic thresholds of *T. vaporariorum* on the same crops.

1.4.1 Tomato, *Solanum lycopersicum* (Solanaceae)

Gusmão *et al.* (2006) reported that four *B. tabaci* nymphs and one adult per tomato leaf, caused crop damage which was equal to the cost of control. These authors therefore suggested three scenarios on which the decision to apply chemical control

should be based. Firstly, no need to apply control, which is when the whitefly population density is low and does not require a decision in terms of chemical control. Secondly, control should be implemented when the whitefly population density reaches a level that will result in economic damage, and thirdly, an in-between scenario where a population is present at a low density but needs to be monitored to be aware when it reaches damaging levels and control is needed.

1.4.2 Soybean, *Glysine max* (Fabaceae)

Soybean is one of the most important crops consumed worldwide with 36.9 million hectares cultivated in Brazil, which supplies 37% of the global demand (Padilha *et al.*, 2021). A study conducted on *B. tabaci* on Brazilian soybean crops indicated that whitefly populations peaked during the reproductive stage of soybeans (R1–R5), which is the most susceptible stage (Padilha *et al.*, 2021). Under the conditions that prevailed during this study and taking into consideration a US$ 20.00 ha^{-1} cost of control, and crop value of US$ 400.00 ton^{-1}, the EIL level for *B. tabaci* was reached with an average population density of 1.465 ± 0.195 individuals per trifoliate. There is a linear relationship between the number of *B. tabaci* present on a plant and grain yield. A single *B. tabaci* per trifoliate reduced grain yield on average by 0.16 ± 0.01 g $plant^{-1}$ or 31.24 ± 4.21 kg ha^{-1} (Padilha *et al.*, 2021).

1.4.3 Melon, *Cucumis melo* (Cucurbitaceae)

Melons are among the most popular fruits consumed worldwide. Global production reached 28.467 million tonnes, with Brazil accounting for 613 933 tonnes in 2020 (FAOSTAT, 2022). *Bemisia tabaci* is an important pest of melon crops (Sharma *et al.*, 2016; Costa *et al.*, 2019), but limited information regarding the EIL of *B. tabaci* on melon crops is available (Costa *et al.*, 2019). However, in a study by Costa *et al.* (2019), the EIL for *B. tabaci* on melon was established at 0.38 and 0.22 adults per melon sample unit (the fifth most apical leaf of one branch) for the vegetative and reproductive stages, respectively (Costa *et al.*, 2019). The number of leaf and pod samples required for an accurate estimation and decision-making was determined to be 16 during the vegetative stage and 25 during the reproductive stage, respectively. Resampling should be implemented after 6–7 days (Costa *et al.*, 2019).

1.5 Integrated pest management - Whiteflies

Integrated pest management (IPM) can be defined as a pest control system that considers environmental and insect pest population dynamics, to implement appropriate management strategies that maintain pest population densities below economically damaging levels (Dent, 2000a). The management strategies employed in modern agriculture for the control of pest infestations include chemical-, biological-, and cultural control as well as genetic host plant resistance (USDA-ARS, 2018; Dara, 2019).

1.5.1 Cultural Control

Cultural control is a management strategy that can be defined as the manipulation of an insect's environment, rendering it unfavourable for the pest (Dent, 2000b). Various methods are employed in this regard and are discussed below.

1.5.1.1 Ultra-violet reflective and absorbing films

An example of a cultural control method implemented for control of insect pests is the installation of reflective or absorbing ultraviolet (UV) films (Fig. 1.4) (Shimoda & Honda, 2013).



Figure 1.4: Silver mulching sheets covering a soybean field (Shimoda & Honda, 2013).

UV-absorbing films block and alter part of the solar spectrum that is perceived by insects, and affect behavioural traits such as spatial orientation, navigation, feeding, and mating behaviour (Coombe, 1982; Scherer & Kolb, 1987; Amorós-Jiménez *et al.*,

2020). Some free-flying insects generally stabilise their horizontal orientation by light shining on their dorsal side when the sun shines from above during flight (Jander, 1963; Goodman, 1965). When light is reflected from the ground, insects become disorientated and unable to continue with their normal flight (Shimoda & Honda, 2013). Such a disruption of flight can be facilitated by using highly reflective UV-films to cover the ground and provide light reflection from below (Shimoda & Honda, 2013). Ultra-violet reflective films and white plastic films were also reported by Latora *et al.* (2022) to significantly repel *B. tabaci* in yellow squash crops.

1.5.1.2 Intercropping

Intercropping is also used as a cultural control practice to manipulate and disorient pest distribution, behaviour and density (Zhang *et al.*, 2020). Intercropping is an agronomic practice of planting and growing two or more crops in the same field simultaneously (Asseng *et al.,* 2014). An example of greenhouse vegetable intercropping for control of whiteflies is provided in Fig. 1.5.



Figure 1.5: Greenhouse intercropping to control whiteflies (Haseeb, 2021).

Furthermore, some intercropping approaches may also utilise the added advantage of the ‘push-pull’ strategy (Cook *et al.*, 2007; Zhang *et al.*, 2020). This strategy uses a combination of behaviour-altering stimuli, such as plant semiochemicals, to manipulate the distribution and abundance of pests and/or beneficial insects (Cook *et al.*, 2007). Intercropping also improves natural enemy abundance, which results in increased pest herbivory, aiding in pest control (Cook *et al.*, 2007). Cotton plants

intercropped with cantaloupe or sunflower as trap crops hosted significantly fewer *B. tabaci* adults and nymphs, according to Zhang *et al*. (2020)., with cantaloupe the preferred host (Zhang *et al.*, 2020). Cotton yield in control plots that were not intercropped was markedly lower compared to cotton yields in plots intercropped with cantaloupe or sunflower (Zhang *et al*., 2020).

1.5.2 Biological Control

Biological control is another element of IPM that focuses on the interactions between insect pests and their natural enemies, aiming to reduce pest population densities below economically damaging levels. Biological control has almost no risks associated with it (Dent, 2000c). Some of the common biological control agents used to control *B. tabaci* and *T. vaporariorum* include parasitoids, entomopathogenic fungi (EPF) and entomopathogenic nematodes (EPN) (Sani *et al.*, 2020).

1.5.2.1 Parasitoids

Current research on parasitoids of whiteflies has increased due to the impact of these pests on various vegetable and ornamental crops (Liu *et al.*, 2015b). Parasitoids can serve as effective biological control agents, and together with their contribution to the development of new biological pesticides, can aid in sustainable and eco-friendly agriculture (Gelman *et al.*, 2005; Kumari *et al.*, 2023). Over 500 species of parasitoids from 23 different genera are known to parasitise whiteflies (Liu *et al.*, 2015b). *Encarsia formosa* (Hymenoptera: Aphelinidae) (Fig. 1.6) is a popular biological control agent used globally to control whitefly infestations in greenhouses on crops such as tomatoes and cucumbers (van Lenteren & Woets, 1988; van Lenteren *et al.*, 1996; Hoddle *et al.*, 1998). This parasitoid generally parasitises all pre-adult stages of whiteflies, excluding eggs, but prefers second instar nymphs and pupae (Hoddle *et al.*, 1998).



Figure 1.6: Adult, *Encarsia formosa* (Osborne, 2021).

1.5.2.2 Entomopathogenic fungi

Entomopathogenic fungi (EPF's) are important biological control agents of various sap-sucking insect pests, including whiteflies (Sani *et al.*, 2020). They can infect insects directly through their cuticles (Khan, 2012; Mascarin *et al.*, 2013; Sani *et al.*, 2020). Insects generally become infected when they come into contact with fungal spores which reside on plants, soil, cadavers of other insects and as airborne particles (Sinha *et al.*, 2016). Currently, approximately 700 species of EPF's have been identified to have pathogenic effects on insects (Abdelghany, 2018; Sani *et al.*, 2020). Species specifically from the genera *Verticillium*, *Isaria* and *Aschersonia* genera are known to kill all life stages of whiteflies (Núñez Del Prado *et al.*, 2008; Sani *et al.*, 2020).

The general process of fungal infection of insects provided by Sinha *et al.* (2016) is shown in Fig. 1.7. Infection starts with the germination of fungal spores, also called conidia, on the cuticle of their insect host, followed by penetration of the insect body through the cuticle, generally in between joints (Sinha *et al.*, 2016). Once inside the body of the host, the fungal spores rapidly reproduce and grow in the hemocoel of the insect, generally resulting in insect mortality (Sinha *et al.*, 2016). However, some fungi can also produce toxins which increase the rate of killing (Sinha *et al.*, 2016). After the insect has died, fungal growth will continue outside the exoskeleton and begin to produce spores, subsequently restarting the life cycle (Sinha *et al.*, 2016).

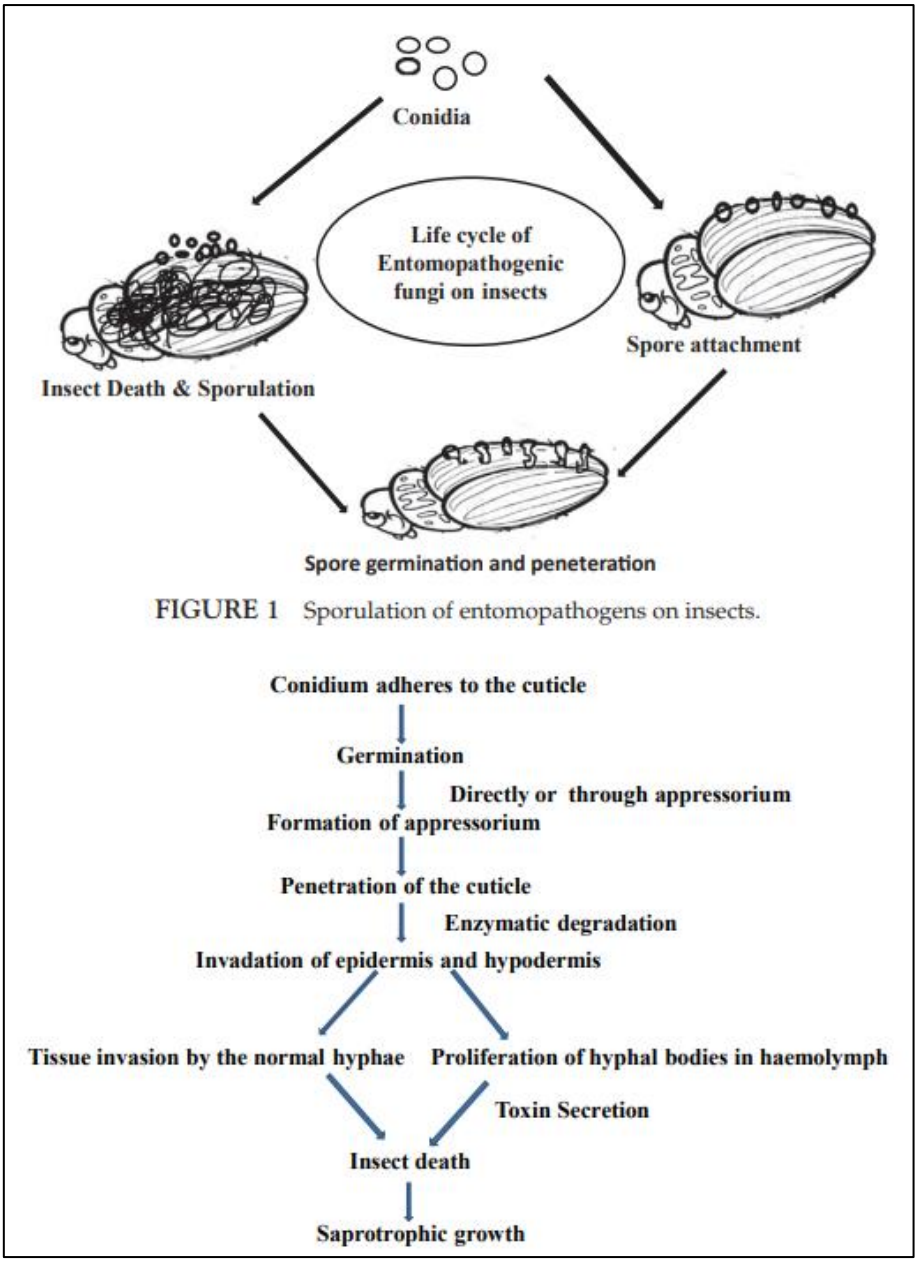


Figure 1.7: Phases of entomopathogenic fungal infection (Sinha *et al.*, 2016).

Isolates of *Beauveria bassiana* (Ascomycota: Hypocreales) and *Lecanicilium muscarium* (Hypocreales: Cordycipitaceae) have been used as formulated foliar sprays to control whitefly infestations (Espinosa *et al.*, 2019). Some *B. bassiana* and *L. muscarium* isolates were reported by Espinosa *et al*. (2019) to be highly effective in controlling 3rd instar whitefly nymphs, with a very high mortality rate of 97%, and more than 80% *B. tabaci* egg mortality. These EPF's can therefore serve as biological control agents that can be integrated into a whitefly management program (Espinosa *et al.*, 2019).

1.5.2.3 Entomopathogenic nematodes

Entomopathogenic nematodes (EPN's) are biological control agents used to manage a variety of insect pests including whiteflies. *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae) (Fig. 1.8) is one of the important nematode species utilised for its pathogenic activity towards insect pests (Wright *et al.*, 2005; Li *et al.*, 2021a).



Figure 1.8: *Heterorhabditis bacteriophora* (Natural Enemies, 2022).

Heterorhabditis bacteriophora penetrates the cuticle or spiracles of their insect hosts whereafter it releases its associated bacterium, *Photorhabdus luminescens* into the hemocoel of the insect. This bacterium is then responsible for rapid killing of the insect host (Bedding & Molyneux, 1982; Ciche, 2007; Li *et al.*, 2021a). After the insect has died, the nematodes feed on the decaying carcass, reproducing for 2-3 generations (Ciche, 2007). When nutrition provided by the decaying insect has been depleted, newly developed infective juveniles (IJs) burst free from the insect carcass searching for their next host, with *H. bacteriophora* IJ's emerging from a greater wax moth larva (Fig. 1.9) (Greb, 2003).



Figure 1.9: *Heterorhabditis bacteriophora* IJs emerging from greater wax moth larva (Greb, 2003).

The life stages of *B. tabaci* most susceptible to EPN infestations, are the second and third instar nymphs (Cuthbertson *et al.*, 2007; Li *et al.*, 2021a). This was confirmed by Li *et al.* (2021a), who reported 66% nymph mortalities caused by *H. bacteriophora.* Therefore, EPNs, specifically *H. bacteriophora,* offer an alternative to chemical control and greatly reduces the negative impact thereof on the environment (Li *et al.*, 2021a).

1.5.3 Host Plant Resistance

Host plant resistance (HPR) can be defined as the genetically inherited characteristics that regulate the amount of damage experienced by a plant due to the herbivory of an insect pest (Smith & Clement, 2012; Stout, 2014). Host plant resistance against whiteflies is an economical and environmentally safe approach to manage whitefly infestations (Suhag *et al.*, 2021). It can either directly (antibiosis), or indirectly (antixenosis) affect an insect pest (Smith & Clement, 2012).

1.5.3.1 Antibiosis

Antibiosis is defined as the negative effects of a resistant host plant on the survival, development, or fertility of an insect pest (Smith & Clement, 2012). Da Silva Oliveira *et al.* (2021) reported first instar *B. tabaci* nymph development time to be extended by a third on resistant cotton genotypes, resulting in fewer generations per cropping season. The prolongation of these developmental stages is a result of increased concentrations of biochemical compounds in the cotton leaves due to increased insect damage (Da Silva Oliveira *et al.*, 2021).

1.5.3.2 Anitixenosis

Antixenosis is a term that describes the non-preference response of an insect to the physical or biochemical characteristics of a resistant host plant. It causes a negative behavioural response resulting in delayed acceptance or rejection of a host plant (Smith & Clement, 2012). It can, for example, be attributed to unfavourable physical or biochemical characteristics of these plants that result in a deterrent effect to the insect pest as reported by Baldin *et al.* (2022) for resistant collard genotypes.

1.5.4 Chemical Control

Chemical control is the application of synthetic chemical compounds or insecticides to control pest infestations (Pimentel, 2009; Dara, 2019). Some of the most popular chemical compounds that are registered for the control of *T. vaporariorum,* include cyantraniliprole, diafenthiuron, flupyradifurone, imidacloprid, pymetrozine and spirotetramat (Harrewijn & Kayser, 1997; Nauen *et al.*, 2007; Gorman *et al.*, 2010; Ovčarenko *et al.*, 2014; Nauen *et al.*, 2015; Núñez-López *et al.*, 2015; Moreno *et al.*, 2018; Farina *et al.*, 2023).

1.5.4.1 Cyantraniliprole

Cyantraniliprole is a novel group 28 anthranilic diamide commonly utilised for the control of sucking insect pests such as whiteflies (Hopkinson & Pumpa, 2019). Cyantraniliprole activates ryanodine receptors and provides cross-spectrum activity against a wide range of insect orders (Selby *et al.*, 2013; Barry *et al.*, 2015; Hopkinson & Pumpa, 2019; Guo *et al.*, 2021a). These ryanodine receptors facilitate the uncontrolled release of calcium ion reserves present in the muscle cells of insects causing muscles to weaken resulting in a rapid decrease in feeding, ultimately inducing insect mortality (Selby *et al.*, 2013; Guo *et al.*, 2021a). Compared to chlorantraniliprole, cyantraniliprole presents enhanced systemic properties, namely lower log*P* values resulting in increased water solubility which ultimately supports improved plant uptake and translocation (Selby *et al.* (2013). Since the mode of action of diamides is different from other insecticide groups, chances for cross-resistance of cyantraniliprole with other insecticides is limited. Cyantraniliprole interferes with the feeding behaviour of the pest, causing transmission of tomato yellow leaf curl virus

(TYLCV), commonly vectored by *T. vaporariorum,* to be reduced with the use of cyantraniliprole (Civolani *et al.*, 2014; Caballero *et al.*, 2015; Guo *et al.*, 2021a).

Cyantraniliprole applied as a foliar spray and as a soil drench, provide control of *B. tabaci* individuals by reducing female oviposition and nymph survival Li *et al.* (2021b), cited in (Caballero *et al.*, 2015). However, Ahumada and Chorbadjian (2019) reported *T. vaporariorum* to be more tolerant to cyantraniliprole applied as a direct foliar spray, compared to *B. tabaci*. Susceptibility of *T. vaporariorum* to cyantraniliprole also varies between strains and localities (Moreno *et al.*, 2018). For example, the susceptibility to cyantraniliprole of *T. vaporariorum* strains collected across the Mediterranean Basin varied 11.4-fold.

1.5.4.2 Diafenthiuron

Diafenthiuron is a group 12A insecticide, first introduced in 1988 to mainly control a wide range of sucking insect pests, especially whiteflies and aphids (Kayser & Eilinger, 2001; Saleem *et al.*, 2021). Diafenthiuron inhibits oxidative phosphorylation, resulting in disruption of adenosine triphosphate (ATP) formation (Kumar *et al.*, 2019). However, diafenthiuron itself does not exert a pesticidal effect and must be activated/transformed into a carbodiimide - diafenthiuron-derived carbodiimide (Steinmann *et al.*, 1989; Ruder *et al.*, 1991; Ruder & Kayser, 1992; Ruder & Kayser, 1993; Ruder & Kayser, 1994; Kayser & Eilinger, 2001). Activation is generally mediated by sunlight after application to crops, thus environmental factors ultimately have an effect on the efficacy of this compound (Kayser & Eilinger, 2001).

Despite the 100% *B. tabaci* adult mortality within 48 hours, and significant reduction in pupal formation and adult emergence caused by diafenthiuron, only a minor reduction in female fecundity and egg hatch rates was reported (Ishaaya *et al.*, 1993). Similar results were reported for *T. vaporariorum*, with nymphs highly susceptible to diafenthiuron-treated tomato seedlings (de Cock *et al.*, 1995). It was, however, found not to be effective against eggs, which was in contrast to the findings of Ishaaya *et al.* (1993) who reported 30-35% reduction in egg hatch for *B. tabaci*. Results from field experiments in Burkina Faso confirmed the efficacy of this product against whiteflies

with diafenthiuron-treated cotton that hosted significantly fewer whiteflies compared to the untreated control cotton (Otoidobiga *et al.*, 2003).

1.5.4.3 Flupyradifurone

Flupyradifurone is a novel group 4B butanolide nicotinic acetylcholine receptor (nAChR) competitive modulator, generally causing a range of symptoms from rapid excitation to inactivity. It leads to cessation of feeding, paralysis and ultimately insect death (Nauen *et al.*, 2015; IRAC, 2022a). Flupyradifurone also exhibits effective translaminar activity with a log*P* value of 1.2, readily translocating from leaves, stems or roots after application (Nauen *et al.*, 2015; Colares *et al.*, 2016; Pesticide Properties Database, 2016; Barbosa *et al.*, 2017; Wang *et al.*, 2020; Zhou *et al.*, 2020). A benefit of flupyradifurone is that it can be applied during flowering of crops since it does not have any adverse toxic effects towards honeybees (Nauen *et al.*, 2015). However, recent studies have shown that although direct exposure of flupyradifurone may not cause bee mortality, post-exposure effects may have a debilitating effect on bee survival and behaviour (Tan *et al.*, 2017; Al Naggar & Baer, 2019; Chakrabarti *et al.*, 2020; Guo *et al.*, 2021b; Harwood *et al.*, 2022; Kablau *et al.*, 2023; Chen *et al.*, 2024). Foliar applications of flupyradifurone trigger a knockdown and feeding cessation effect in *B. tabaci* populations, which further reduces their ability to transmit TYLCV (Roditakis *et al.*, 2017). Additionally, both lethal and sub-lethal concentrations of flupyradifurone effectively reduce the relative TYLCV loads in treated plants by reducing whitefly feeding time (Liu *et al.,* 2021). Field populations of *B. tabaci* exhibited high susceptibility towards flupyradifurone in China (Wang *et al.*, 2020). It also exhibits strong efficacy in controlling *T. vaporariorum* infestations (Nauen *et al.*, 2015). Additionally, cross-resistance is not possible between flupyradifurone and imidacloprid in resistant aphid and whitefly populations, due to metabolic restrictions (Nauen *et al.*, 2015).

1.5.4.4 Imidacloprid

Imidacloprid is a first-generation group 4A neonicotinoid insecticide, introduced in 1991, with contact and systemic activity (Mullins, 1993; Nauen, 1995; Buckingham *et al.*, 1997; Bass *et al.*, 2015). Similar to other nicotinic acetylcholine receptor (nAChR) competitive modulators mentioned previously, imidacloprid triggers the uncontrolled

firing of excitatory neurotransmitters in insects, leading to convulsions and ultimately death (Simon-Delso *et al.*, 2015; Casida, 2018; Zhou *et al.*, 2022). Imidacloprid also offers the added advantage of having low soil persistence at a low application rate and a long residual activity, providing prolonged protection against pests (Elbert *et al.*, 1998; Abdel-Razik, 2019). Several studies reported high levels of susceptibility by *B. tabaci* towards imidacloprid, further supporting its efficacy for controlling whitefly populations (Zhang *et al.*, 2011b; Chaitanya & Kumar, 2018). Since its introduction in 1991, imidacloprid provided exceptional efficacy in controlling sucking pests, which resulted in its high popularity for use. However, its constant application ultimately lead to the evolution of insecticide resistance (Basit, 2019). Current studies on imidacloprid resistance by *T. vaporariorum* are limited, however, Kapantaidaki *et al.* (2018) reported Greek populations of *T. vaporariorum* to exhibit moderate levels of resistance to imidacloprid.

1.5.4.5 Pymetrozine

Pymetrozine is a group 9B pyridine azomethine derivate systemic and contact insecticide that targets and binds to and disrupts the transient receptor potential vanilloid (TRPV) channel complexes in invertebrates, which is essential for sensing gravity, balance, acceleration, proprioception, hearing and kinaesthesia (IRAC, 2022b). Disruption results in immediate and permanent termination of feeding ultimately leading to starvation and death of the insect (Fuog *et al.*, 1998; Polston & Sherwood, 2003; Gill & Chong, 2021). Similar to other systemic insecticides, pymetrozine exhibits adequate translaminar activity after foliar application, while its strong translocation characteristics following soil application provide effective protection to new plant growth (Fuog *et al.*, 1998). Pymetrozine resistance in *B. tabaci* populations have been reported globally (Taquet *et al.*, 2020). Furthermore, induced resistance studies conducted in China found that *B. tabaci* resistance to pymetrozine increased linearly with increased pymetrozine concentration (Wang *et al.*, 2021). Karatolos *et al.* (2010) also reported low levels of resistance by *T. vaporariorum* to pymetrozine, with a resistance factor (RF) of 20 reported in only one strain.

1.5.4.6 Spirotetramat

Spirotetramat is a group 23 systemic insecticide that belongs to the chemical class of tetronic and tetramic acid derivatives, used for control of a range of sucking insect pests, including whiteflies (Bojan *et al.*, 2009; Brück *et al.*, 2009; IRAC, 2022c). Spirotetramat mainly disrupts lipid biosynthesis causing insects not to completely shed their exoskeleton, which hampers their growth, and ultimately results in death (Nauen, 2005; Ehrenfreund *et al.*, 2012; Lümmen *et al.*, 2014; Lueke *et al.*, 2020). Once spirotetramat enters the leaves, it is converted to spirotetramat-enol, which translocates up-and-down in a plant through the phloem, allowing it to spread throughout the plant and control pests that are difficult to reach by traditional foliar applicators (Nauen *et al.*, 2007). Under aerobic soil environments, spirotetramat breaks down quickly (1–2 days), with more than 90% dissipated (Babczinski & Hellpointner, 2008). Spirotetramat is therefore environmentally safe and considered to be harmless to most beneficial insects (Brück *et al.*, 2009). Spirotetramat controls both *B. tabaci* immatures and adults, with high toxicity to immature stages, but less toxic to adult whiteflies (Abbas *et al.*, 2022). Cross-resistance studies confirmed spiromesifen-resistant *T. vaporariorum* strains to be also resistant to spirotetramat (Karatolos *et al.*, 2012b). Resistance to spirotetramat by 2nd instar *B. tabaci* nymphs has recently been reported in populations in the United States of America (Dimase *et al.*, 2024).

1.6 Insecticide Resistance

Its wide host range, short life cycle and high reproductive potential enable *T. vaporariorum* to rapidly evolve resistance to insecticides. Specifically, insecticide resistance of *T. vaporariorum* to a variety of insecticide classes is widespread (Gorman *et al.*, 2007; Kapantaidaki *et al.*, 2018; Erdogan *et al.*, 2021). *Trialeurodes vaporariorum* resistance has been reported for cyantraniliprole, diafenthiuron, imidacloprid, pymetrozine and spirotetramat (Harrewijn & Kayser, 1997; Nauen *et al.*, 2007; Gorman *et al.*, 2010; Ovčarenko *et al.*, 2014; Nauen *et al.*, 2015; Núñez-López *et al.*, 2015; Moreno *et al.*, 2018; Costa *et al.*, 2019; Farina *et al.*, 2023). Continued reliance on insecticides with the same modes of action increases selection pressure for resistance, ultimately resulting in increased control failure frequencies (Guedes, 2017). No information is available regarding the insecticide resistance of *T. vaporariorum* in South Africa.

1.6.1 Mechanism of resistance evolution

Arthropod pests pose a significant threat to global agriculture, the subsequent use of insecticides, therefore, greatly aid in the management of pest infestations (Panini *et al.*, 2016). Applications at higher dosage rates than the recommended label rate or continued application of insecticides with the same modes of action, ultimately results in increased selection for resistance evolution. Individuals possessing the resistance genes evolve over generations, emanating into an increased number of resistant individuals present in a susceptible population (Figure 1.10) (Panini *et al.*, 2016; Ye *et al.*, 2022). Over the past decades malpractice of insecticide application have resulted in resistance in more than 500 arthropod pest species (Nauen *et al.*, 2019). The ability of an arthropod to evolve resistance to insecticides depends on a variety of factors such as genetics, ecology, biochemical composition, generation rate, fecundity rate, dispersal ability, fitness costs, frequency and persistence of insecticide application (Brattsten *et al.*, 1986; Hemingway *et al.*, 2002; Kliot & Ghanim, 2012; Liu, 2015; Panini *et al.*, 2016).

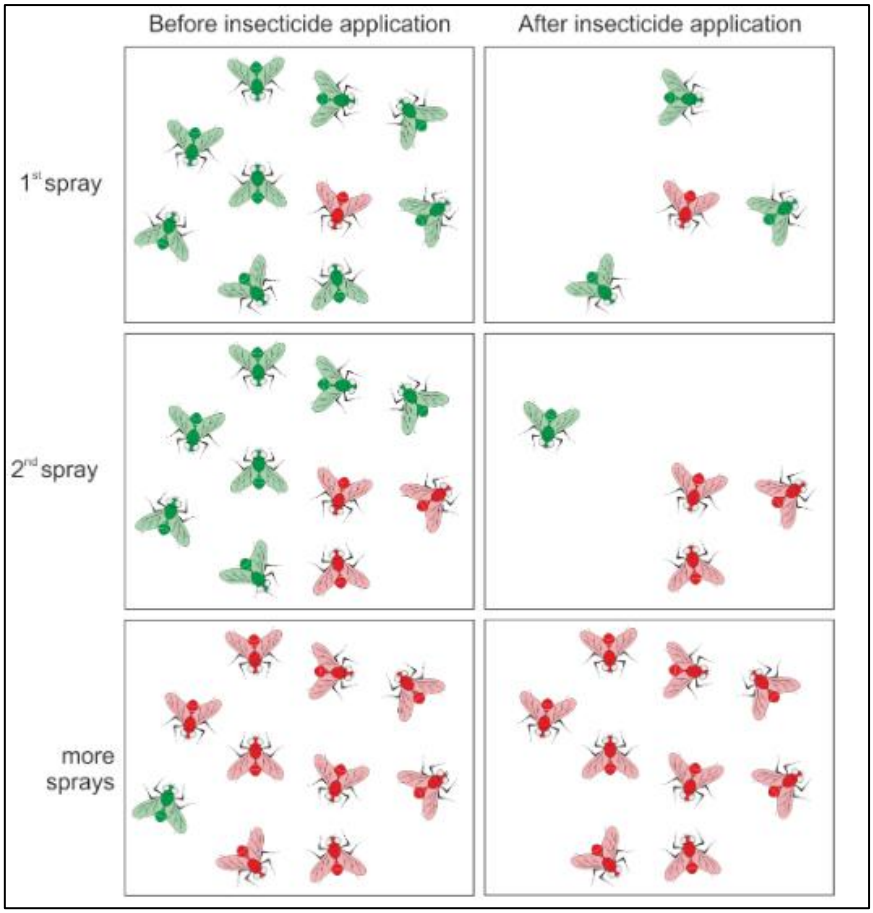


Figure 1.10: Illustration of a hypothetical increase of insecticide resistance levels within a pest population (Panini *et al.*, 2016). The insects indicated in red represent resistant individuals in the population.

Persistent insecticide application will increase selection pressure, resulting in the survival of resistant individuals (red) to carry resistant traits/genes to the next generation, to a point where these individuals dominate in a population (Panini *et al.*, 2016). Insecticide resistance can manifest within an arthropod by various mechanisms that include, metabolic detoxification, target-site modification/resistance, epidermal penetration resistance (reduced penetration of an insecticide or increased excretion of detoxification by-products) and behavioural resistance (Plapp, 1976; Mullin & Scott, 1992; Feyereisen *et al.*, 2015; Pavlidi *et al.*, 2018; Ye *et al.*, 2022).

Metabolic resistance is one of the more frequent defence mechanisms that evolve in insect pests against insecticides (Panini *et al.*, 2016). Enzymatic reactions either detoxify or sequester damaging compounds. These enzymes are usually acquired by means of previous exposure to defensive plant chemicals (Panini *et al.*, 2016). These

enzymes detoxify harmful foreign compounds into less damaging forms that can readily be secreted or sequestered (Panini *et al.*, 2016). Insect pests that exhibit resistance to insecticides can metabolise harmful compounds faster since their detoxifying enzymes catalyse at a higher rate compared to susceptible individuals (Panini *et al.*, 2016). Insecticide detoxification takes place in two stages, stage one primarily consists of hydrolysis or oxidation while stage two involves the conjugation of stage one products with endogenous compounds whereafter they are excreted from the insect body (Panini *et al.*, 2016). One of the prominent enzymes that are involved in stage one of insecticide detoxification are P450 monooxygenases (P450s) (Panini *et al.*, 2016). These cytochromes are especially effective since they can catalyse a variety of reactions, some of which include, O-dealkylation, desulphurisation, N-dealkylation, hydroxylation and epoxidation (Panini *et al.*, 2016). The compounds of a variety of insecticidal classes, namely neonicotinoids, pyrethroids, carbamates and organophosphates can therefore be metabolised by P450 cytochromes (Panini *et al.*, 2016).

Another common resistance mechanism identified in arthropod pests is target-site resistance, where high levels of insensitivity to an insecticide are achieved with mutations to the target protein (Panini *et al.*, 2016). Penetration of the cuticle of an insect by an active ingredient happens before it can reach a target-site. Insect pests with penetration resistance evolve cuticles that reduce the absorption/penetration rate of insecticides (Panini *et al.*, 2016). Penetration resistance usually occurs together with other resistance mechanisms, subsequently resulting in a greater insensitivity towards insecticides (Panini *et al.*, 2016).

Behavioural resistance did not receive much attention from researchers to date. Nevertheless, some studies have found that resistant insects can identify harmful compounds because of previous exposure, and subsequently avoid these compounds (Georghiou, 1972; Lockwood *et al.*, 1984; Sparks *et al.*, 1989; Panini *et al.*, 2016).

1.6.2 Insecticide resistance in whiteflies

Since whiteflies are phloem feeding insects, metabolic resistance is a primary mechanism involved in insecticide resistance evolution in these pests (Karunaratne, 1998; WHO, 1998; Marasinghe & Karunaratne, 2021). Insecticide resistance in *T.*

vaporariorum is largely mediated by the overexpression and induction of P450 cytochrome monooxygenases (Karatolos *et al.*, 2012a; Liu *et al.*, 2015a; Zhou *et al.*, 2020).

These cytochromes have a significant capability to detoxify insecticides through the increased production of proteins and activity of enzymes (Liu *et al.*, 2015a). The interaction of various insecticide-resistant P450 cytochrome enzymes are probably responsible for the evolution of resistance (Liu *et al.*, 2015a). P450 cytochrome enzymes bind to a wide range of substrates, subsequently promoting insects to evolve cross-resistance to other insecticides through metabolic detoxification (Ye *et al.*, 2022). Prior or continued exposure to defensive secondary plant metabolites influences sensitivity of arthropods to insecticides (Johnson, 1999; Francis *et al.*, 2001; Després *et al.*, 2007; Govind *et al.*, 2010; Schuler, 2011; Alon *et al.*, 2012; De La Paz Celorio-Mancera *et al.*, 2013; Vogel *et al.*, 2014; Ragland *et al.*, 2015; Xu *et al.*, 2015; Pym *et al.*, 2019; Zhang *et al.*, 2021).

1.7 Aims and objectives

There is currently no information available regarding the susceptibility of *T. vaporariorum* to insecticides in South Africa. The aim of this study was, therefore, to estimate the susceptibility of *T. vaporariorum* nymphs and adults to insecticides in South Africa.

Specific objectives of the study were to:

1. Estimate the susceptibility of adults and 2nd instar *T. vaporariorum* nymphs to cyantraniliprole, diafenthiuron, flupyradifurone, imidacloprid, pymetrozine, and spirotetramat by conducting IRAC approved leaf- and plant dip bioassays.
2. Compare the susceptibility of *T. vaporariorum* adults exposed to the contact and systemic action of diafenthiuron, flupyradifurone and imidacloprid, respectively.

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Chapter 2:
Susceptibility of *Trialeurodes vaporariorum* to insecticides in South Africa

Abstract

The greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae), is a polyphagous, phloem feeding insect pest. Producers regularly manage infestations in both field and greenhouse settings using systemic and contact insecticides. Its wide host range, short life cycle and high reproductive potential enable *T. vaporariorum* to rapidly evolve resistance to insecticides. To date, no data are available on the susceptibility of *T. vaporariorum* populations to insecticides in South Africa. The aim of this study was to assess its susceptibility to six insecticides, *viz*., cyantraniliprole, diafenthiuron, flupyradifurone, imidacloprid, pymetrozine and spirotetramat. Bioassays were conducted to evaluate the responses of adults and nymphs from two South African populations (WSC-1-24 and BLF-1-25) to these insecticides. Adults from both the WSC-1-24 and BLF-1-25 populations were susceptible to five insecticides tested, although responses to certain compounds varied. Both populations exhibited low adult susceptibility to cyantraniliprole, whereas nymphs were susceptible. Flupyradifurone was effective against both life stages in both populations, except for BLF-1-25 nymphs, whose LC_{80} exceeded the recommended application rate. Susceptibility to pymetrozine was highly variable across both populations and life stages, with particularly low responses. Control failure is therefore likely in the WSC-1-24 population for both adults and nymphs, although BLF-1-25 adults remained susceptible. Imidacloprid also poses a concern, especially for nymph control. Nymphs in the WSC-1-24 population showed low susceptibility, with LC_{80} values of the BLF-1-25 nymphs nearing the recommended rate, though adults remained susceptible to imidacloprid. In contrast, both life stages in both populations were susceptible to diafenthiuron and spirotetramat. These differences in susceptibility between populations likely reflect differing local spray programs, underscoring the need for effective insecticide resistance management strategies.

Key words: Bioassays, insecticide resistance, integrated pest management, LC_{50}, LC_{80}, South Africa

2.1 Introduction

Trialeurodes vaporariorum (Westwood) (Hemiptera: Aleyrodidae), also known as the greenhouse whitefly, is a highly polyphagous phloem feeding insect pest, with a worldwide distribution (Mound & Halsey, 1978; Martin *et al.*, 2000; Mandušić *et al.*, 2019). *Trialeurodes vaporariorum* causes extensive damage to vegetable and ornamental crops (Nissar *et al.*, 2022). It also vectors several plant viruses such as tomato infectious chlorosis virus (TICV), tomato chlorosis virus (ToCV), potato yellow vein virus (PYVV), beet pseudo yellow virus (BPYV) and strawberry pallidosis associated virus (SPaV) (Wisler *et al.*, 1998ab; Tzanetakis *et al.*, 2003; Tzanetakis & Martin, 2004; Roopa *et al.*, 2012; Khamis *et al.*, 2021). Chemical control is frequently implemented to control *T. vaporariorum* (Gorman *et al.*, 2002; Matu *et al.*, 2021). It includes insecticides with a contact, systemic or translaminar action. Continuous application of insecticides with the same mode of action increases selection pressure for insecticide resistance (Mahapatro, 2017; Siddiqui *et al.*, 2023).

Numerous reports around the world have indicated that *T. vaporariorum* evolved resistance to the insecticide classes that are commonly applied for its control, such as neonicotinoids and pyrethroids (Wardlow *et al.*, 1972; Bi *et al.*, 2002; Wang *et al.*, 2003; Bi & Toscano, 2007ab; Gorman *et al.*, 2007; Karatolos *et al.*, 2010; Karatolos *et al.*, 2012ab; Longhurst *et al.*, 2013; Ovčarenko *et al.*, 2014; Kapantaidaki *et al.*, 2018; Erdogan *et al.*, 2021; Ayelo *et al.*, 2022). Resistance is largely due to the over expression of P450 cytochrome monogeneses where it is ascribed to metabolic resistance (Ranson *et al.*, 2002; Xie *et al.*, 2020). P450 cytochromes can readily metabolise insecticides by converting active insecticide compounds into more soluble or less damaging compounds that can easily be excreted or sequestered by an insect (Feyereisen, 2012; Nauen *et al.*, 2022).

Neonicotinoids are applied for the control of sucking insect pests such as whiteflies. Neonicotinoids target the nicotinic acetylcholine receptors (nAChR) that are responsible for rapid neurotransmission in arthropods (Bai *et al.*, 1991; Tomizawa & Yamamoto, 1992, 1993; Yamamoto, 1999). Neonicotinoids bind to and activate nAChR, after which these receptors are unable to deactivate, resulting in continuous firing of neurons that overstimulate the nervous system, eventually leading to the death of an insect (Chang *et al.*, 2013; Buszewski *et al.*, 2019). Due to the application of

neonicotinoids, *T. vaporariorum* evolved resistance to imidacloprid in the United Kingdom as early as 2007 (Gorman *et al.*, 2007; Erdogan *et al.*, 2021). Subsequent cases have also been reported (Longhurst *et al.*, 2013; Pappas *et al.*, 2013; Ovčarenko *et al.*, 2014). Neonicotinoids registered for whitefly control include imidacloprid, acetamiprid and flupryradifurone (Yamamoto, 1999; Bi & Toscano, 2007a; Nauen *et al.*, 2015; Liang *et al.*, 2019; Wang *et al.*, 2020; Bansal *et al.*, 2023).

Another systemic insecticide applied for control of whitefly infestations is cyantraniliprole (Li *et al.*, 2012; Caballero *et al.*, 2013; Grávalos *et al.*, 2015; Wang *et al.*, 2017; Moreno *et al.*, 2018). Cyantraniliprole is a second-generation anthranilic diamide that targets ryanodine receptors in arthropods, causing the continuous release of calcium ions that cause rapid and uncontrolled muscle contraction resulting in paralysis and death (Lahm *et al.*, 2005; Cordova *et al.*, 2006; Lahm *et al.*, 2009; Moreno *et al.*, 2018). Field strains of *T. vaporariorum* collected from the Mediterranean basin exhibited a 11.4-fold variation in the cyantraniliprole concentration needed to kill 50% of the population (LC_{50}) (Moreno *et al.*, 2018). The latter authors also noted a minor increase in LC_{50} values among other collected *T. vaporariorum* strains, which confirmed the evolution of cyantraniliprole resistance (Moreno *et al.*, 2018). Cyantraniliprole resistance was also confirmed in Chilean *T. vaporariorum* populations (Ahumada & Chorbadjian, 2019).

Pymetrozine is a chordotonal organ TRPV channel modulator (Group 9B) (IRAC, 2025). It is a systemic compound with high selectivity to sucking pests, and low toxicity to mammals and beneficial insects (Fuog *et al.*, 1998). Unlike other insecticides used for whitefly control, pymetrozine does not produce a knockdown effect and is not directly toxic to insect pests (Fuog *et al.*, 1998). However, feeding of insects exposed to this insecticide is immediately ceased due to them being unable to insert their stylets into the plant tissue. Treated insects therefore die from starvation. The starvation process is gradual and depending on climatic conditions, treated insects may appear unaffected for several days before death (Fuog *et al.*, 1998).

Since whiteflies are known vectors of plant viruses, the specific mode of action of pymetrozine will therefore greatly reduce the transmission rate of viruses. Limited information is available on the effect of pymetrozine on virus transmission in *T.*

vaporariorum populations. However, transmission studies have been conducted on populations of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (Garzo *et al.*, 2020). *Bemisia tabaci* individuals exposed to pymetrozine-treated tomato plants exhibited reduced probing duration, along with decreased acquisition and transmission rates of tomato yellow leaf curl virus (TYLCV) (Garzo *et al.*, 2020). Resistance to both pymetrozine and neonicotinoids have been reported for European and Asian strains of *T. vaporariorum*, confirming the presence of intragroup cross-resistance (Karatolos *et al.*, 2010). Similarly, resistance and cross resistance to pymetrozine and imidacloprid in a European *T. vaporariorum* population have also been reported by Ovčarenko *et al.* (2014).

Spirotetramat is a systemic insecticide for control of a wide variety of sucking insect pests (Brück *et al.*, 2009; Kay & Herron, 2010; Smiley *et al.*, 2011; Chen *et al.*, 2019; Liu *et al.*, 2022). It disrupts lipid biosynthesis in arthropods, a process essential for cuticle moulting (Brück *et al.*, 2009; Smiley *et al.*, 2011), and therefore controls the immature/ juvenile stages of sucking insect pests (Nauen *et al.*, 2007). Spirotetramat also decreases the fecundity and fertility of adult female sucking pests, leading to a reduction in overall insect populations (Nauen *et al.*, 2007; Brück *et al.*, 2009). Limited information is available regarding the resistance of *T. vaporariorum* to spirotetramat. However, studies have investigated *B. tabaci* resistance to spirotetramat and serves as complimentary information for *T. vaporariorum*.

Spirotetramat resistance has been identified in Spanish field strains of *B. tabaci* with resistance ratios of up to 130-fold (Bielza *et al.*, 2019). Peng *et al.* (2017) reported that the resistance levels of field-collected *B. tabaci* strains to spirotetramat in China, increased from low to high levels of resistance between 2012 and 2016. These authors also found that the nymphal stages of *B. tabaci* were more sensitive to spirotetramat than eggs.

Diafenthiuron is primarily a contact insecticide with some stomach activity and translaminar movement in plants (Brits & Vickers, 1990). It is a pro-insecticide that is converted by light or in *vivo*, by cytochrome P450 in insects, to the corresponding carbodiimide, which is an inhibitor of mitochondrial respiration (Ruder & Kayser, 1994; Kayser & Eilinger, 2001; Tomlin, 2009). This carbodiimide is more toxic than its pro-

insecticide (diafenthiuron) and is a very strong inhibitor of mitochondrial adenosine triphosphate (ATP) synthesis which greatly affects arthropod mitochondrial respiration (Ruder *et al.*, 1991; Ishaaya *et al.*, 1993). No information on resistance of *T. vaporariorum* to diafenthiuron is available.

No data on the susceptibility of *T. vaporariorum* to the insecticides discussed above is currently available in South Africa. The aim of this study was therefore to estimate the baseline susceptibility of *T. vaporariorum* adults and nymphs to selected insecticides in South Africa.

2.2 Material & Methods

2.2.1 *Trialeurodes vaporariorum* collection and rearing

At least 500 *Trialeurodes vaporariorum* adults were collected from various ornamental flowers in a greenhouse in Bloemfontein (BLF-1-25) (29°03'43.3"S 26°08'02.7"E), Free State province and from cucumber and tomato plants at Trawal (WSK-1-24) (31°53'31"S 18°38'28"E), Western Cape province, South Africa. Collected colonies were reared separately on bush bean, *Phaseolus vulgaris* L. var. Contender, in net cages (Fig. 2.1), in a greenhouse at 26±4 °C, 60% RH at the North-West University, Potchefstroom Campus, South Africa.



Figure 2.1: *Trialeurodes vaporariorum* was reared on bush bean in net cages.

To ensure that adult whiteflies used in bioassays were of a uniform age, mating and oviposition on potted bean plants were allowed for 24 hours. Afterwards, the plants

were removed and kept in insect-free cages for approximately 28 days, allowing for the completion of their life cycle. The plants were watered every second day.

2.2.2 Bioassays

2.2.2.1 Adults

The Insecticide Resistance Action Committee (IRAC) susceptibility test method 015 was used to evaluate the susceptibility of *T. vaporariorum* adults (IRAC, 2016). The latter test method required small Petri dishes (diameter of 3-5 cm) to be filled halfway with 1% w/w agar solution. After the agar solution had set, severed bean leaves were cut into discs to a diameter similar to that of the Petri dishes. A 1 L stock solution of each insecticide was prepared from which 10-13 serial dilutions (250 ml) were made. Furthermore, to ensure optimal coverage of insecticides on treated leaves, Triton X-100, a non-ionic surfactant was also added (0.25 ml L^{-1}) to the serial dilutions to reduce the surface tension of the leaves. Leaf discs were then dipped and slightly agitated in the serial dilutions of insecticides and allowed to air dry on a sterilised wire rack. Another agar solution was prepared and allowed to cool to 65 ºC. A thin layer of this solution was poured into the Petri dishes, after which the treated leaves were placed, with their adaxial sides down, onto the agar before it solidified. The additional layer of agar acted as a 'glue' to keep the leaves in position once the Petri dishes were inverted after whitefly inoculation. One hundred adult whiteflies, all uniformly aged and no older than 48 hours, were collected from the rearing colony using an aspirator and transferred into individual 50 mL Falcon® conical tubes (Fig. 2.2).

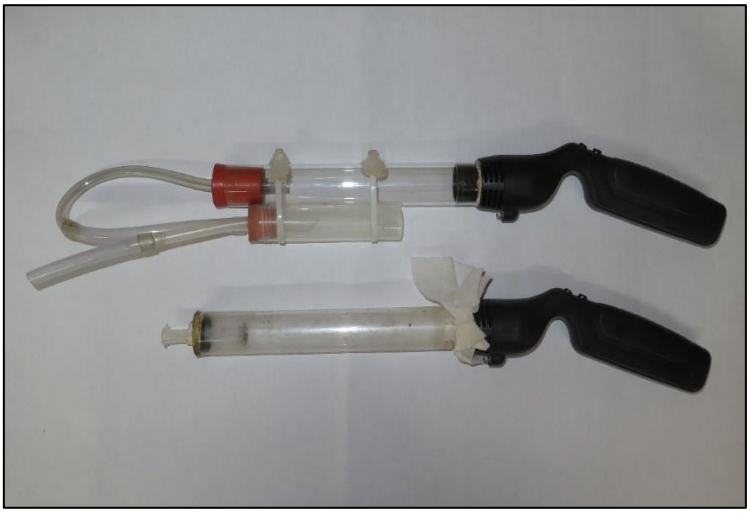


Figure 2.2: Aspirators with falcon tube attached used to collect adult whiteflies.

The whiteflies were lightly anesthetised with medical grade CO_2 for 20-25 s. From the collected adult whiteflies, forty individuals were carefully inoculated with a fine paint brush onto the treated leaves in Petri dishes, whereafter the Petri dishes were inverted to mimic the undersides of leaves. The insecticides tested were cyantraniliprole, diafenthiuron, flupyradifurone, imidacloprid, and pymetrozine. Adult whiteflies were not tested against spirotetramat since it primarily targets nymphs. Mortality caused by cyantraniliprole and diafenthiuron was recorded 48 hours after inoculation onto leaves. Mortality for flupyradifurone and imidacloprid was recorded 72 hours after inoculation and for pymetrozine, 120 hours after inoculation. Mortality was assessed by lightly touching adult whiteflies with a fine paintbrush. Individuals that moved their wings or flew away were recorded as alive, while those that remained motionless were recorded as dead. Each insecticide treatment was replicated three times, with a fresh stock solution prepared for each replicate.

2.2.2.2 Nymphs

IRAC susceptibility test method 016 was used to evaluate the susceptibility of 2nd-instar *T. vaporariorum* nymphs to insecticides. The insecticides tested were cyantraniliprole, diafenthiuron, flupyradifurone, imidacloprid, pymetrozine and spirotetramat (IRAC, 2009). Eighty adult whiteflies were collected with a vacuum aspirator (Fig. 2.2). After collecting, adult whiteflies were anesthetised with CO_2 for 20-25 seconds before being transferred to clip-on cages, which were then attached to a leaf of a bean plant. Twelve to 14 plants were used per replicate. These plants were at a growth stage with at least three fully developed true leaves. Confining the whiteflies in this manner ensured that eggs were laid on the specific leaf area (Fig. 2.4) enclosed by the clip-on cages (Fig. 2.3). The clip-on cages were removed after 24 hours.



Figure 2.3: Whiteflies were confined on bush bean leaves with clip-on cages.

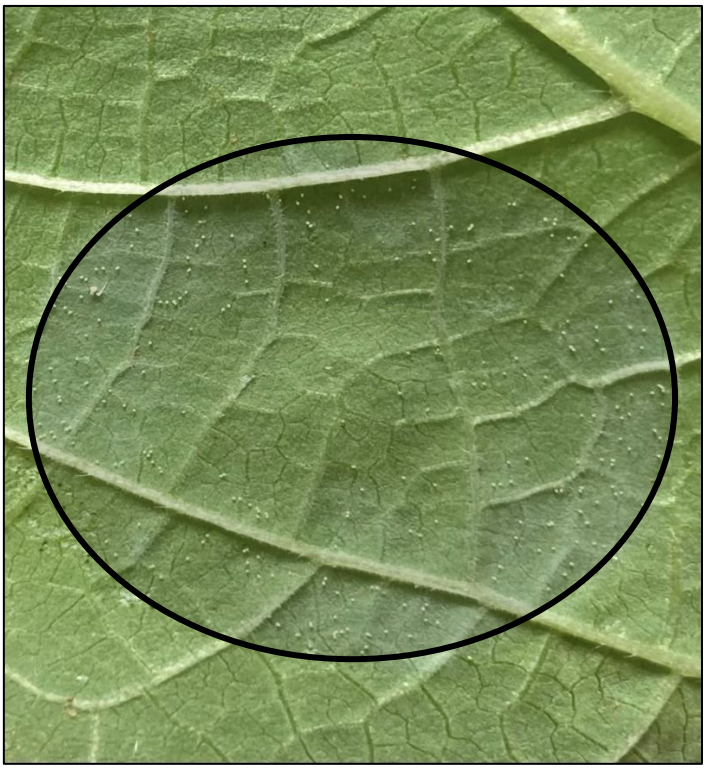


Figure 2.4: Whitefly eggs laid only on the area of a bush bean leaf where adults were confined for 24 hours.

Plants with eggs were kept for ±11 days in a climate-controlled chamber at 26 ºC, 60% RH and a 16L:8D photoperiod until the nymphs reached the 2nd-instar. Serial dilutions of insecticides were prepared (refer to 2.2.2.1). Plants containing 2nd-instar nymphs were dipped into the insecticide solutions, and the leaves were gently agitated during

immersion. After treatment, the plants were allowed to air dry and were left for an additional 9 days. Nymph mortality was then assessed under a ZEISS Stemi 305 stereo microscope. A grid was used to ensure unbiased counting. Forty nymphs per replicate were evaluated and mortality data recorded (Fig. 2.5). Nymphs that reached their 3rd-instar were recorded as alive, while nymphs which remained in the 2nd-instar were recorded as dead (Fig. 2.6)

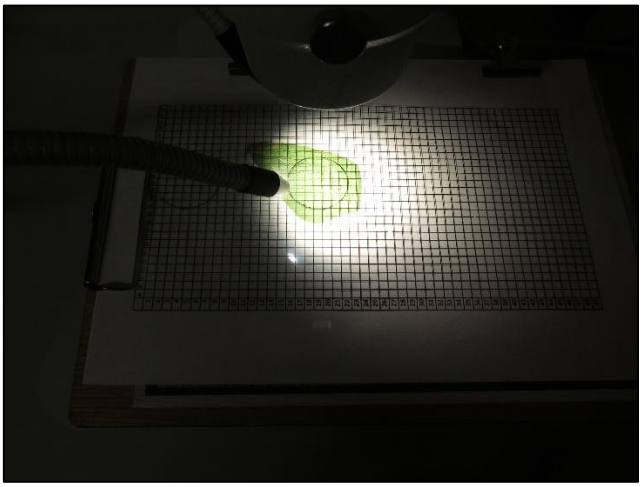


Figure 2.5: A 1×1 cm grid was used under a microscope to assess mortality and count whitefly nymphs.

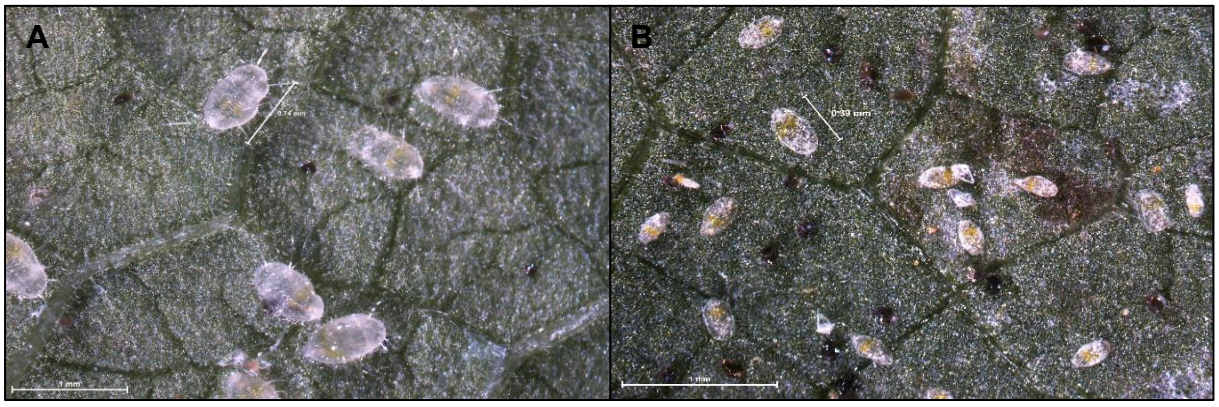


Figure 2.6: Assessment of whitefly nymph mortality following insecticide application, showing (A) surviving nymphs and (B) dead nymphs.

2.3 Statistical Analysis

Whitefly mortality data were subjected to Abbot's formula to correct for control mortalities (Abbott, 1925). A probit dose-response analysis was conducted for each insecticide per insect population to provide LC_{50} and LC_{80} estimates as well as the

corresponding confidence limits (CL 95%). The analyses were conducted with PoloSuite® software (version 1.8) (LeOra Software). Lethal concentrations (LCs) with the corresponding 95% confidence limits (CLs) for relationships between two populations were also calculated. The latter responses were considered significantly different when the 95% CLs of the 50% lethal concentration ratios did not include the value 1 (Robertson *et al.*, 2017).

No baseline insecticide susceptibility data from a *T. vaporariorum* reference population 1675 is currently available in South Africa. Control failure likelihood was therefore assessed according to a method by Silva *et al.* (2011). The estimated LC_{80} value for a specific insecticide tested against each insect population was compared with the recommended label rate of the insecticides to recognise control failure. Where the estimated LC_{80} value was higher than the recommended label rate of the insecticide, control failure is expected. The concentration of the recommended label rates for the respective insecticides was calculated using the following formula:

$$\text{Recommended label rate (ppm)} = \frac{\text{Recommended amount of insecticide (mL OR g) x Insecticide Concentration (mL/L OR g/l OR g/kg)}}{\text{Recommended tank water volume (L)}}$$

Since a range of spray volumes is recommended for some insecticides, an average recommended water volume was calculated for each insecticide individually (Table 2.1).

Table 2.1: Recommended field application rates for the respective insecticides for adults and nymphs of *Trialeurodes vaporariorum* in South Africa.

Active Ingredient	Formulation (g/L) OR (g/kg)	Recommended dosage (mL/ha) OR (g/ha)	Recommended water spray volume (L/ha)	Recommended label rate (converted to concentration) (ppm)		
				Range		
				Low	Mid	High
[1]Cyantraniliprole	100	500	500-2000	25	40	100
[1]Diafenthiuron	500	600	1000		-	300
[1]Flupyradifurone	200	750	1000		-	150
[1]Imidacloprid	700	250	1000		-	175
[2]Pymetrozine	500	400-500	1000	200	225	250
[3]Spirotetramat	240	300-400	1000	72	84	96

The recommended dosage rate calculated according to recommendation for [1]tomato, [2]fruiting vegetables and [3]cucurbits.

2.4 Results

Results of the corresponding probit analyses for the different insecticides are summarised in Table 2.2. Both populations (except for adults from WSC-1-24 and nymphs from the BLF-1-25 population against pymetrozine) responded uniformly and fitted the log(dose)/probit(mortality) model, and linearity of data was obtained. Susceptibility of *T. vaporariorum* adults and nymphs to cyantraniliprole, diafenthiuron flupyradifurone, imidacloprid, pymetrozine and spirotetramat are further discussed below.

2.4.1 *Trialeurodes vaporariorum* adults

2.4.1.1 Cyantraniliprole

The adults of both *T. vaporariorum* populations exhibited very low susceptibility to cyantraniliprole. The LC_{50} and LC_{80} values for the WSC-1-24 population were 1304.72 ppm and 4658.41 ppm, respectively, while those for the BLF-1-25 population were 514.32 ppm and 1043.16 ppm (Table 2.2). The response slopes for both *T. vaporariorum* populations were high (>1), indicating consistent responses (Table 2.2), with the BLF-1-25 population showing a steeper slope than the WSC-1-24 population. The 2.4-fold higher response of the WSC-1-24 population to cyantraniliprole was significantly different to the response of the BLF-1-25 population (Table 2.3). The LC_{80}

values for adults from the WSC-1-24 and BLF-1-25 were 116.46-fold and 26.08-fold higher than the recommended label rate of 100 ppm, respectively.

2.4.1.2 Diafenthiuron

Adult whiteflies from both the WSC-1-24 and BLF-1-25 *T. vaporariorum* populations exposed to diafenthiuron were highly susceptible to diafenthiuron, with LC_{50} values of 6.41 ppm and 9.04 ppm and LC_{80} values of 19.39 ppm and 15.46 ppm, respectively (Table 2.2). The BLF-1-25 population showed a 1.42-fold lower susceptibility compared to the WSC-1-24 population (Table 2.3). The response slopes of both populations were high (>1), with the steepest slope being that of the BLF-1-25 population. The LC_{80}s for adults from both *T. vaporariorum* populations exposed to diafenthiuron were below the recommended field rate of 300 ppm (Table 2.1).

2.4.1.3 Flupyradifurone

Adults from both the WSC-1-24 and BLF-1-25 populations were highly susceptible to flupyradifurone. The LC_{50} and LC_{80} values for the WSC-1-24 population were 3.20 ppm and 8.70 ppm, respectively, while those for the BLF-1-25 population were 2.57 ppm and 4.27 ppm (Table 2.2). The 1.24-fold lower susceptibility of the WSC-1-24 population to flupyradifurone was not significantly different from that of the BLF-1-25 population (Table 2.3). The response slopes of both populations were high (>1), with the steepest slope that of the BLF-1-25 population (3.83) (Table 2.2). The LC_{80}s for adults from both *T. vaporariorum* populations exposed to flupyradifurone were below the recommended field rate of 150 ppm (Table 2.1).

2.4.1.4 Imidacloprid

The LC_{50} and LC_{80} values of adults from the WSC-1-24 and BLF-1-25 *T. vaporariorum* populations exposed to imidacloprid were 4.42 ppm and 21.83 ppm, respectively, while those for the BLF-1-25 population were 9.92 ppm and 24.94 ppm (Table 2.2). The 2.24-fold lower susceptibility of the WSC-1-24 population to imidacloprid was not significantly different from that of the BLF-1-25 population (Table 2.3). The response slopes of both populations were high (>1), with the steepest slope being that of the BLF-1-25 population (2.10) (Table 2.2). The LC_{80}s for adults from both *T. vaporariorum*

populations exposed to imidacloprid were below the recommended field rate of 175 ppm (Table 2.1).

2.4.1.5 Pymetrozine

The responses of *T. vaporariorum* adults from both the WSC-1-24 and BLF-1-25 populations to pymetrozine were highly variable. In particular, the low susceptibility of individuals from the WSC-1-2024 population resulted in inconsistent bioassay outcomes, making it impossible to generate reliable dose-response curves for LC_{50} and LC_{80} estimations. Mortality in the WSC-1-24 population remained below 45%, even at the highest concentration tested (2467.88 ppm). Raw data are presented in Table 2.6. For the BLF-1-25 population, the LC_{50} and LC_{80} values were estimated at 3.23 ppm and 42.52 ppm, respectively, with a response slope of <1 (0.75) (Table 2.2). Notably, the LC_{80} for this population exposed to pymetrozine, remained well below the recommended field application rate of 200 ppm (Table 2.1).

Table 2.2: Log dose probit mortality data for *Trialeurodes vaporariorum* adults from populations collected in two regions in South Africa.

Population	n	LC_{50}	CL (95%)	LC_{80}	CL (95%)	Slope	SE	X^2	df
Cyantraniliprole									
WSC-1-24	480	1304.72	942.44-1949.05	4658.41	2859.78-11300.87	1.52	0.17	4.53	2
BLF-1-25	960	514.32	454.06-627.52	1043.16	906.20-1220.39	2.95	0.23	7.95	6
Diafenthiuron									
WSC-1-24	480	6.41	4.93-7.88	19.39	16.13-24.03	1.75	0.17	2.04	2
BLF-1-25	960	9.01	7.59-10.27	15.46	13.82-17.43	3.59	0.35	7.02	6
Flupyradifurone									
WSC-1-24	720	3.20	2.40-4.02	8.70	7.15-10.63	1.94	0.17	3.99	4
BLF-1-25	600	2.57	2.14-3.05	4.27	3.58-5.33	3.83	0.30	7.20	3
Imidacloprid									
WSC-1-24	840	4.42	0.70-10.39	21.83	8.76-35.47	1.21	0.18	8.26	5
BLF-1-25	600	9.92	7.90-12.10	24.94	20.01	2.10	0.18	5.07	3
Pymetrozine									
*WSC-1-24	-	-	-	-	-	-	-	-	-
BLF-1-25	840	3.23	0.32-10.57	42.52	14.18-84.44	0.75	0.09	10.94	5

*Very low susceptibility of adults. 100% mortality could not be obtained.

Table 2.3: Relative potency ratio and the 95% confidence limits of the WSL-1-24 vs BLF-1-25 populations of *Trialeurodes vaporariorum* adults exposed to different insecticides.

ADULTS		
Insecticides	**Ratio**	**CL 95%**
Cyantraniliprole	2.40	1.842 -3.216*
Diafenthiuron	1.42	1.083-1.851*
Flupyradifurone	1.24	0.943-1.637
Imidacloprid	2.24	0.898-5.604

*Responses of the respective populations compared were significantly different when the 95% confidence limits of the relative potency ratio did not include the value 1

2.4.2 *Trialeurodes vaporariorum* nymphs

2.4.2.1 Cyantraniliprole

The LC_{50} and LC_{80} values for nymphs from the WSC-1-24 population were 3.19 ppm and 8.26 ppm, respectively compared to the LC_{50} and LC_{80} of nymphs from the BLF-1-25 population, which were 13.57 ppm and 42.60 ppm (Table 2.4). The susceptibility of *T. vaporariorum* nymphs of the WSC-1-24 population to cyantraniliprole was 4.25-fold higher than that of the BLF-1-25 population, resulting in a significant difference in susceptibility to this insecticide (Table 2.5). The response slopes of both populations were high (>1), with the steepest slope being that of the BLF-1-25 population (1.69) (Table 2.4). The LC_{80}s for nymphs from both *T. vaporariorum* populations exposed to cyantraniliprole were below the recommended field rate of 100 ppm (high range) (Table 2.1).

2.4.2.2 Diafenthiuron

Trialeurodes vaporariorum nymphs from both the WSC-1-24 and BLF-1-25 populations exposed to diafenthiuron were highly susceptible to this insecticide with LC_{50} and LC_{80} values of 25.58 and 46.46 ppm (WSK-1-24) and 5.82 and 15.27 ppm (BLF-1-25). The susceptibility of *T. vaporariorum* nymphs from the WSC-1-24 population to cyantraniliprole was 4.25-fold lower than that of nymphs from the BLF-1-25 population, resulting in a significant difference in susceptibility to this insecticide (Table 2.5). The response slopes of both populations were high (>1), with the steepest slope being that of the WSC-1-24 population (3.99) (Table 2.4). The LC_{80}s for nymphs from both *T. vaporariorum* populations exposed to diafenthiuron were below the recommended field rate of 300 ppm (Table 2.1).

2.4.2.3 Flupyradifurone

The LC_{50} and LC_{80} values for *T. vaporariorum* nymphs of the WSC-1-24 population exposed to flupyradifurone were 20.07 ppm and 52.16 ppm, respectively, while those for the BLF-1-25 population were much higher at 106.27 ppm and 217.30 ppm (Table 2.4). This reflects a 5.3-fold lower susceptibility in the BLF-1-25 population compared to WSC-1-24, a statistically significant difference (Table 2.5). Both populations exhibited steep dose-response slopes (>1), with the BLF-1-25 population showing the steepest slope (2.10) (Table 2.4). The LC_{80} value for the WSC-1-24 population

remained below the recommended application rate of 150 ppm, whereas that of the BLF-1-25 population exceeded it (Table 2.1), suggesting reduced efficacy against the latter population.

2.4.2.4 Imidacloprid

The LC_{50} (40.45 and 119.73 ppm) and LC_{80} values (172.98 and 483.47 ppm) of nymphs exposed to imidacloprid from both the WSC-1-24 and BLF-1-25 populations, respectively, were high. The susceptibility of *T. vaporariorum* nymphs from the WSC-1-24 population to imidacloprid was 2.96-fold higher than that of the nymphs of the BLF-1-25 population, resulting in a significant difference in susceptibility to this insecticide (Table 2.5). The response slopes of the populations were 1.33 and 1.39 for the WSC-1-24 and BLF-1-25 populations, respectively (Table 2.4). The LC_{80}s for nymphs from the WSC-1-24 population were slightly below the recommended application rate, while the LC_{80} for the BLF-1-24 population exceeded the recommended application rate of 175 ppm (Table 2.1).

2.4.2.5 Pymetrozine

The nymphs from the BLF-1-25 population showed inconsistent responses to pymetrozine, which prevented the data from being fitted to the log(dose)/probit(mortality) model. The LC_{50} and LC_{80} could therefore not be estimated. Raw data of these tests are provided in Table 2.7. Only 41.7% nymphs died at a concentration of 3844.38 ppm, while the recommended application rate (high range) is 225 ppm. Nymphs from the WSC-1-24 population responded more uniformly to pymetrozine, and the data fitted the log(dose)/probit(mortality) model. The LC_{50} and LC_{80} values for nymphs from this population were 41.05 ppm and 341.17 ppm, respectively (Table 2.4). The slope of this response line was low (0.92) and the LC_{80} value exceeded the recommended application rate of 250 ppm (high range) (Table 2.1).

2.4.2.6 Spirotetramat

The LC_{50} and LC_{80} values of nymphs from the WSC-1-24 and BLF-1-25 *T. vaporariorum* populations exposed to spirotetramat were 15 ppm and 38.22 ppm, respectively, while those for the BLF-1-25 population were 20.91 and 69.28 ppm (Table

2.4). The 1.39-fold higher susceptibility of the WSC-1-24 population to imidacloprid was significantly different from that of the BLF-1-25 population (Table 2.5). The response slopes of both populations were steep (>1), with the steepest slope being that of the WSC-1-24 population (2.07) (Table 2.4). The LC_{80}s for nymphs from both *T. vaporariorum* populations exposed to spirotetramat were below the recommended field rate of 96 ppm (high rate) (Table 2.1).

Table 2.4: Log dose probit mortality data for *Trialeurodes vaporariorum* nymphs from populations collected in two regions within South Africa.

Population	n	LC_{50}	CL (95%)	LC_{80}	CL (95%)	Slope	SE	X^2	*df*
Cyantraniliprole									
WSK-1-24	480	3.19	2.26-4.15	8.26	6.29-12.13	2.04	0.20	4.90	2
BLF-1-25	1080	13.57	10.99-16.23	42.60	36.90-49.32	1.69	0.13	4.37	7
Diafenthiuron									
WSK-1-24	840	25.58	26.38-30.66	46.46	43.63-49.68	3.99	0.26	4.17	5
BLF-1-25	840	5.82	4.85-6.87	15.27	12.90-18.39	2.01	0.14	4.56	5
Flupyradifurone									
WSK-1-24	1200	20.07	18.40-21.81	52.16	47.57-57.65	2.03	0.10	5.38	8
BLF-1-25	1200	106.27	83.85-126.75	217.30	184.48-264.99	2.71	0.27	12.61	8
Imidacloprid									
WSK-1-24	720	40.45	29.53-53.98	172.98	127.91-244.86	1.33	0.08	7.17	4
BLF-1-25	960	119.73	86.34-154.47	483.47	372.08-681.77	1.39	0.15	6.47	6
Pymetrozine									
WSK-1-24	720	41.05	27.25-58.72	341.17	216.62-649.43	0.92	0.07	9.82	4
*BLF-1-25	-	-	-	-	-	-	-	-	-
Spirotetramat									
WSK-1-24	720	15.00	11.85-18.37	38.22	31.41-47.55	2.07	0.13	7.21	4
BLF-1-25	1200	20.91	17.95-24.11	69.28	59.42-82.26	1.62	0.09	7.04	8

*Very low susceptibility of adults. 100% mortality could not be obtained.

Table 2.5: Relative potency ratio and the 95% confidence limits of the WSL-1-24 vs BLF-1-25 populations of *Trialeurodes vaporariorum* nymphs exposed to different insecticides.

NYMPHS		
Insecticides	**Ratio**	**CL 95%**
Cyantraniliprole	4.25	3.127-5.782*
Diafenthiuron	4.30	3.503-5.271*
Flupyradifurone	5.30	4.383-6.398*
Imidacloprid	2.96	2.084-4.203*
Spirotetramat	1.39	1.114-1.745*

*Responses of the respective populations compared were significantly different when the 95% confidence limits of the relative potency ratio did not include the value 1

Table 2.6: Raw data of adults from the WSC-1-24 population exposed to pymetrozine.

Concentration (ppm)	Number tested (n)	Mortality (n)
0.00	120	12
10.00	120	21
16.50	120	27
27.23	120	26
44.92	120	31
74.12	120	45
122.30	120	36
201.79	120	28
332.96	120	37
549.38	120	35
906.74	120	47
1495.68	120	55
2467.88	120	49

Table 2.7: Raw data of nymphs from the BLF-1-25 population exposed to pymetrozine.

Concentration (ppm)	Number tested (n)	Mortality (n)
0.00	120	1
2.00	120	10
4.26	120	15
9.07	120	23
19.33	120	27
41.17	120	25
87.69	120	30
186.77	120	34
397.82	120	33
847.36	120	34
1804.87	120	40
3844.38	120	50

2.5 Discussion

This study is the first to provide data on insecticide susceptibility of the greenhouse whitefly, *T. vaporariorum*, in South Africa. It is a much-needed contribution, given the numerous country-wide complaints from farmers and reports of control failures. Currently, *T. vaporariorum* is the predominant whitefly species in both greenhouse and open-field crops in the country (personal observation, unpublished data). Globally, most existing literature focuses on the susceptibility of *B. tabaci* (Caballero *et al*., 2015; Chaitanya & Kumar, 2018; Basit, 2019; Costa *et al*., 2019; Dimase *et al*., 2024).

The current study showed that *T. vaporariorum* nymphs were highly susceptible to cyantraniliprole, while adults from both the WSC-1-24 and BLF-1-25 populations exhibited low susceptibility. The LC_{80} value for adults from the WSC-1-24 population was 116 times higher than the recommended field rate, and 26 times higher for the BLF-1-25 population. As such, no effective adult control with cyantraniliprole is expected in either population, with WSC-1-24 being the least susceptible. The latter findings align with those of Zeinali and Ahmadi (2025), who observed only 53% mortality in *T. vaporariorum* nymphs treated with cyantraniliprole at 100 ppm. Although the nymphs are still regarded as highly susceptible to cyantraniliprole, based on their LC_{80} values being below the recommended application rate, the LC_{50} values for nymphs in the WSC-1-24 and BLF-1-25 populations were 31.4-fold and 7.4-fold higher than those reported in Iran (Zeinali and Ahmadi, 2025). This indicates reduced susceptibility of the populations evaluated in this study, compared to those evaluated by Zeinali and Ahmadi (2025). Additionally, nymphs from these two South African populations were 3.2 times less susceptible than Chilean *T. vaporariorum* nymphs, as reported by Ahumada and Chorbadjian (2019). However, nymphs from the WSC-1-24 population were significantly more susceptible to cyantraniliprole (4.25-fold) than nymphs from the BLF-1-25 population.

The LC_{80} values for diafenthiuron for both nymphs and adults from the WSC-1-24 and BLF-1-25 populations were below the recommended application rate, suggesting that effective control of *T. vaporariorum* can be achieved using this insecticide. However, the LC_{50} value for nymphs from the WSC-1-24 population (25.58 ppm) was 4.3 times higher than that of the BLF-1-25 population, indicating a potential shift in susceptibility in the former. The LC_{50} for BLF-1-25 nymphs was comparable to those of adults from

both populations (6.41 ppm and 9.01 ppm, respectively). Published data on *T. vaporariorum* susceptibility to diafenthiuron are limited. However, de Cock *et al.* (1995) reported LC_{50} values of 13.9 ppm for adults and 14.4 ppm for 1st-instar nymphs from a Belgian population. The LC_{50} for adults is 1.5 to 2 times higher than the corresponding LC_{50} values observed in the WSC-1-24 and BLF-1-25 populations, suggesting greater sensitivity in the South African strains. Although *B. tabaci* 2nd-instar nymphs from a Mediterranean population showed comparable susceptibility to the WSC-1-24 nymphs, with a mean LC_{50} of 32.92 ppm for diafenthiuron (Alvarez *et al.*, 2024). Furthermore, a recent study on a Pakistani *B. tabaci* population demonstrated the potential for rapid resistance evolution, with LC_{50} values for adults increasing from 84 to 123 ppm over a six-year period (Hussain *et al.*, 2025).

Adults from both populations were highly susceptible to flupyradifurone, with LC80 1890 values of 8.70 ppm (WSC-1-24) and 4.27 ppm (BLF-1-25). The LC_{50} values of 3.20 ppm (WSC-1-24) and 2.57 ppm (BLF-1-25) are also consistent with findings by Nauen *et al.* (2015) who reported an average LC_{50} of 4.55 ppm for *T. vaporariorum* adults. Effective control of nymphs from the WSC-1-24 population is therefore expected with flupyradifurone applications. However, the LC_{80} for BLF-1-25 nymphs exceeded the recommended application rate of 150 ppm, indicating likely control failure in this population. Flupyradifurone tolerance in *B. tabaci* has been shown to increase in the presence of certain plant flavonoids (Zhang *et al.*, 2021). Compounds such as rutin, quercetin, kaempferol, myricetin, and catechin, commonly found in bean plants, have been shown to reduce the insecticide's toxicity to *B. tabaci* (López *et al.*, 2013; Ganesan & Xu, 2017; Yang *et al.*, 2018; Zhang *et al.*, 2021). While flupyradifurone is widely used against *B. tabaci*, limited data are available on the evolution of resistance in *T. vaporariorum*, underscoring the need for continued monitoring.

Imidacloprid was highly effective against *T. vaporariorum* adults. However, nymphs were less susceptible. The LC_{80} value for nymphs from the WSC-1-24 population was close to the recommended application rate, while that for the BLF-1-24 population exceeded it, suggesting a high likelihood of control failure in the latter. In comparison, Ovčarenko *et al.* (2014) reported very low LC_{50} values (0.42 - 4.34 ppm) for adults from *T. vaporariorum* populations in Finland. This indicates that the adults evaluated

in the current study were slightly less susceptible to imidacloprid than was reported in Finland. The host plant's influence on *T. vaporariorum* susceptibility to imidacloprid has been demonstrated by Salehi *et al.* (2013), who recorded LC_{50} values of 71.50 ppm on tomato plants and 72.18 ppm on cucumber. These values are notably higher than those recorded for adult whiteflies in both South African populations tested in this study, indicating greater susceptibility in the latter. LC_{50} values reported for two field strains of *B. tabaci* nymphs from China of 68.90 ppm and 92.00 ppm (Liu *et al.*, 2023) were higher than those recorded for the WSC-1-24 population but lower than those of the BLF-1-25 population. Imidacloprid was therefore more effective in controlling *T. vaporariorum* adults. Overall, imidacloprid proved more effective against *T. vaporariorum* adults than nymphs, suggesting that control efforts should focus on targeting adults rather than nymphs.

Responses of both adults and nymphs from the WSC-1-24 and BLF-1-25 populations to pymetrozine were highly variable, and overall susceptibility was very low. Adult whiteflies from the WSC-1-24 population could not be effectively controlled, even at concentrations as high as 2468 ppm. Similarly, the LC_{80} value for nymphs exceeded the recommended field application rate. The very shallow slopes of the dose-response lines observed for nymphs from WSC-1-24 and adults from BLF-1-25 further suggest significant genetic variation among individuals within both populations (Robertson *et al.*, 2017). The findings of this study are consistent with those of Karatolos *et al.* (2010), who reported that LC_{50} values for adult whiteflies exposed to pymetrozine ranged from 38.80 ppm to 484 ppm. The slopes of their dose-response lines, ranging from 1.19 to 2.03, are also comparable to those recorded in the present study. The extremely low susceptibility of *T. vaporariorum* to pymetrozine in South Africa represents a significant loss for producers, as this insecticide serves a dual purpose. It not only kills the pest by disrupting its ability to insert its stylet into plant tissue and impairing nervous system function, followed by starvation and death, but also helps reduce virus transmission due to its unique mode of action.

Control of *T. vaporariorum* nymphs at the recommended application rates appears achievable in both populations, although nymphs from the BLF-1-25 population showed lower susceptibility compared to those of the WSC-1-24 population to spirotetramat. Recently, low levels of resistance to spirotetramat in *B. tabaci* have

been reported in China (Liu *et al.*, 2025), and Dai *et al.* (2023) also noted reduced susceptibility in *B. tabaci* individuals that transmit tomato chlorosis virus (ToCV). Plant viruses have been shown to influence the expression of genes involved in insecticide detoxification (Ding *et al.*, 2019; Dai *et al.*, 2023). The observed decline in susceptibility to spirotetramat, particularly in the BLF-1-25 population, warrants attention, and timely implementation of insecticide resistance management strategies is essential to prevent the evolution of irreversible resistance.

2.6 Conclusions

Adults of *T. vaporariorum* from both the WSC-1-24 and BLF-1-25 populations were generally susceptible to the five insecticides evaluated in this study, *viz*. cyantraniliprole, diafenthiuron, flupyradifurone, imidacloprid and pymetrozine. However, the responses to certain compounds varied. Both populations exhibited low adult susceptibility to cyantraniliprole, whereas nymphs were susceptible. Flupyradifurone was effective against both life stages of both populations, except for BLF-1-25 nymphs, whose LC_{80} exceeded the recommended application rate. Susceptibility to pymetrozine was highly variable across all groups, with notably poor responses. Control failure is likely in the WSC-1-24 population for both adults and nymphs, although BLF-1-25 adults remained susceptible. Imidacloprid also poses a concern, especially for nymph control. Nymphs in the WSC-1-24 population showed low susceptibility, with LC_{80} values for the BLF-1-25 nymphs nearing the recommended rate, though adults remained susceptible. In contrast, both life stages of individuals of both populations were susceptible to diafenthiuron and spirotetramat. The observed differences in susceptibility between populations most likely reflect differences in local spray programs, underscoring the need for effective insecticide resistance management strategies.

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Chapter 3:

Comparative evaluation of two toxicological bioassays for assessing the susceptibility of *Trialeurodes vaporariorum* adults to systemic insecticides

Abstract:

Trialeurodes vaporariorum is a major pest of vegetable crops in South Africa, primarily managed through the use of insecticides. To support effective resistance management strategies, accurate information regarding insecticide resistance is needed. Bioassays are essential tools used to determine the levels of resistance of pests to insecticides. Globally, two standardised bioassay methods are commonly used to evaluate insecticide efficacy against whitefly adults and nymphs. The leaf dip method is used to assess resistance in adults, while immersion of potted plants into serial dilutions of insecticides is done for nymph evaluations. We hypothesised that the systemic action achieved by immersing potted plants in insecticide solutions would lead to lower LC_{50} and LC_{80} values compared to using detached leaves dipped in the same solutions. In this study, both methods were used to evaluate three insecticides, namely diafenthiuron (IRAC Group 12A), flupyradifurone (Group 4D), and imidacloprid (Group 4A). The results differed between bioassay methods. The plant immersion method resulted in higher lethal dose values than the detached leaf dip method across all three insecticides, regardless of their mode of action or action within the plants. The LC_{80} for *T. vaporariorum* exposed to diafenthiuron, flupyradifurone, and imidacloprid, estimated using both bioassay methods, was below their recommended field application rates. This suggests that no control failure of adult *T. vaporariorum* is currently expected with any of these insecticides in South Africa. These significant differences in lethal dose estimations by different bioassay methods highlight the importance of using consistent and standardised bioassay methods in monitoring programs to ensure reliable and comparable results over time.

Key words: Contact, insecticides, IRAC test methods, systemic*, Trialeurodes vaporariorum*

3.1 Introduction

Trialeurodes vaporariorum Westwood (Hemiptera: Aleyrodidae), commonly known as the greenhouse whitefly, is a highly polyphagous pest that causes significant damage to various crops globally (Byrne & Bellows Jr, 1991; Salazar *et al.*, 2000; Paschapur *et al.*, 2023). Direct damage is inflicted through feeding, leading to stunted growth and leaf wilting (Kim *et al.,* 2023), and indirect damage by transmitting plant viruses (Wisler *et al.*, 1998a,b; Wintermantel, 2004; Wintermantel & Wisler, 2006; Navas-Castillo *et al.*, 2011; Fiallo-Olivé & Navas-Castillo, 2023). This pest can also inflict indirect damage by producing honeydew, which serves as a substrate for the growth of sooty mould on plant surfaces. This mould interferes with the plant's photosynthetic capacity (Lloyd, 1922; Hussey *et al.*, 1969; Lindquist *et al.*, 1972; Johnson *et al.*, 1992; Liu *et al.*, 1993; Nasruddin & Mound, 2016).

Chemical control is widely used to control whitefly infestations (Zaidem *et al.*, 2023). Insecticides are generally categorised into contact- and systemic insecticides. Before the development of modern insecticides, producers relied only on contact insecticides such as organophosphates and carbamates for the control of insect pests (Wardlow *et al.*, 1972; Michelakis, 1986). Contact insecticides require direct physical contact with the target insect to be effective (Abubakar *et al.*, 2020). These active ingredients can also enter the insect through cuticular diffusion, inhalation through spiracles, or ingestion of treated plant material (Abubakar *et al.*, 2020). An example of such an insecticide is diafenthiuron, which is designed to target phytophagous insects, including whiteflies (Ishaaya *et al.*, 1993).

Systemic insecticides are absorbed by the roots, stems and leaves of plants (Wedding, 1953), making them effective against pests that are difficult to reach with conventional spray applications. Sucking insect pests such as *T. vaporariorum,* which feed on the underside of leaves, can be challenging to manage using foliar contact insecticides (Bennett, 1957; Jeschke *et al.*, 2013; Jeschke, 2019; Vojvodić & Bažok, 2021; Guedes *et al.*, 2023). Systemic insecticides translocate within plant tissues, allowing for control of phloem-feeding insects such as whiteflies (Horowitz *et al.*, 2004). However, the prolonged residual activity of systemic insecticides such as neonicotinoids may have adverse effects on beneficial insects, including honeybees (Arce *et al.*, 2017; Baron *et al.*, 2017; Siviter *et al.*, 2018; Muth *et al.*, 2019; Siviter & Muth, 2020).

Three of the insecticides currently registered for control of whiteflies in South Africa are diafenthiuron, imidacloprid, and flupyradifurone. These insecticides belong to three different insecticide groups. Flupyradifurone is a butenolide, classified under IRAC group 4D, acting on nicotinic acetylcholine receptors. Imidacloprid is a neonicotinoid, categorised under IRAC group 4A, also targeting nicotinic acetylcholine receptors. Diafenthiuron is a thiourea, classified under IRAC group 12A, with a unique mode of action that primarily inhibits mitochondrial respiration (Ruder & Kayser, 1994).

Monitoring for insecticide resistance should be a key component of insect resistance management (IRM) programs. The use of bioassays to monitor the levels of resistance in pest populations provides information that can be used proactively in IRM, rather than reactive responses to unexpected control failure (Forrester & Cahill, 1987).

Currently, the Insecticide Resistance Action Committee (IRAC) has developed only two standardised bioassay methods to evaluate the efficacy of insecticides against whitefly adults and nymphs, i.e., test methods 008 and 015 (IRAC, 2009, 2016). Test method 015, commonly used to assess adult whitefly susceptibility, involves dipping detached leaves into serial dilutions of insecticides. Because the leaves are removed from the plant prior to dipping, this method is presumed to primarily evaluate the contact activity of insecticides against adults. However, many insecticides used to control whiteflies are systemic, and their efficacy is influenced by biotic and abiotic factors (Thompson *et al.*, 2020; Li, 2025). For nymph susceptibility assessments, test method 008 is recommended. This method involves immersing leaves of potted plants into insecticide solutions to account for systemic uptake (IRAC, 2009).

The Insecticide Resistance Action Committee (IRAC) therefore recommends different bioassay test methods for the adult and nymphal stages of whiteflies, despite both stages being phloem feeders. Additionally, insecticides vary in their mode of action, with some acting through contact and others systemically. We hypothesised that the systemic action achieved by immersing potted plants into insecticide solutions would lead to lower LC_{50} and LC_{80} values compared to using detached leaves dipped in the same solutions. The objective of this study was therefore to compare these two bioassay methods that are used to evaluate the susceptibility of *T. vaporariorum* adults to diafenthiuron, flupyradifurone, and imidacloprid.

3.2 Material and Methods

3.2.1 *Trialeurodes vaporariorum* collection and rearing

Adult whiteflies were collected from Trawal (31°53'31"S 18°38'28"E) in the Western Cape province of South Africa to establish a rearing colony. Rearing was done on bush bean, *Phaseolus vulgaris* L. var. Contender, in a greenhouse at 26±4 °C, 60% RH at the North-West University, Potchefstroom Campus, South Africa.

3.2.2 Bioassays

To obtain whiteflies for the bioassays, plants were put into cages containing whitefly adults for a 24-hour period to allow for oviposition. To obtain same-aged adult whiteflies, the plants were removed after 24 hours after which they were maintained in climate-controlled chambers at 26 ºC, 60% RH and a 16L:8D photoperiod.

An adapted version of IRAC test method 015 was used to test the susceptibility of *T. vaporariorum* adults. The standard method prescribes that leaves are detached and dipped into serial dilutions of an insecticide as summarised in Chapter 2 (see 2.2.2.1). In the adapted method potted bean plants at the three-leaf stage, with their leaves intact, were dipped into the serial dilutions of insecticides. Serial dilutions of insecticides were prepared as described in Chapter 2 (see 2.2.2.1). The control treatment was water. Treated plants were allowed to air dry for 30 minutes before 40 two-day-old adult whiteflies were transferred into clip-on cages on the treated plants. These clip-on cages were then attached to treated leaves and kept in place by a clip connected to a skewer (Fig. 3.1).

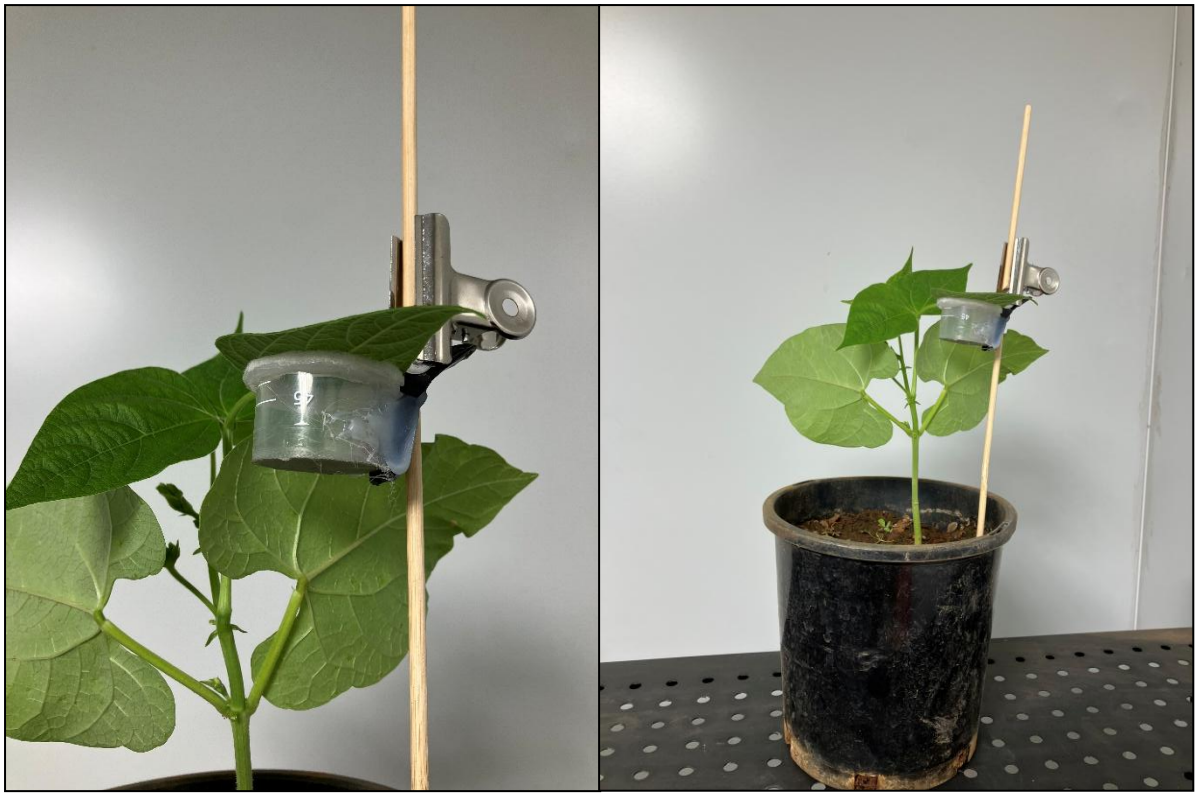


Figure 3.1: Clip-on cages used to confine adult whiteflies after the plants were immersed into an insecticide solution.

The insecticides used were diafenthiuron (Pegasus® 500 SC, Syngenta Global AG), flupyradifurone (Sivanto Prime® SC, Bayer CropScience), and imidacloprid (Confidor® 70 WG, Bayer CropScience). Whitefly mortality was recorded 48 hours after exposure to diafenthiuron and 72 hours after exposure to flupyradifurone and imidacloprid. Each insecticide was tested in triplicate. Each replicate consisted of 12-14 plants (depending on the number of insecticide dilutions used), each fixed with three clip-on cages. Freshly prepared stock solutions were used for each replicate.

3.3 Statistical Analyses

To account for mortality in the control treatment, data were corrected using Abbott's formula (Abbott, 1925), followed by log-probit analysis with PoloSuite® (version 8.1 LeOra Software). Results from the log-probit analysis were used to estimate the LC_{50} and LC_{80} and the corresponding confidence limits (CL 95%) for each insecticide tested. The responses of the whiteflies to the respective insecticides, as determined by the

two test methods, were considered significantly different when the 95% confidence intervals of the relative potency ratio excluded the value 1. The likelihood of control failure was assessed using a method described by Silva *et al.* (2011). The estimated LC_{80} value for a specific insecticide tested against each insect population was then compared with the recommended label rate of that insecticide (see Table 2.1). Where the estimated LC_{80} value was higher than the recommended label rate of the insecticide, control failure is expected. The concentration of the recommended label rate of the respective insecticides was calculated using the following formula:

$$\text{Recommended label rate (ppm)} = \frac{\text{Recommended amount of insecticide (mL OR g) x Insecticide Concentration (mL/L OR g/l OR g/kg)}}{\text{Recommended tank water volume (L)}}$$

3.4 Results

The results of the probit analyses from the leaf detached method (from Table 2.2) and plant immersion bioassays to diafenthiuron, flupyradifurone and imidacloprid are presented in Table 3.1. The responses of the whiteflies to these insecticides were homogeneous and fitted the log-dose/ Probit model. The susceptibility levels of *T. vaporariorum* to diafenthiuron, flupyradifurone, and imidacloprid using different bioassay application methods are described below.

3.4.1 Diafenthiuron

The LC_{50} and LC_{80} estimates for whiteflies exposed to diafenthiuron using immersed potted bean plants were 36.88 and 65.09 ppm respectively, compared to those of the detached leaf-dip method of 6.41 and 19.39 ppm (Table 3.1). The LC_{50} estimate (commonly used to calculate the fold-increase) that was obtained using the plant immersion method was therefore 5.75 times higher than that of the leaf-dip method (Table 3.2). The responses of the population to diafenthiuron therefore differed significantly between the two bioassay methods (Table 3.2). The slope of the probit-response line of the leaf-dip method was steeper compared to the plant immersion method (Table 3.1). The LC_{80} for *T. vaporariorum* exposed to diafenthiuron estimated with both test methods, were below the recommended field rate of 300 ppm (Fig. 3.2).

3.4.2 Flupyradifurone

The LC_{50} and LC_{80} estimates for whiteflies exposed to bean plants immersed into flupyradifurone solutions were 5.31 and 13.57 ppm respectively, compared to the estimates of the detached leaf-dip method, with LC_{50} and LC_{80} values of 3.20 and 8.70 ppm, respectively (Table 3.1). The LC_{50} estimate obtained using the plant immersion method was 1.66 times higher than the estimates obtained with the detached leaf-dip method (Table 3.2). The responses of the population to flupyradifurone, obtained using two bioassay methods, were significantly different (Table 3.2). The LC_{80} for *T. vaporariorum* exposed to flupyradifurone estimated with both test methods were below the recommended field rate of 150 ppm (Fig. 3.3).

3.4.3 Imidacloprid

The LC_{50} and LC_{80} estimates for whiteflies exposed to plants immersed into imidacloprid solutions were 32.96 and 87.64 ppm respectively, compared to the estimates of the detached leaf-dip method, with LC_{50} and LC_{80} values of 4.42 and 21.83 ppm, respectively (Table 3.1).The LC_{50} estimate obtained using the plant immersion method was 7.46 times higher than the estimate obtained with the detached leaf-dip method (Table 3.2). These responses of the population therefore differed significantly between the two bioassay methods (Table 3.2). The slope of the probit-response line of the leaf-dip method was steeper than that of the plant immersion method (Table 3.1). The LC_{80} for *T. vaporariorum* exposed to imidacloprid estimated with both test methods were below the recommended field rate of 175 ppm (Fig. 3.4).

Table 3.1: Log-dose probit mortality data for *Trialeurodes vaporariorum* adults exposed to insecticides using the leaf-dip and plant immersion methods

Population	**n**	**LC_{50}**	**CL (95%)**	**LC_{80}**	**CL (95%)**	**Slope**	**SE**	**X^2**	***df***
Diafenthiuron									
Leaf-Dip	480	6.41	4.93-7.88	19.39	16.13-24.03	1.75	0.17	2.04	2
Plant-immersion	720	36.88	31.47-42.03	65.09	57.26-75.74	3.41	0.30	5.56	4
Flupyradifurone									
Leaf-Dip	720	3.20	2.40-4.02	8.70	7.15-10.63	1.94	0.17	3.99	4
Plant-immersion	600	5.31	3.23-7.24	13.57	10.24-19.27	2.06	0.20	9.86	3
Imidacloprid									
Leaf-Dip	840	4.42	0.70-10.39	21.83	8.76-35.47	1.21	0.18	8.26	5
Plant-immersion	1200	32.96	26.31-39.31	87.64	74.73-104.16	1.98	0.15	9.08	8

df = Degrees of freedom (n-2)

Table 3.2: Differences in the estimation of LC_{50} values of three insecticides using two different bioassay methods.

Insecticide	Ratio	CL 95%
Diafenthiuron	5.75	4.44-7.45*
Flupyradifurone	1.66	1.20-2.30*
Imidacloprid	7.45	2.97-18.71*

*Responses of the population tested with two bioassay methods were significantly different since the 95% confidence limits of the relative ratio did not include the value 1.

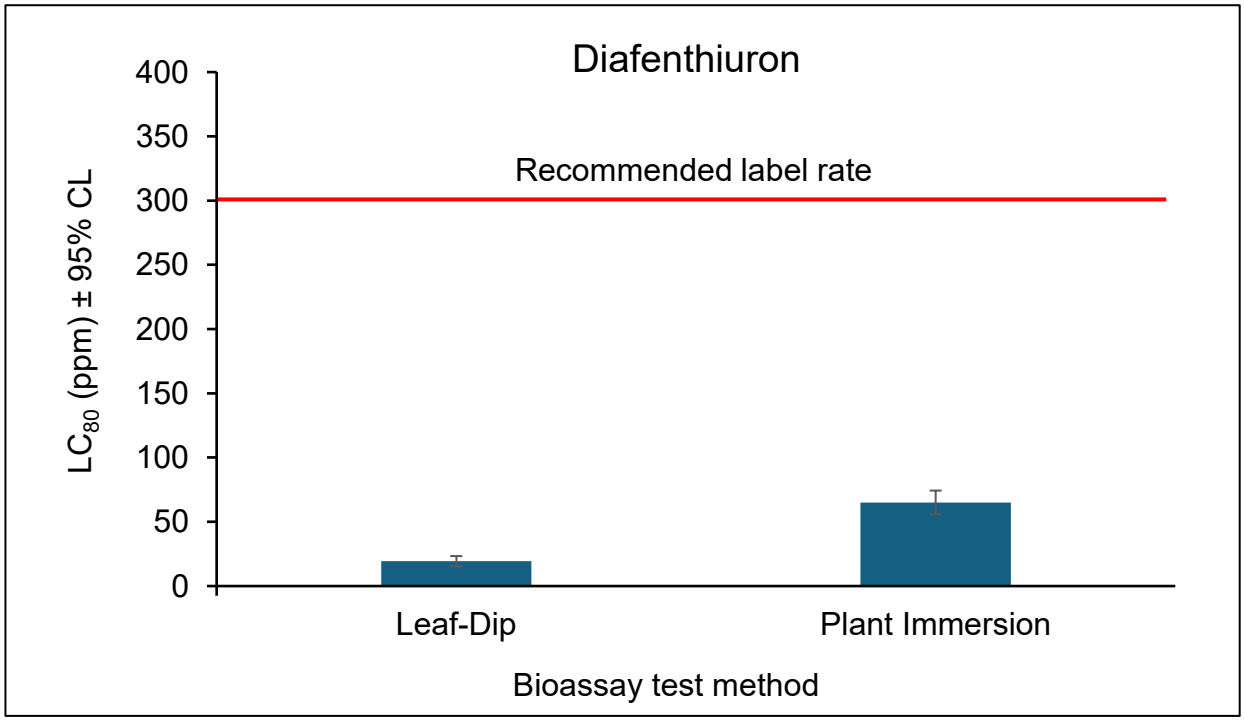


Figure 3.2: Lethal concentration 80 (LC_{80}) of *Trialeurodes vaporariorum* for diafenthiuron estimated by means of leaf dip and plant immersion bioassays in relation to the recommended label rate. Bars indicate 95% Confidence limits.

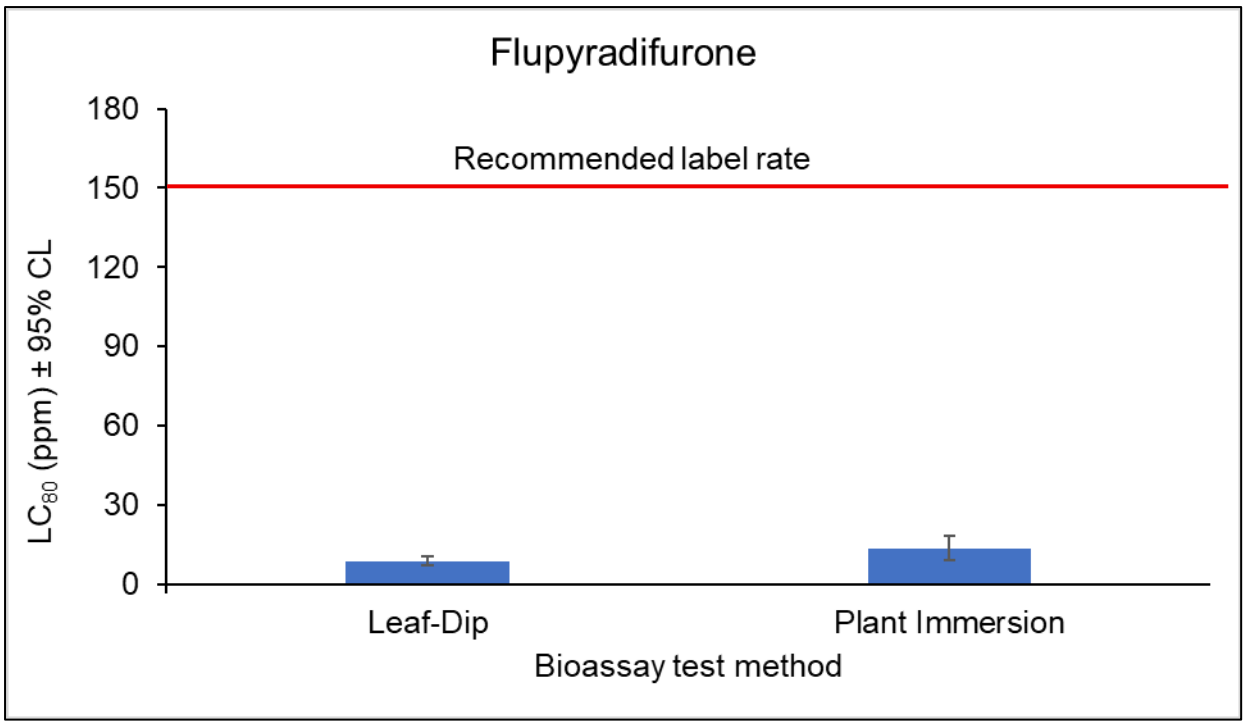


Figure 3.3: Lethal concentration 80 (LC_{80}) of *Trialeurodes vaporariorum* for flupyradifurone estimated by means of the leaf dip and plant immersion bioassays in relation to the recommended label rate. Bars indicate 95% Confidence limits.

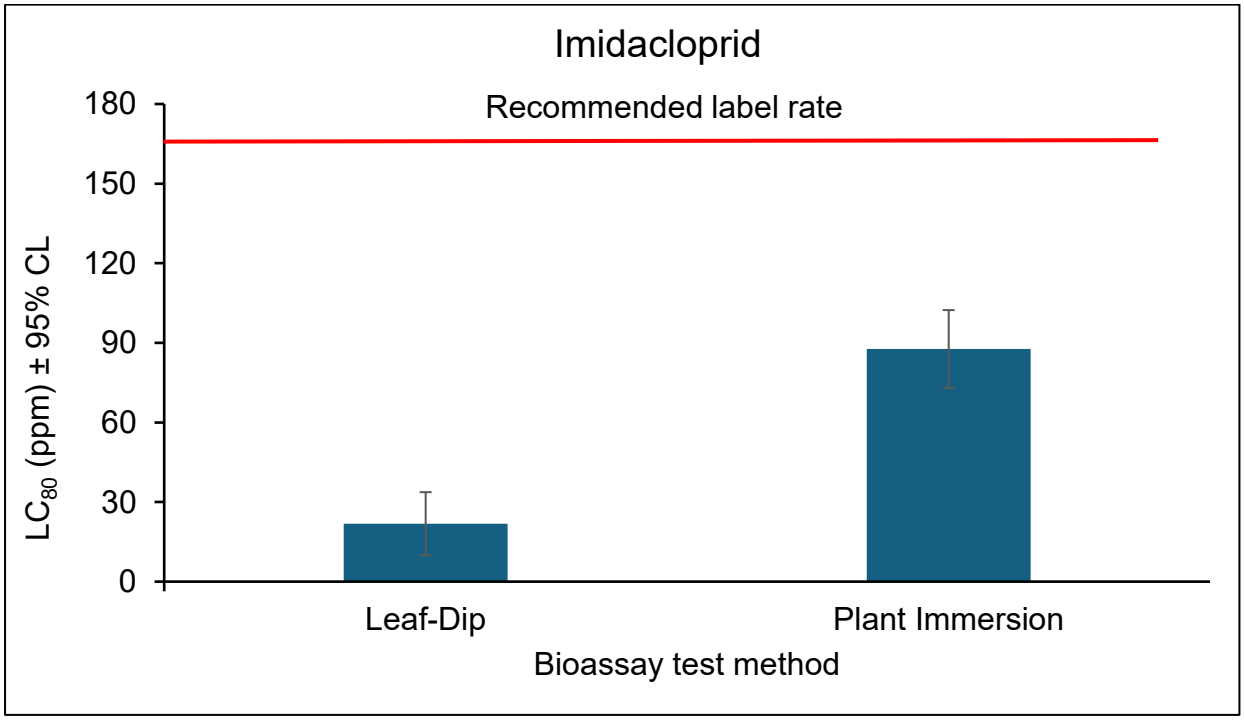


Figure 3.4: Lethal concentration 80 (LC_{80}) of *Trialeurodes vaporariorum* for imidacloprid estimated by means of leaf dip and plant immersion bioassays in relation to the recommended label rate. Bars indicate 95% Confidence limits.

3.5 Discussion

The LC_{50} and LC_{80} values of diafenthiuron estimated for *T. vaporariorum* adults were higher when potted plants were immersed into the insecticide solutions compared with detached leaves being dipped into these solutions. Diafenthiuron is a pro-insecticide that is converted by light or *in vivo*, by cytochrome P450 in insects, to the corresponding carbodiimide, which is an inhibitor of mitochondrial respiration (Ruder & Kayser, 1994; Kayser & Eilinger, 2001; Tomlin, 2009). In addition to its contact and stomach action, it also exhibits a translaminar activity (Brits & Vickers, 1990). Although it was expected that the toxicity in intact leaves would be higher, and therefore that whiteflies would be more sensitive to the insecticide, the opposite was observed. Although the insecticide's effectiveness was limited to contact activity on detached leaves, the lethal concentration values estimated by the detached leaf dip method were lower than those obtained from potted plants immersed in diafenthiuron solutions. The LC_{80} values estimated for *T. vaporariorum* exposed to diafenthiuron were below the recommended field rate of 300 ppm in both bioassays, suggesting that control failure in the tested population is unlikely.

Similar results were obtained for the two systemic insecticides, flupyradifurone and imidacloprid. The estimated LC_{50} and LC_{80} values for *T. vaporariorum* adults were also higher after feeding on potted plants that were immersed into the insecticide solutions compared with detached leaves that were dipped into these solutions. A possible explanation may be that insecticides are readily absorbed and metabolised by plants (Jan *et al.*, 2020; Zhang *et al.*, 2024). Plants use various detoxification mechanisms, primarily enzymatic processes such as hydrolysis, oxidation, and conjugation, to break down these compounds (Schäffner *et al.*, 2002; James & Strand, 2009; Kawahigashi, 2009; Zhang *et al.*, 2017; Su *et al.*, 2019; Lu *et al.*, 2020; Zhang & Yang, 2021). Through metabolism, the chemical structure of the insecticide is altered, often reducing its toxicity and hydrophobicity, and producing more water-soluble metabolites at lower concentrations. This metabolic transformation may have contributed to the reduced toxicity observed in potted plants compared to leaves that were dipped into these insecticide solutions. However, detailed studies measuring insecticide concentration levels within treated plants are needed to confirm this explanation.

Although a significant difference between the lethal concentrations of flupyradifurone was recorded for the leaf dip and plant immersion methods, both methods indicated high susceptibility of adult whiteflies to flupyradifurone. Similar to imidacloprid, flupyradifurone acts as an agonist of nicotinic acetylcholine receptors (nAChRs), disrupting neural transmission and ultimately leading to paralysis and death (Nauen *et al.*, 2015). However, unlike other insecticides with the same mode of action (e.g. imidacloprid), flupyradifurone is chemically distinct (Nauen *et al.*, 2015). Flupyradifurone is the first nAChRs insecticide to feature a stemofoline-derived butenolide pharmacophore, which enables it to bind strongly to insect nAChRs. This structural difference may help flupyradifurone evade certain metabolic resistance mechanisms that commonly affect neonicotinoids such as imidacloprid (Nauen *et al.*, 2015). Additionally, flupyradifurone has been shown to act more rapidly than other neonicotinoids (Nauen *et al.*, 2015). The generally low lethal dosages recorded for both flupyradifurone and imidacloprid in this study align with the findings of Cremonez *et al.* (2023). These low LC_{80} values estimated with both application methods were well below the recommended label rates of 150 and 175 ppm, for flupyradifurone and imidacloprid, respectively. No control failure of *T. vaporariorum* adults is therefore currently expected with either of these two systemic insecticides.

A study by Perier *et al.* (2023) reported that imidacloprid absorption by cotton plants does not reach a saturation point, resulting in continuous accumulation of the insecticide within the plant. However, Ahmed *et al.* (2024) found that imidacloprid uptake by lettuce plants follows a saturable pattern, reaching an equilibrium point beyond which further absorption is minimal. The time required to reach the equilibrium may, however, vary depending on plant species and the method of application (Ahmed *et al.*, 2024). For example, the concentration of imidacloprid in lettuce leaves peaks five days after treatment (Ahmed *et al.*, 2024). In the present study, mortality was assessed after only three days of exposure to imidacloprid, which may have contributed to the lower observed mortality rates.

The results of this study are in contrast with the findings of Sparks *et al.* (2020), who compared the detached leaf bioassay with a root/petiole drenching assay and reported that the former provided the poorest dose response data for adult whiteflies. The differences in adult mortality responses reported in their study between the methods

were, however, minimal, leading to the conclusion that various bioassays for assessing whitefly insecticide response are comparable. In contrast, Sain *et al.* (2019) found that using intact leaves was more advantageous than detached leaf bioassays in petri dishes for identifying the most virulent entomopathogenic fungal strains for controlling *B. tabaci*.

Bioassays play a vital role in the development and implementation of IRM strategies (Sparks *et al.*, 2020). However, differences in adult mortality responses have been reported across bioassay methods. To ensure consistency and comparability of results within a monitoring program, it is therefore essential to use a standardised method.

3.6 Conclusions

Bioassays are essential tools for detecting changes in a pest's susceptibility to insecticides. However, the limited number of studies comparing different bioassay methods for testing adult whiteflies has produced inconsistent results. In this study, comparison of the leaf dip method, using detached leaves in petri dishes, resulted in significantly higher mortality rates than the method involving confinement of adults in clip-on cages after whole plant immersion. Given these significant differences in lethal dose estimates between bioassay methods and the variation in responses across bioassay methods, it is important that monitoring programs adopt a standardised method to ensure consistency and comparability of results over time.

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Chapter 4: Conclusions and recommendations

Food insecurity remains a critical global issue. In 2023, an estimated 2.33 billion people experienced difficulties in accessing sufficient food on a regular basis, while 864 million faced severe food insecurity with the large majority of these people located in Africa (FAO, 2024). Climate change plays a significant role in aggravating food insecurity, as rising global temperatures alter rainfall patterns and increase the frequency of extreme weather events, ultimately impacting crop yield and quality (Nelson *et al.*, 2009; Zhang *et al.*, 2017; Ribeiro *et al.*, 2021; Pius Awhari *et al.*, 2024). Sub-Saharan African producers are particularly vulnerable to these impacts (Masipa, 2017).

Climate change also influences the distribution, abundance, and resistance dynamics of agricultural pests (Bale *et al.*, 2002; Ma *et al.*, 2021; Srinivasa Rao *et al.*, 2022; Subedi *et al.*, 2023). In response to growing environmental concerns, the European Union has proposed reducing the use of conventional chemical insecticides by 50% and transitioning toward more sustainable agricultural practices such as integrated pest management (IPM) by 2030 (EU, 2020). Despite these initiatives, many regions continue to depend on chemical insecticides for pest control. A prominent pest is the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae), a highly polyphagous, phloem feeding insect that feeds on more than 859 plant species, including economically important greenhouse crops (CABI, 2021; Dearlove *et al.*, 2024). Damage results from both direct feeding and the transmission of plant viruses (Duffus *et al.*, 1996; Navas-Castillo *et al.*, 2014; Walia *et al.*, 2015; Perring *et al.*, 2018; Leskey *et al.*, 2021; Albornoz *et al.*, 2023). Although various strategies are employed to manage whitefly populations, chemical control remains the dominant control method (Perring *et al.*, 2018; Horowitz *et al.*, 2020; Abubakar *et al.*, 2022; Dai *et al.*, 2024). However, the continued and excessive use of insecticides increases selection pressure, promoting resistance evolution and reducing the efficacy of available active ingredients. This underscores the necessity of implementing effective insecticide resistance management (IRM) strategies.

Detecting and managing resistance is crucial for ensuring the long-term effectiveness and sustainability of pest control strategies (Kranthi, 2005). Bioassays are widely used

to assess insecticide resistance levels, providing a standardised, quantitative approach to evaluate the relationship between insecticide potency and its effectiveness against target insect species (Bliss, 1957; Hoskins & Craig, 1962; Zhu, 2008). Because it is not always possible to determine the exact dose ingested or absorbed by individual insects, the LC_{50} (lethal concentration required to kill 50% of a test population) is commonly used to denote the concentration of the insecticide required to cause 50% mortality of the test population, and therefore as an indicator of susceptibility (Paramasivam & Selvi, 2017). Shifts in LC_{50} values over time or between populations signal changes in susceptibility and can be used to detect and monitor the evolution of resistance (Paramasivam & Selvi, 2017). In the absence of a baseline LC_{50} value for comparison, the LC_{80} value (lethal concentration causing 80% mortality) can be used to assess the likelihood of control failure. By comparing the estimated LC_{80} value to the insecticide's recommended application rate, one can infer potential efficacy issues. If the LC_{80} exceeds the recommended label rate, a failure in field-level control is likely (Silva *et al.*, 2011). Currently, no data are available on the susceptibility of *T. vaporariorum* to insecticides in South Africa. The aim of this study was, therefore, to estimate the susceptibility of *T. vaporariorum* nymphs and adults from two different populations to six insecticides in South Africa.

In Chapter 2, susceptibility tests were conducted on adults and 2nd-instar *T. vaporariorum* nymphs using leaf-dip and plant immersion bioassays. Results showed that nymphs were highly susceptible to cyantraniliprole, whereas adults from both the WSC-1-24 and BLF-1-25 populations exhibited low susceptibility. Consequently, control failure with cyantraniliprole is anticipated in adult populations, despite good efficacy against nymphs. In contrast, diafenthiuron and flupyradifurone provided effective control across both populations, although reduced susceptibility was noted in BLF-1-25 nymphs to flupyradifurone, suggesting possible control failure. The efficacy of diafenthiuron can be enhanced when applied under sunny conditions, as UV exposure facilitates conversion of the parent molecule into a more active carbodiimide form, which possesses both insecticidal and acaricidal properties.

The low susceptibility to flupyradifurone in the BLF-1-25 population highlights the need for proactive resistance management strategies. While imidacloprid provided effective control of adults in both populations, it was less effective against nymphs. Caution is

warranted, as resistance and high tolerance to imidacloprid have been documented globally (Silva *et al.*, 2009; Schuster *et al.*, 2010; Kontsedalov *et al.*, 2012; Wang *et al.*, 2020; Zhang *et al.*, 2024; Du *et al.*, 2025).

Responses to pymetrozine were inconsistent across both life stages and populations, with overall susceptibility remaining extremely low. This is of particular concern, as pymetrozine not only targets pest insects but also interferes with virus transmission by infected whiteflies. Previous studies have shown that tomato plants treated with pymetrozine exhibited significantly lower incidence of tomato yellow leaf curl virus (Polston & Sherwood, 2003). *Trialeurodes vaporariorum* is also a known vector of tomato torrado virus (ToTV) (Pospieszny *et al.*, 2007), with recent ToTV outbreaks reported in Limpopo province, South Africa (Moodley *et al.*, 2020). Although these outbreaks were linked to weedy hosts, Budziszewska *et al.* (2025) confirmed that *T. vaporariorum* can acquire and transmit ToTV. Viral variants were detected in the stylets of whiteflies, indicating a potential transmission pathway. Further research is warranted to evaluate the impact of pymetrozine on ToTV transmission in *T. vaporariorum* populations feeding on treated tomato plants.

Although spirotetramat was effective against nymphs at the recommended application rate in both tested populations, the reduced susceptibility observed in BLF-1-25 warrants further investigation. Differences in susceptibility between life stages were also evident. Dai *et al.* (2024) reported that *T. vaporariorum* nymphs and eggs were less susceptible to flupyradifurone and imidacloprid compared to adults, while adults exhibited resistance to cyantraniliprole.

Whitefly responses also varied depending on the bioassay method used (Chapter 3). LC_{50} values obtained from the plant-immersion method were 1.6-4.0 times higher than those from the leaf-dip method, demonstrating the influence of bioassay type. Additionally, maintaining a consistent host plant for whitefly rearing is essential, as host-specific secondary metabolites can alter gene expression and subsequently affect insecticide sensitivity. According to Pym *et al.* (2019), *T. vaporariorum* susceptibility can vary by more than 40-fold depending on the host plant.

4.1 Future recommendations

This study provides data on the susceptibility of *T. vaporariorum* to cyantraniliprole, diafenthiuron, flupyradifurone, imidacloprid, pymetrozine, and spirotetramat in South Africa. Future studies can use these findings to monitor shifts in susceptibility over time. Investigating how different host plants affect whitefly susceptibility may also yield valuable insights for resistance management. Such data will support researchers, agrochemical companies, and producers in optimising insecticide application, delaying resistance evolution and integrating chemical and non-chemical approaches within broader integrated pest management (IPM) strategies.

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NORTH-WEST UNIVERSITY
NOORDWES-UNIVERSITEIT
YUNIBESITI YA BOKONE-BOPHIRIMA

Private Bag X1290, Potchefstroom
South Africa 2520

Tel: 018 299-1111/2222
Fax: 018 299-4910
Web: http://www.nwu.ac.za

Senate Committee for Research Ethics
Tel: 016 910 3446
Email: Feziwe.Mseleni@nwu.ac.za

ETHICS APPROVAL LETTER OF STUDY

Based on the review by the **Faculty of Natural and Agricultural Sciences Ethics Committee (FNASREC)**, the Committee hereby clears your study as no ethical risk. This implies that the FNASREC grants permission that, provided the general conditions specified below are met, the study may be initiated, using the ethics number below.

Study title: Susceptibility of Trialeurodes vaporariorum (Hemiptera: Aleyrodidae) to insecticides in South Africa
Study Leader/Supervisor: Prof MJ du Plessis
Student: BC Smit

Ethics number:	N	W	U	-	0	1	2	8	7	-	2	2	-	A	9
	Institution				Study Number						Year			Status	

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Application type: Single **Risk Category:** **No Risk**

Commencement date: 01/02/2022
Expiry date: 13/09/2025

General conditions:
The following general terms and conditions apply:

- *The commencement date indicates the date when the study may be started.*
- *In the interest of ethical responsibility, the NWU-SCRE and FNASREC reserves the right to:*
  - *request access to any information or data at any time during the course or after completion of the study;*
  - *to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process;*
  - *withdraw or postpone approval if:*
    - *any unethical principles or practices of the study are revealed or suspected;*
    - *it becomes apparent that any relevant information was withheld from the FNASREC or that information has been false or misrepresented;*
    - *submission of the annual (or otherwise stipulated) monitoring report, the required amendments, or reporting of adverse events or incidents was not done in a timely manner and accurately; and / or*
    - *new institutional rules, national legislation or international conventions deem it necessary.*
- *FNASREC can be contacted for further information or any report templates via Roelof.Burger@nwu.ac.za 018 299 4269*

The FNASREC would like to remain at your service as scientist and researcher, and wishes you well with your study. Please do not hesitate to contact the FNASREC or the NWU-SCRE for any further enquiries or requests for assistance.

Yours sincerely,



Prof Roelof Burger
Chairperson Faculty of Natural and Agricultural Sciences Ethics Committee (FNASREC)