Effect of plant growth stimulants on the response of *Phthorimaea absoluta* (Lepidoptera: Gelechiidae) larvae and moths to tomato plants

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Dissertation accepted in fulfilment of the requirements for the degree *Master of Science in Environmental Sciences with Integrated Pest Management* at the North-West University

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Graduation: July
2025

Acknowledgements

From the opportunity to do a master's degree, to the physical, emotional and spiritual support throughout the two years and the supernatural provision in the final three months, our Lord and Savior deserves all the glory. Thank You, Jesus!

He blessed me with two astounding supervisors, Professor Johnnie van den Berg and Professor Hannalene du Plessis which guided me through the minutiae of completing a master's degree effectively. Thank you prof. Johnnie for having allot of grace with me from the early days, teaching me the steps to success in life and stretching me into new capacity. Prof. Tekkies, your kind heart has reached life's even outside the eco-rehab circle. Thank you for your patience and servant heart which is a daily encouragement to me. God bless these two people.

I would like to thank dr. Johan van der Waals for the guidance and mentorship throughout the project, for all your effort put in and the support from RealIPM. God intentionally used you to show me what Faith can achieve. Thanks to RealIPM for the financial support.

Thanks to the Eco-rehab team which helped in all the practical facets of this project. Especially Mischa and Zunel, two God fearing women which became true friends.

Thanks to all the friendships which sharpened me to cut through the gruelling times contained within this two-year period, you know who you are. A special thanks to Stephan and Ruben for your leadership, for my area leaders and this amazing church governance.

To Ouma Lente for encouraging me to build upon the rock and not the sand and to my brother for his willingness to help me rest effectively when not busy with studies.

Finally, to my two wonderful parents for being only a phone call away, for being a safe refuge in the loneliest of times and allowing me to chase after my passions.

May the peace of God, which surpasses all understanding, guard your hearts and minds in Christ Jesus.

Abstract

Phthorimaea absoluta (Lepidoptera: Gelechiidae), the tomato leaf miner, is the most serious pest of tomato in the world and a major threat to cultivation of this crop. Moths of this pest lay their eggs mostly on plants leaves and stems, and the endophytic larvae feed on the parenchyma of leaves. Insecticide applications are the most common strategy used for control of *P. absoluta* larvae. However, pesticides have detrimental effects on biological control agents and disrupt the use of IPM programs. *Phthorimaea absoluta* populations have also evolved resistance to chemical insecticides wherever tomatoes are cultivated. Other management strategies for *P. absoluta* include mating disruption, changes in agronomic practices and the general management of plant health. Plant growth stimulants are commonly used in tomato production to benefit plant health and are reported to result in increased availability of nutrients and increases in various plant growth parameters. One of the benefits of plant growth stimulants is increased plant °Brix, which is considered a measure of plant health and the indicator of total soluble solids in plants. Various plant growth- and biostimulants are used in tomato production in South Africa. These products include Seabrix (RealIPM, Thika, Kenya) and RealTrichoderma (RealIPM) formulated from *Trichoderma asperellum* and registered for the control of pathogenic fungi. Another such product is NewSil (RealIPM), a product that is applied as foliar fertilizer treatment. The adverse effects of these products on pests are often ascribed to increased plant °Brix. The potential of using watering regimes and plant growth stimulants to manipulate °Brix of crops as a pest management strategy have been investigated in various crop species such as potato and other vegetables, raspberry and grapes. The aim of this study was to determine whether plant °Brix could be manipulated by application of plant growth stimulants, and different soil moisture conditions, and if such treatments result in differences in °Brix which may influence the oviposition and larval preferences of *P. absoluta*. Experiments were conducted with potted tomato plants maintained in greenhouses and *P. absoluta* moths and larvae, sourced from an insect rearing colony I maintained throughout the course of this study. Results of the three experiments during which °Brix readings were taken showed that neither soil water conditions nor growth stimulant treatments had pronounced effects on leaf °Brix. °Brix varied significantly over weeks and growth stimulants had only minor effects on °Brix. The differences in °Brix resulting from growth stimulant treatments in this study was, however, masked by the high variation recorded in °Brix at different sampling times. No significant correlation existed between the numbers of eggs laid on plants in no-choice ($r = -0.41$; $p = 0.086$) and two-choice ($r = 0.039$; $p = 0.711$) tests and larvae only showed preference for leaves of plants in two of the 15 combinations that they were provided with. Results from the study illustrate the difficulty to manipulate °Brix by means of growth stimulants, and showed no relationship between leaf °Brix of tomato plants and the preference of *P. absoluta* moths, or larval response to plants that were treated with stimulants for prolonged periods. The leaf °Brix of tomato plants in this study was much higher than those reported for tomato in the literature. A lack of knowledge of the relationship between leaf and fruit °Brix was identified during this study. While moths of this pest lays their eggs on plant leaves and larvae feed predominantly on leaf parenchyma tissue,°Brix of tomato plants that were reported to influence the life history parameters of *P. absoluta* were based on fruit °Brix. The results therefore, provide no support for either a positive or negative relationship between leaf °Brix of tomato plants and the preference of *P. absoluta* larvae or moths for plants with higher or lower °Brix. °Brix levels alone cannot be used to indicate host plant suitability for this pest.

Key words: pest management, plant growth stimulants, plant health, tomato leaf miner.

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Chapter 1

Literature review

1.1 History of tomatoes

Tomatoes originate from the region between Ecuador and Chile, in the Andes Mountain range (Kelley *et al.*, 2010; Nakazato & Housworth, 2011; Peralta *et al.*, 2008). The region where domestication of tomatoes was done is uncertain, but it is most likely done in Mexico (Kelley *et al.*, 2010; Klee & Resende Jr, 2020).

1.2 Economic value of tomatoes

Tomatoes are the most consumed vegetable in the world with 189.13 million tons produced globally in 2021 (Shahbandeh, 2023). In South Africa, the value of tomato production in 2021 was US$ 170.785 million (Figure 1) (FAO, 2023). The value of South African tomato production increased by approximately 300% over the past 60 years, from US$ 49 million in 1962 to US$ 216 million in 2017.

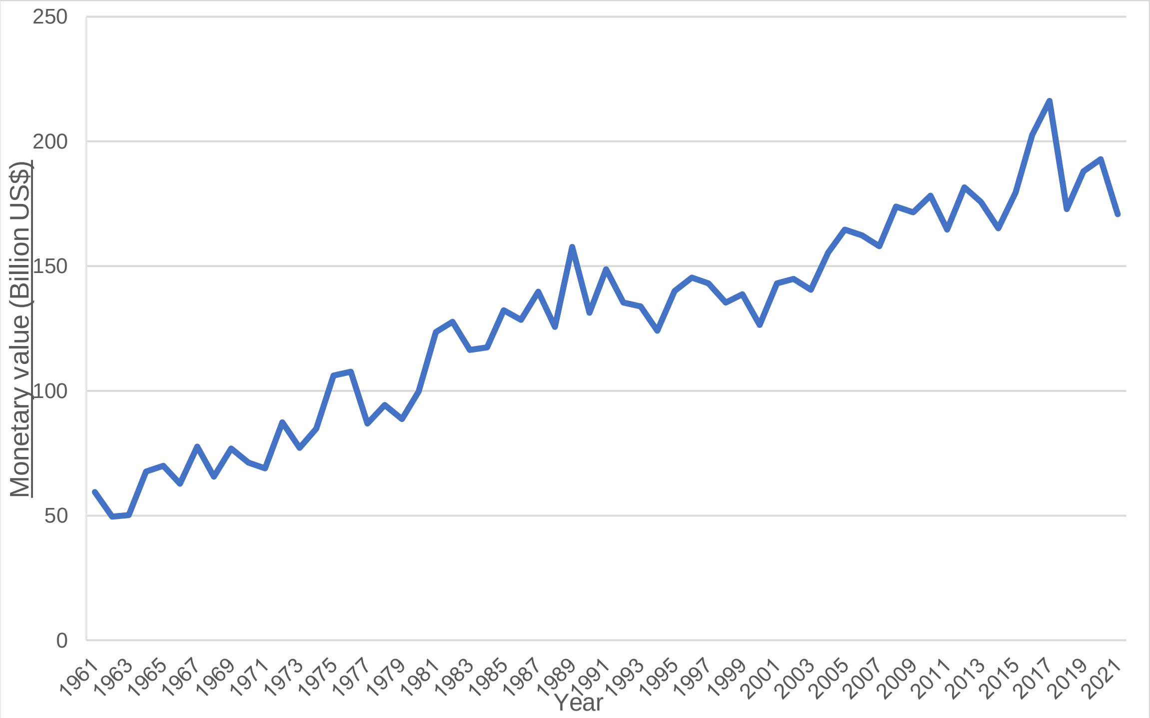

Figure 1: The value of South African tomato production (US$) from 1961 to 2021 (FAO, 2023).

Tomato production in Africa is done mainly under unfavourable conditions, resulting in low yields (Msangi, 2007). Comparison of agricultural efficiency between regions of the world show that West- and East-Africa production systems are the least efficient (Sikora *et al.*, 2017). Compared to the rest of Africa and the world, tomato yields in South Africa are comparatively high. The world average tomato yield between 2015 - 2017 was 37 169 kg/ha, while in South Africa, it was 75 353 kg/ha (Sikora *et al.*, 2017).

While tomatoes are grown across South Africa, the Limpopo province is best suited for tomato cultivation due to its warm climate. The Limpopo province is the largest producer of tomatoes in the country, making up more than 75% of the total production area (3 590 ha) (DALRRD, 2020). Within the province, the northern Lowveld production region comprises 2 700 ha, while the far northern regions of Limpopo encompass 890 ha. Other notable tomato-growing regions include the Border area of the Eastern Cape province, which has 450 ha dedicated to tomato production, and the Onderberg region of Mpumalanga, which has 770 ha (DALRRD, 2020).

1.3 Agronomy of tomatoes

1.3.1 Basic cultivation principles

While wild tomatoes are herbaceous plants that grow upright as perennial plants, cultivated varieties tend to behave like annual plants due to their susceptibility to frost and drought (Peralta & Spooner, 2007). Tomato cultivation methods largely depend on the growing style of the variety (Dam *et al.*, 2005).

Tomatoes have five main growth stages, namely the germination and initial leaf stage, followed by the vegetative-, flowering-, early fruiting- and mature fruiting stage (Shamshiri *et al.*, 2018). The duration of each of the stages is dependent on several environmental factors, for example, temperature and soil conditions (Figure 2). The timespan from planting to the first harvest ranges from 45 to 100 days (Jones, 2013).

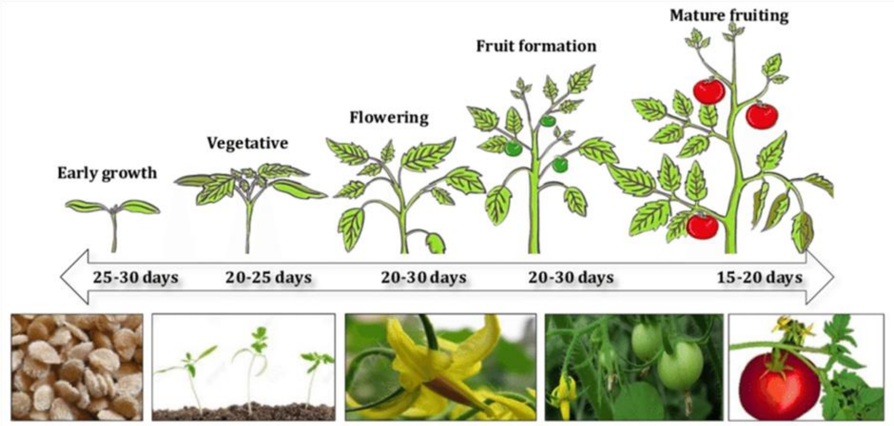

Figure 2: Illustration of tomato growth stages and approximate duration of each stage (Shamshiri *et al.*, 2018).

1.3.2 Climatic factors

Tomatoes are adapted to various climates (Dam *et al.*, 2005; Gould, 2013). The optimal temperatures for the different developmental stages of tomato plants are provided in Table 1.1.

Table 1.1: The minimum, optimum and maximum temperatures for tomato plant development (Dam *et al.*, 2005).

	Temperatures (°C)		
Stage	**Minimum**	**Optimal**	**Maximum**
Seed germination	11	16-29	34
Seedling growth	18	21-24	32
Fruit set	18	20-24	30
Fruit colour development	10	20-24	30

Since day length does not influence the growth or fruit set of tomatoes but rather the temperatures accompanying the change in day length, tomatoes can be described as day-neutral plants (Gould, 2013). Demers and Gosselin (2000) stated that the optimal photoperiod for the best quality yield and growth is 14 hours per day and that too much light may decrease the yield and overall quality of growth.

1.3.3 Soil quality

Tomatoes are cultivated in various types of soil. Well aerated soils with a moderate water holding capacity are preferred (Brady *et al.*, 2016; Dam *et al.*, 2005; Gould, 2013). Soils with a high-water holding capacity, such as peat soils or certain heavy clayey soils, are not ideal for tomato cultivation. The most ideal soil for tomatoes is deep sandy loam soil (Dam *et al.*, 2005; Gould, 2013). According to Gould (2013) the ideal soil pH for tomato cultivation is 6.0 - 6.5.

1.3.4 Soil fertility

Fertilization of the soil can be done using inorganic fertilizer or organic manures. Organic manure encapsulates horse manure, cattle manure, pig manure, chicken manure and sheep or goat manure combined to form a compost (Dam *et al.*, 2005; Gould, 2013). The benefits of using composts or manure, which is produced on-farm, are that it increases soil fertility, improves soil structure and increases soil organic carbon (Dam *et al.*, 2005).

Inorganic (chemical) fertilizer improves the soil nutrient status but does not improve soil structure (Dam *et al.*, 2005). Lime application is widely used to increase soil pH if necessary (Brady *et al.*, 2016). Although compost or manure is good practice, it will not always address all nutrient deficiencies (Dam *et al.*, 2005). Inorganic fertilizers can improve certain nutrient deficiencies (Dam *et al.*, 2005). The application of chemical fertilizer is required when nitrogen leaches out of the soil due to an overabundance of rain (Brady *et al.*, 2016).

1.4 Pests of tomato

Several pest species impact tomato production. These include tomato leaf miner, *Phthorimaea absoluta* (Meyrick) (Lepidoptera: Gelechiidae), white flies, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae), root-knot nematodes, *Meloidogyne* spp. (Tylenchida: Heteroderidae), African bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), and red spider mite *Tetranychus urticae* (Koch) (Trombidiformes: Tetranychidae) (Brévault *et al.*, 2014; Sadashiva *et al.*, 2017).

Phthorimaea absoluta is a major threat to the cultivation of tomatoes (Biondi *et al.*, 2018; Soares & Campos, 2020) and is considered to be the most serious pest of tomatoes in the world (Brévault *et al.*, 2014).

1.4.1 Phthorimaea absoluta

Phthorimaea absoluta, was previously known as *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). This species has undergone several genera changes from 1967 until the last change in 2021 by Chang and Metz (2021), which reinstated the original name from Meyrick (1925).

After the first collection of *P. absoluta* specimens in 1917, this species was present throughout South America (Biondi *et al.*, 2018). It was found for the first time outside of its area of origin in 2006 in Spain, and later on South Africa in 2016 (Soares & Campos, 2020; Visser *et al.*, 2017). The rapid spread of *P. absoluta* was not necessarily due to the migration of moths but also by human-assisted spread, most likely in the form of eggs or larvae present in plant material or fruit (Biondi *et al.*, 2018).

Phthorimaea absoluta causes damage to tomato plants by feeding on the fruit and leaves, reducing the photosynthetic potential (Biondi *et al.*, 2018). The destruction by larvae may cause 100% damage to tomato plants and lead to large economic losses (Biondi *et al.*, 2018). The damage caused by *P. absoluta* to tomatoes results in increased costs of control measures and restrictions on tomato exports (Biondi *et al.*, 2018; Han *et al.*, 2019c).

1.4.2 Biology and damage symptoms of *Phthorimaea absoluta*

1.4.2.1 Eggs of *Phthorimaea absoluta*

Eggs are mainly deposited at night and on the above-ground parts of the plant (Sannino & Espinosa, 2010). Eggs are deposited in batches of 2–5 eggs on the apical branches of plants, leaves (Figure 3) and green, unripe fruits (Desneux *et al.*, 2010). Leaf contact and plant volatiles are key factors which induce oviposition by female moths (Proffit *et al.*, 2011). The number of eggs deposited depends on temperature and varies between 120-170 eggs per female (Sannino & Espinosa, 2010). The incubation period ranges between 4-5 days and 10-11 days at 30 °C and 15 °C, respectively (Sannino & Espinosa, 2010).

Phthorimaea absoluta has been reported to exhibit oviposition preferences for different host plant species (Jiang *et al.*, 2023). For example, the average number of eggs per female on four different host plants ranged from 219.3 on *Lycopersicon esculentum* to 26.08 on *Nicotiana tabacum,* with 163.1 and 99.5 on *Solanum tuberosum* and *Solanum melongena* respectively (Jiang *et al.*, 2023).

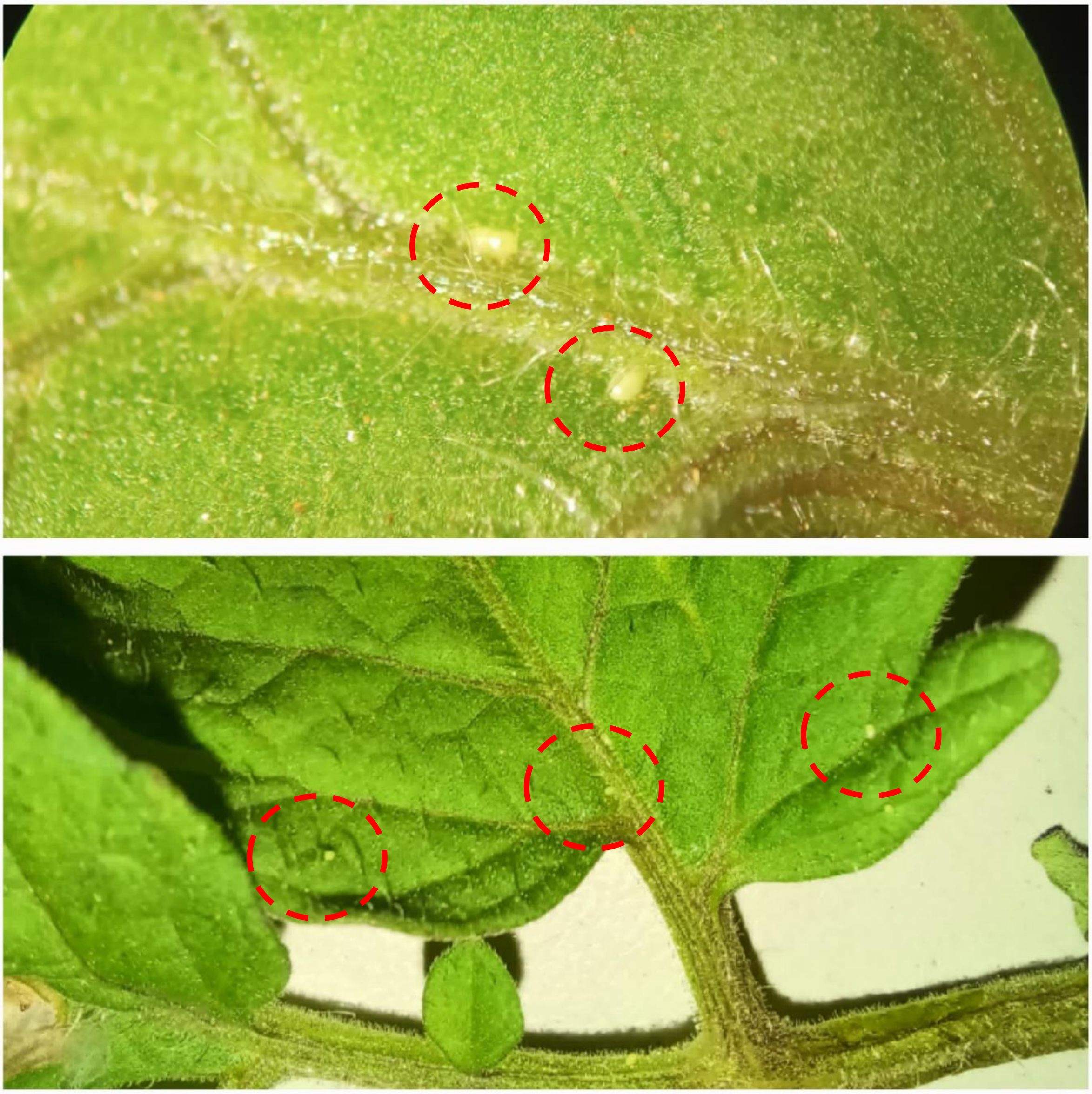

Figure 3: *Phthorimaea absoluta* eggs on tomato leaves. Photo: Regardt Rademan, 2023.

1.4.2.2 Larval stages of *Phthorimaea absoluta*

Phthorimaea absoluta has three larval instars (Table 1.2) (Jiang *et al.*, 2023). The duration of each stage is dependent on environmental factors, notably humidity and temperature, because they are heterothermic insects (El-Shafie, 2020; Sannino & Espinosa, 2010). The larval period ranges from 20 days at 18.5 °C to 13 days at 27 °C (Sannino & Espinosa, 2010). The minimum temperature at which development occurs is 6 °C (Sannino & Espinosa, 2010), and the optimal temperature for development is 30 °C (Biondi *et al.*, 2018).

After hatching, neonate larvae move around on the plant leaf surface for about 40 minutes before mining into the leaf tissue (Sannino & Espinosa, 2010). Larvae are endophytic and feed on the parenchyma of leaves (Figure 4), creating blister-like tunnels in which the granular excrement can be observed when held against light (Sannino & Espinosa, 2010). The suitability of the host plant for larval development is indicated by the size and length of the mines created by the larva (Sannino & Espinosa, 2010). The larger the mines created within a specific time, the more suitable the hostplant. The mines found inside stems and petioles lead to desiccation of the vegetative tissue above the mined tissue (Sannino & Espinosa, 2010).

Figure 4: *Phthorimaea absoluta* larvae feeding on tomato leaves create blister-like damage symptoms on leaves. Photo: Johan van der Waals & Regardt Rademan, 2023.

Once the larvae mature, the majority leaves the plant by spinning off to the ground, forming sticky cocoons that allow debris to stick to it (Sannino & Espinosa, 2010). Some larvae may pupate on plants (Figure 5). The pupal stage can last between eight and 18 days, while hibernation can extend the pupal period up to 65 days (Sannino & Espinosa, 2010). *Phthorimaea absoluta* does not go into diapause (Sannino & Espinosa, 2010). In South America up to 12 generations have been reported annually and these usually overlap.

Figure 5: *Phthorimaea absoluta* pupa inside a silk cocoon on a tomato plant. Photo: Regardt Rademan, 2023.

1.4.2.3 Moths of *Phthorimaea absoluta*

The longevity of moths ranges between nine and 11 days if larvae develop on tomato leaf tissue and if a 10% honey solution is provided as food (Prasannakumar *et al.*, 2023). Females generally live longer than males, and moths that have mated live longer than unmated ones (Sannino & Espinosa, 2010). This is due to the preference of female moths towards males based on weight, age and sexual status (Gonçalves *et al.*, 2024). Therefore, female moths prefer mating with younger and heavier virgin male moths, most likely due to the genetic benefits of increased size (Gonçalves *et al.*, 2024; Parker & Birkhead, 2013). The thermal requirements of *P. absoluta* fit the range for optimal tomato cultivation (14 - 35 °C), which also ensures optimal development and survival of the pest on tomato plants (Krechemer & Foerster, 2015).

Mating usually takes place one night after moth emergence with egg-laying usually commencing one day later (Sannino & Espinosa, 2010). Females can only mate once daily and up to six times during their lifetime (Figure 6). Mating may last up to five hours and most of the eggs are laid over a seven-day period after mating (Desneux *et al.*, 2010; Sannino & Espinosa, 2010). Oviposition continues for about a week, peaking between the third and fifth day (Sannino & Espinosa, 2010).

Figure 6: A male and female *Phthorimaea absoluta* moths mating.

The overall lifecycle of *P. absoluta* may range from 26 days (33 °C) to 75 days (17 °C) (Martins *et al.*, 2016).

Table 1.2: Duration of different life stages of *Phthorimaea absoluta* feeding on *Lycopersicon esculentum* and a honey solution (10%) at 26 ± 2 °C and a relative humidity of 65 ± 5 (%) with a light-dark period of 16:8 h (Prasannakumar *et al.*, 2023).

Stage	Mean duration (days ± S.E.)	Overall duration (days)
Egg	4 ± 0	-
First instar	1.8 ± 0.2	1-2
Second instar	2.6 ± 0.4	1-3
Third instar	2.6 ± 0.2	1-3
Fourth instar	2.0 ± 0.3	1-3
Larval period	9.0 ± 1.7	8-11
Pupal period	5.3 ± 1.8	4-7
Adult period	10.0 ± 0.6	9-11
Total developmental period	**27.6 ± 0.5**	**27-28**

1.4.2.4 Management of *Phthorimaea absoluta*

Integrated pest management (IPM) is a decision-support system for the selection and application of pest control strategies, either alone or in harmony with one another as part of a management strategy, based on cost-benefit evaluations that take into consideration the interests of and impacts on producers, society, and the environment (Kogan, 1998).

The goal of implementing IPM practices is to minimize pesticide use, protect the health of humans and the environment, and increase profitability for the farmer (Ehler, 2006). The practical application of IPM implies monitoring pest numbers and abundance of natural enemies, integrating suppressive tactics and managing multiple pests by using economic and action thresholds (Ehler, 2006).

IPM strategies rely on four main pillars, namely, host plant resistance and biological, chemical, and cultural control (Ehler, 2006). Management strategies for *P. absoluta* include monitoring of pest numbers, mass trapping, mating disruption and the altering of agronomic practices (Biondi *et al.*, 2018; Mansour *et al.*, 2018).

1.4.3 Monitoring pest abundance

There are several methods through which *P. absoluta* infestation levels in tomato fields are monitored.

Phthorimaea absoluta numbers in tomato crops are often monitored by trapping male moths or by sampling leaves to determine the number of eggs and larvae that are present on plants. When utilizing female pheromones, delta traps and bucket traps can be used to catch the males (Benvenga *et al.*, 2007). The level of a *P. absoluta* infestation in a particular environment is estimated by counting the number of moths per trap (Mansour *et al.*, 2018). Although the numbers of moths per trap or eggs per plant provide an indication of infestation levels, they do not appear to be good predictors of the level of damage they will cause to a particular tomato crop (Benvenga *et al.*, 2007). A more effective sampling strategy that permits timeous decision-making is to monitor for the presence of mines within the leaves in the middle third of tomato plants (Cocco *et al.*, 2015). Decision-making based on accurate monitoring of leaf mines and timely implementation of management strategies was reported to keep fruit loss below 1% (Cocco *et al.*, 2015). Studies have shown that a significant relationship exists between leaf infestation levels and subsequent damage to the crop. Therefore, action thresholds based on larval numbers in leaves can be used as a guideline of what to expect regarding economic damage to fruits, two weeks ahead of time. Yield losses can be limited to1 - 3% if an action threshold of 0.6 - 1.8 mines per leaf, two weeks prior to spray application is used (Cocco *et al.*, 2015). Therefore, monitoring leaf damage caused by larvae indicates possible damage to fruits two weeks later.

1.4.4 Cultural control

Various cultural control strategies have been implemented against *P. absoluta*. These include the manipulation of agronomical factors such as irrigation regimes, fertilizer, timely planting, host eradication, destroying of crop residues, covering of soil with plastic sheets. Insect proof greenhouses and pheromone-based tools such as mating disruption and mass trapping, are also commonly used (Mansour *et al.*, 2018). The use of sticky traps was reported to reduce *P. absoluta* infestation levels, although it also affects non-target species by trapping them on the trap surface (Biondi *et al.*, 2018). Implementation of sterile insect techniques has been reported to be effective in greenhouses at a 15:1 sex ratio (Cagnotti *et al.*, 2016).

For mass trapping and early detection, a synthetic female sex pheromone is used. Pheromone dispensers inside traps and light sources can attract and mass trap male moths in water traps (Caparros Megido *et al.*, 2013). Similarly, the pheromones are utilized in field efforts to mass trap moths and disrupt mating by saturating the surrounding area with the synthetic female pheromone (Caparros Megido *et al.*, 2013). The number of traps implemented for effective trapping of *P. absoluta* in Tunisia was 32 delta traps or 36 water traps with pheromone lures per hectare and traps upwind from the tomato fields were reported to trap more moths (Lobos *et al.*, 2013; Mansour *et al.*, 2018). Such trapping systems have been reported to lead to reduced infestation levels in comparison to the application of insecticides in Argentina, although success is largely dependent on the severity of the infestation (Caparros Megido *et al.*, 2013; Lobos *et al.*, 2013). However, mass trapping is ineffective as a standalone method and should be integrated with other management practices (Mansour & Biondi, 2021).

Mating disruption refers to the saturation of the environment with the female sex pheromone to reduce the efficiency of a male to locate a female moth (Desneux *et al.*, 2022). Mating disruption was shown to be successful in greenhouses where 30 g of active ingredient/ha was implemented with 500 traps/ha. The active ingredient contained a 90:10 rate blend of (3E,8Z,11Z)-tetradecatrien-1-yl acetate and (3E,8Z)-tetradecadien-1-yl acetate, respectively (Cocco *et al.*, 2013; Vacas *et al.*, 2011). The implementation of 500 – 1000 synthetic pheromone dispensers ensures significantly reduced crop damage (Cocco *et al.*, 2013). The downfall of mating disruption is, however, the high cost of producing pheromone lures (Desneux *et al.*, 2022).

Removal of host plants of *P. absoluta* is also employed as a cultural control method (Cherif & Verheggen, 2019). Implementable practices include crop rotation with plants from other families and removing old infested Solanaceae species crop residues from fields (Cherif &

Verheggen, 2019). Weedy host plants of *P. absoluta* in South Africa include *Datura stramonium* and *Solanum nigrum,* which are both in the Solanaceae family (Cherif & Verheggen, 2019; Garcia & Espul, 1982; Ingegno *et al.*, 2017). Cultivated host plants which should not be rotated with tomato crops to reduce pest infestation include *Nicotiana rustica, Nicotiana tabacum, Solanum melongena, Solanum aethiopicum, Solanum muricatum* and *Solanum tuberosum* (Bawin *et al.*, 2016; Cherif & Verheggen, 2019; Ingegno *et al.*, 2017; Sawadogo *et al.*, 2022).

The nutritional status of tomato plants has also been reported to influence the host suitability for *P. absoluta*. Decreasing the amount of irrigation water that tomatoes receive, together with lower nitrogen levels, was reported to reduce the survival rate from egg to pupa, pupal weight and increased the overall development time of *P. absoluta* (Han *et al.*, 2014; Han *et al.*, 2019b). Pseudo-resistance of tomatoes against *P. absoluta*, which includes induced resistance, increases with lower availability of nitrogen and water (Han *et al.*, 2014). Furthermore, severe drought conditions trigger bottom-up defense effects of tomato plants against *P. absoluta* (Han *et al*., 2014). These effects result in shorter larval development times because of the reduced amount of moisture taken in by the larvae. This is consistent with the nitrogen limitation hypothesis, which states that larvae feeding on nitrogen deficient plants will have reduces growth rates due to impaired metabolism when they experience a lack in organic nitrogen (White, 1993). The effect of decreased nitrogen availability on *P. absoluta* was ascribed to the change in leaf nutrition and the increase in leaf chemical defences (Han *et al.*, 2014). Another reason why *P. absoluta* experienced bottom-up effects from tomato plants grown under drought stress conditions was ascribed to the growth differentiation balance hypothesis (Herms & Mattson, 1992). The hypothesis states that plants that experience growth limitations induced by external factors accumulate increased levels of not only carbohydrates, but also soluble phenolics which are used as defense mechanism against herbivorous insects (Stout *et al.*, 1998). Implementing lower nitrogen or moisture levels to manage pests implies critical evaluation of the precise amount of each factor, in order to balance agronomical and pest control outcomes. It is important not to reduce yield at the cost of improved pest control, and to ensure that the amount of fertilizer that optimizes the IPM strategy is determined (Desneux *et al.*, 2022).

Potassium deficiency in tomato plants may also cause bottom-up effects on *P. absoluta* (Sung *et al*., 2015). It has been reported that the metabolite profile of potassium deficient plants exhibit an increased amount of soluble sugar and overall reduced levels of amino acids (Flores *et al.*, 2016). There is, however, very little information available on the bottom-up effects of higher or lower potassium levels on *P. absoluta*.

1.4.5 Biological control

Biological control strategies, which include conservation and augmentative biocontrol, has been developed after *P. absoluta* invaded Europe (Biondi *et al.*, 2018). Biological control agents which suppress *P. absoluta* populations in both native and invaded environments include hemipteran predators, such as Anthocoridae, Miridae, Nabidae, and Pentatomidae (Biondi *et al.*, 2018). The use of *Trichogramma* egg parasitoids have shown promise, and augmentative release of *Trichogramma pretiosum* (Riley) (Hymenoptera: Trichogrammatidae) has shown promise in greenhouse systems in Brazil (El-Arnaouty *et al.*, 2014). Larval parasitism of up to 40% has been reported in South America with *Pseudapanteles dignus* (Meusebeck) (Hymenoptera: Braconidae) and *Dineulophus phthorimaeae* (de Santis) (Hymenoptera: Eulophidae) (Desneux *et al.*, 2010).

The use of *Bacillus thuringiensis* (Bacillales: Bacillaceae) var. *kurstaki* and *aizawaii* has shown successful microbial control against *P. absoluta* larvae (González-Cabrera *et al.*, 2011). Host plants which were infected with *P. absoluta* and treated with *Bacillus thuringiensis* (Berliner) as a foliar application showed a significant decrease on the amount of damaged area per leaflet as well as decreased adult emergence (González-Cabrera *et al.*, 2011). When *Steinernema* spp. (Rhabditida: Steinernematidae) and *Heterorhabditis* spp. (Rhabditida: Heterorhabditidae) nematodes were evaluated under laboratory conditions, 87 - 95% control of *P. absoluta* was recorded (Batalla-Carrera *et al.*, 2010). An example of a biocontrol strategy is the combined use of *Schizosaccharomyces japonicus* (Schizosaccharomycetales: Schizosaccharomycetaceae) and *Macrolophus pygmaeus* (Riley) (Hemiptera: Miridae) (Chailleux *et al.*, 2017; Chailleux *et al.*, 2014).

The use of entomopathogenic fungi (EPFs) as part of IPM strategies carries much potential. Fungal isolates of *Beauveria bassiana* (Hypocreales: Cordycipitaceae) and *Metarhizium robertsii* (Hypocreales: Clavicipitaceae) act as biopesticides against *P. absoluta* and affect certain life history parameters (Zheng *et al.*, 2023). *Metarhizium robertsii,* formally known as *M. anisopliae,* resulted in 100% mortality of second-instar larvae of *P. absoluta* fed on tomato leaves treated with this pathogen *(*Zheng *et al., 2023).* As an endophyte, *M. anisopliae* also promotes the growth of tomato plants by increasing the biomass (Zheng *et al.*, 2023). The use of entomopathogenic fungi to control *P. absoluta* proved effective in increasing plant biomass and because it results in delayed larval development, shorter adult longevity, and reduced fecundity (Zheng *et al.*, 2023). *Metarhizium anisopliae* (Sorokin) can cause up to 100% mortality of second-instar larvae, whereas *Metarhizium flavoviride* (Roszypal), *Beauveria bassiana* (Vuilemin) and *Cordyceps fumosorosea* (Kepler) caused 92.6%, 92.6% and 92.1% second-instar mortality respectively (Zheng *et al.*, 2023). *Metarhizium anisopliae* have been reported as a pathogen to larvae, eggs, pupae and adults of *P. absoluta (*Zheng *et al.*, 2023*).*

Cordyceps fumosorosea has been reported as the most effective EPF against third-instar larvae. Infection by this pathogen results in a short lethal period, high mortality of *P. absoluta* larvae, and increased tomato plant growth (Zheng *et al.*, 2023). The three main EPF's that have been reported to be effective in field trials were *M. anisopliae, C. fumosorosea* and *B. bassiana.* Each of these species has a significant detrimental effect on the survival rate, development time and fecundity of *P. absoluta* (Zheng *et al.*, 2023)

1.4.6 Chemical control

Chemical control of *P. absoluta* is difficult and compared to similar lepidopteran pests, poor results have been achieved (Biondi *et al.*, 2018). These difficulties resulted in changes in the active ingredients used over the years. Initially, organophosphates and pyrethroids were commonly applied. However, these have been replaced by cartap and abamectin, followed by oxadiazine indoxacarb and chitin synthesis inhibitors (Guedes & Picanço, 2012). Pesticides which are currently applied on tomatoes in South Africa against *P. absoluta* include chlorantraniliprole, emamectin benzoate, flubendiamide indoxocarb as well as spinosad (AgriIntel, 2023).

The evolution of resistance of *P. absoluta* to various insecticides resulted in control failure and significant yield loss in tomato crop yields in South America (Biondi *et al.*, 2018). The evolution of resistance against pesticides makes *P. absoluta* difficult to manage (Biondi *et al.*, 2012; Guedes & Picanço, 2012). In South America this pest evolved resistance against pyrethroids, abamectin, cartap and the organophosphate, methamidophos (Siqueira *et al.*, 2000). Once *P. absoluta* was detected in Europe, high levels of pyrethroid resistance and moderate levels of resistance to indoxacarb and spinosyn resulted in the diversification of insecticide use (Gontijo *et al.*, 2013).

The use of insecticides has a detrimental effect on biological control agents of *P. absoluta*. It disrupts the use of IPM programs (Martinou *et al.*, 2014). Similarly, spinosad, used in organic farming to control *P. absoluta,* causes a high mortality rate of predatory agents in Brazil (Barros *et al.*, 2015).

1.4.7 Resistant varieties

Host-plant resistance is the inherent genetic capacity of a crop to limit, slow down, or overcome pest infestations, resulting in decreased yield loss and improved quality of the harvested product (Dent, 2000). Primed resistance, on the other hand, is the exposure of the plant to a specific stimulus, such as chemicals, non-pathogenic bacteria, or pathogens to activate and enhance a plant's defence (Ramírez-Carrasco *et al.*, 2017). Therefore, native host plant resistance differs from primed resistance because it is inherent and genetically derived. Native host plant resistance does not need to be activated or enhanced by a certain stimulus while primed resistance requires a stimulus.

The most diverse gene pool for resistant tomato cultivars can be found in wild tomato varieties (Maluf *et al.*, 2010). Tomato cultivar improvement programs focus on breeding for constitutive resistance against *P. absoluta* with a focus on, for example, allelochemicals or leaf trichome density (Guedes & Picanço, 2012). The increased number of trichomes on the leaves of certain tomato cultivars decreases leaf damage and the number of holes made in the stem by *P. absoluta* larvae (Sohrabi *et al.*, 2017). High numbers of leaf trichomes and the presence of repellent allelochemicals decrease oviposition and larval feeding, leading to reduced larval numbers on plants and less plant damage (Bleeker *et al.*, 2012; Sohrabi *et al.*, 2017). Some wild tomato varieties have been reported also to reduce the fecundity and survival rate of whitefly and spider mites (Maluf *et al.*, 2001).

Examples of South American tomato varieties with resistance to *P. absoluta* is *Solanum pennellii* (Solanales: Solanaceae) (LA716) and *Solanum peruvianum* (Solanales: Solanaceae) (NAV29) (Maluf *et al.*, 2010). *Solanum pennellii* (LA716) is known for its high total acyl sugar content, and the variety is utilized in breeding programs that develop modern pest resistant cultivars (Maluf *et al.*, 2010).

Varieties containing zingiberene was reported to reduce overall leaf damage inflicted by *P. absoluta larvae* by 35% (de Azevedo *et al.*, 2003). Zingiberene is reported to be highly heritable and this resistant trait can be easily selected for in breeding programs (de Azevedo *et al.*, 2003). The concentration of zingiberene is associated with the type IV and VI trichome densities of a F2 tomato population (Maluf *et al.*, 2001). Therefore, the production of repellent and toxic compounds from the glandular trichomes of wild tomato species can be utilized as a source of resistance against spider mites and white flies (Bleeker *et al.*, 2012).

1.5 Use of growth stimulants to improve tomato plant health

It has been reported that weak plants attract more pests than healthy plants and that the improvement of soil health reduces pest pressure on plants (Altieri & Nicholls, 2003; Bezerra *et al.*, 2021). Plant growth and defence mechanisms can be enhanced using organic bio-stimulants (Mirmajlessi & Radhakrishnan, 2020; Yakhin *et al.*, 2017). For example, the exogenous application of monosilicic acid acts as a growth stimulant, which results in higher numbers of fruit and increased fruit mass per plant, as well as benefiting fruit quality by increasing the lycopene content and °Brix of fruit (Bansode *et al.*, 2020).

A wide range of extracts and bioactive substances with pesticidal characteristics have been produced as a result of research into cyanobacteria, seaweeds, macroalgae and microalgae species (Asimakis *et al.*, 2022). For example, compounds produced by a brown marine alga species, *Sargassum wightii* (Fucales: Sargassaceae), have shown promise as standalone treatments for *Xanthomonas oryzae* and *Pseudomonas syringae* that infect rice plants (Asimakis *et al.*, 2022). Increased fruit weight and fruit °Brix were achieved with the application of seaweed extracts on tomato plants (Hussain *et al.*, 2021). The full potential of such products can, however, only be realized when they are used in IPM programs (Asimakis *et al.*, 2022).

Rhizophagus irregularis (Glomerles: Glomeraceae) is an arbuscular mycorrhizal fungus (AMF) which is used as a biofertilizer and growth stimulant of tomatoes (Roussis *et al.*, 2022). *Rhizophagus irregularis* improves tomato plant physiology and leads to efficient plant growth, enhancing yield and improving fruit quality (Bona *et al.*, 2017). The use of AMF increases symbiotic relationships between the plant and the rhizosphere (Smith & Read, 2010). The utilization of AMF also increases the bioavailability of phosphorus and the effective use of nitrogen (Shafiq *et al.*, 2023). The use of AMF's *R. irregularis*, *Funneliformis mosseae* (Glomerales: Glomeraceae) and *Trichoderma harzianum* T22 (Hypocreales: Hyporeaceae) is effective in protecting tomato plants against *P. absoluta* under laboratory and field tomato production conditions (Ivanov, 2023) although the mechanism is poorly studied (Shafiei *et al.*, 2022). The inoculation of plants with the abovementioned microbes results in the accumulation of defensive compounds causing primed resistance which contributes to the microbe-induced resistance against *P. absoluta* (Ivanov, 2023). Treatment with *T. harzianum* and *F. mosseae* reduces *P. absoluta* larval survival under laboratory conditions (Aprile *et al.*, 2022; Ivanov, 2023). The application of *Trichoderma asperellum* (Hypocreales: Hypocreaceae) to tomato plants as a standalone application can decrease the °Brix of the tomato fruits while the application as an entomopathogenic fungicide for the control of *Fusarium oxysporum* (Hypocreales: Nectriaceae) increased the °Brix of the tomato fruits (Hasan *et al.*, 2021; Ruiz-Cisneros *et al.*, 2018).

Defensive compounds involved in the priming of resistance include jasmonic acid, which is mainly effective against necrotrophic pathogens and chewing insects (Gruden *et al.*, 2020). *Phthorimaea absoluta* attack have been shown to trigger jasmonic acid pathways in tomato (D'Esposito *et al.*, 2021). Inoculation of tomato seeds with endophytic fungi has been reported to provide effective protection against *P. absoluta* (Agbessenou *et al.*, 2020).

Another plant growth-promoting microorganism, *Aspergillus niger* (Tieghem, 1867) (Eurotiales: Trichocomaceae), is classified as a phosphate solubilizing microorganism (Khan *et al.*, 2009). *Aspergillus niger* contains two growth-promoting compounds, namely, 2-carboxymethyl 3-n-hexyl maleic acid and 2-methylene-3-hexylbutanedioic acid, which increases root and shoot length, as well as biomass of tomato plants (Mondal & Dureja, 2000).

The use of *A. niger* promotes plant health by increasing the chlorophyll content of leaves, lycopene content of fruits, total phenolic content, ascorbic acid content of fruit, the °Brix of fruit as well as the accumulation of salicylic acid (SA) (Anwer & Khan, 2013). Tomato plants treated with *A. niger* was reported to have higher °Brix and SA contents in comparison to untreated plants (Anwer & Khan, 2013). As a measure of plant health, leaf °Brix is an indicator of the balance of nutrients and photosynthates in plants (Mann *et al.*, 2011).

1.6 °Brix

The term °Brix is used as a measurement of the dissolved sugar to water mass ratio of a liquid (Harrill, 1998) and therefore, can provide an indication of the total dissolved sugars in plant tissue. A refractometer, the instrument used to measure the °Brix level of a liquid, is used to determine the degree Brix, which is the amount of refraction that light undergoes while passing through a liquid (Harrill, 1998). A liquid with a higher dissolved sugar content is a denser medium and will have an increased angle of refraction, resulting in a higher °Brix reading (Harrill, 1998).

Quantifying °Brix values is an affordable and valuable tool used in plant breeding programs. For example, the strong correlation between °Brix and inulin content of Jerusalem artichokes and chicory is used as indicator of improved varietal characteristics (Baldini *et al.*, 2004; Van Waes *et al.*, 1998). The method to estimate total dissolved sugar by using a refractometer is much less time-consuming and expensive than laboratory analyses (Van Waes *et al.*, 1998). The measure of °Brix can also be used to estimate tomato yields since higher °Brix is often correlated with higher tomato yields, although common cultivars with increased °Brix tend to be less productive (Garcia & Barrett, 2006).

1.6.1 Plant °Brix

The °Brix of plant sap is a summation of the amount of sucrose, fructose, vitamins, minerals, amino acids, proteins, hormones and other solids (Garcia & Barrett, 2006). The total soluble sugar in a tomato fruit can be utilized as a measure of yield quality. The °Brix of cherry tomatoes, for example, was used together with pH and total titratable acid to evaluate the efficacy of manure substrate combinations for use as organic substrates under greenhouse conditions (Costa *et al.*, 2018). During the latter study, °Brix ranging from 4.5 - 9.3 were obtained and the best performing substrate combination was indicated by the highest °Brix (Costa *et al.*, 2018).

1.6.2 The effect of abiotic and biotic factors on °Brix

Mild water stress during the late stages of tomato fruit growth increases the °Brix of tomato pulp, which decreases the processing costs without affecting the yield (Reid, 2002). Similarly, a decreased frequency of irrigation increases the °Brix without affecting the pH or fruit colour

(Alvino *et al.*, 1979). Excessive soil moisture has been reported to result in decreased °Brix of tomato fruit. For example, (Alordzinu *et al.*, 2021) reported that at a field capacity of 100% water, tomato plants produced 42 fruits per plant at an average °Brix of 4.3, compared 21 tomatoes per plant and 6.8 °Brix at a field capacity of 55% (Alordzinu *et al.*, 2021). Increased irrigation intervals (from daily to every six days) were also reported to increase the °Brix of marigold leaves, improving the quality of Trichodermaproducts derived from marigolds (Yari *et al.*, 2024). Based on the abovementioned reports of the effect of water on °Brix of tomato and marigold leaves, it is highly likely that °Brix of tomato plants will increase at decreased moisture levels.

Harvesting tomatoes earlier in the season, before full ripeness, results in lower °Brix while tomatoes harvested during the pink or red phase have increased °Brix (Chinamale, 2010). Tomatoes cultivated during dry and cool conditions were reported to have a higher °Brix than tomatoes that were cultivated during the rainy growing season, although the yield during the summer was higher than the yield during the winter (Bihon *et al.*, 2022). In vineyards, the time of day was shown to not affect leaf °Brix, irrespective of the time of the season. However, vineyard location was reported to have a significant effect on leaf °Brix which varied across sampling periods by over four degrees (Green, 1996).

1.6.3 Impact of soil nutrients on °Brix

Correlations between certain soil nutrients and °Brix have been reported by several authors. Potassium, calcium, magnesium, lime, organic matter and pH show a positive correlation with °Brix, while a negative correlation was found between °Brix and salt and available sodium (Aydin & Yoltaş, 2002). °Brix of date palm fruits was reported not to be affected by varying nitrogen contents of soil (Dialami & Mohebi, 2010). However, nitrogen has an indirect effect on °Brix by influencing plant health, growth and physiological attributes (Erdal *et al.*, 2006).

The solarization of soil resulted in higher °Brix in lemon and tomato fruits compared to non-solarized fruits, due to lower nematode and weed pressure induced by solarization (Candido *et al.*, 2008). The higher °Brix reported by the latter authors was ascribed to improved plant growth in solarized soils, leading to plants bearing higher number of smaller fruit, yielding higher °Brix (Candido *et al.*, 2008). Low quality fruit with low °Brix may be explained by early infestation by nematodes which reduces plant health (Candido *et al.*, 2008).

An increase in °Brix, vitamin C content and total acidity of tomato fruits with the foliar application of specific growth stimulants such as naphthalene acetic acid (NAA) was reported by (Nagi, 2021). NAA application resulted in a significant increase in chemical fruit quality at a foliar application rate of 200 ppm, followed by spraying with NAA at 100 ppm (Nagi, 2021). The application of NAA also resulted in significant increases in the concentration of nitrogen, phosphorous, potassium and total carbohydrates in the foliage of tomato plants compared to untreated plants (Nagi, 2021).

1.7 Impact of soil nutrients on insect feeding behaviour

A substantial body of data on the impact of nitrogen fertilizer on insect populations has been accumulated, and the general view holds that increasing nitrogen levels in plant tissues lead to increased pest occurrence (Jahn *et al.*, 2005; Mardani-Talaee *et al.*, 2017; Syrovy & Prasad, 2010). *Hysteroneura setariae* (Thomas) (Homoptera: Aphididae) was reported to survive at significantly higher numbers and to have increased fecundity on rice plants with 150% added nitrogen, compared with untreated plants (Jahn *et al.*, 2005).

Studies done on potassium and phosphorous in maize showed that an increase in these nutrient levels results in reduced pest pressure (Facknath & Lalljee, 2005). The application of potassium negatively affects lepidopteran pests of maize. For example, *Sesamia calamistis* (Hampson) (Lepidoptera: Noctuidae), *Eldana saccharina* (Walker) (Lepidoptera: Pyralidae), and *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) have reduced pupal weights, lower survival rates, and lower fecundity when exposed to maize plants fertilized with potassium (Facknath & Lalljee, 2005).

Increased levels of phosphorous were shown to have a negative effect on the number of punctures, mines and adults on leaves, as well as life history parameters such as eclosion, pupation and emergence of *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) on potato plants (Facknath & Lalljee, 2005). A similar tendency was reported for the hemlock woolly adelgid, *Adelges tsugae* (Annand) (Hemiptera: Adelgidae), of which abundance decreased with increased concentrations of phosphorous (Pontius *et al.*, 2006).

Myzus persicae (Sulzer) (Hemiptera: Aphididae) nymphs developed quicker on vermicompost-treated bell pepper plants than on zinc sulfate-treated bell pepper plants (Mardani-Talaee *et al.*, 2017). Nymphal mortality rates of *M. persicae*, was higher with vermicompost fertilization than with zinc sulfate treatments (Mardani-Talaee *et al.*, 2017).

Plutella xylostella (L.) (Lepidoptera: Plutellidae) fitness was significantly affected by fertilizer composition with different ratios of nitrogen, phosphorus and potassium, which had an influence on the host plant, *Brassica napus* (Brassicales: Brassicaceae) (Sarfraz *et al.*, 2009). Female moths chose host plants that provided the best pre-imaginal development and survival, as well as the longest lifespan for the next generation of moths (Heisswolf *et al.*, 2005; Sarfraz *et al.*, 2009). Plants attacked by *P. xylostella* increased the production of sulphur, whereas the levels of nitrogen in damaged leaves decreased (Sarfraz *et al.*, 2009). Regardless of fertilizer application, plants increased their root mass in response to herbivory

(Sarfraz *et al.*, 2009). Female *P. xylostella* moths favored plants with high phosphorus, sulfur, and calcium concentrations, as well as plants with intermediate nitrogen, potassium, and magnesium concentrations (Sarfraz *et al.*, 2009). High concentrations of organic acids and unbalanced amino acid profiles can result from increased levels of nitrogen and other nutrients in plant tissues (Williams & Cronin, 2004). Such diets can be harmful and even lethal to insects (Brodbeck *et al.*, 1990), which tend to feed more to compensate for low nitrogen diet (Berner *et al.*, 2005). *Plutella xylostella* females, which were reared as larvae on plants that received sub-optimal levels of fertilizer survived longer without food compared to those on unfertilized plants (Sarfraz *et al.*, 2009).

1.8 Effect of the varying °Brix on insects and life history parameters

Another nutritional factor of plants that influences the preference of insects is °Brix of the plant. Sugars such as melibiose, lactose, maltose, turanose, sucrose, galactose, glucose, fructose and xylose have been identified as compounds that stimulate the feeding of larvae of several lepidopteran pests species (Juma, 2010).

Lower sugar content of host plants reduces the fecundity of phytophagous insects due to the increased intake of soluble sugar, which leads to an increased amount of α-amylase activity within the gut of the insect (Fang *et al.*, 2019; Yang *et al.*, 2024). Therefore, the development of chewing and sucking insects is promoted by an increase in the sugar and protein content of host plants (Yang *et al.*, 2024). Furthermore, the activity of digestive enzymes, which utilize nutrients and promote insect growth has been reported for *P. absoluta* larvae that feed on nutrient-rich host plants (Yang *et al.*, 2024; Zhi, 2021).

The susceptibility of raspberry fruit to *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) oviposition is influenced by the soluble sugar content of the berries (Lee *et al.*, 2016) and grapes (Entling *et al.*, 2019). The susceptibility of fruit increases due to softening of the fruit's skin during the ripening process. As grapes ripen, the soluble sugar content increases and, therefore, susceptibility to *D. suzukii* increases (Lee *et al.*, 2016). However, analysis done on ten cherry cultivars showed no significant effect of °Brix on the development of *D. suzukii* eggs in adult flies (Wang *et al.* (2019). Studies conducted on grapevine under field conditions showed that there was no significant correlation between leaf °Brix and leafhopper nymph, *Erythroneura variabilis* (Beamer) (Hemiptera: Cicadellidae) densities (Green, 1996).

1.9 Primed host plant resistance

Plants defend themselves against pathogen and insect attacks by controlling numerous inducible defence reactions. Primed host plant resistance is a type of induced resistance in which defence systems are activated in response to earlier exposure to a pathogen or elicitor (Mookiah *et al.*, 2021).

Induced host plant resistance is a temporary increase in resistance caused by changes in plant or environmental factors, such as a change in the amount of water or the nutritional status of soil (Mookiah *et al.*, 2021). These reactions involve the action of signalling molecules induced by pathogenic stimuli or plant exposure to an external physical or chemical stressor. Plants treated with seaweed extracts that contain elicitor molecules can enhance a variety of inducible defensive reactions (Jayaraj *et al.*, 2008). These elicitors increase host plant resistance by mimicking pathogen action, which triggers induced resistance (Jayaraj *et al.*, 2008). Elicitor effects are aided by defensive signalling molecules such as salicylic acid (SA), jasmonic acid (JA), and ethylene (ET), which might result in systemic acquired resistance (SAR) or induced systemic resistance (ISR) (Vlot *et al.*, 2009; Zhang *et al.*, 2011).

The primed resistance of tomato plants can be a valuable tool in pest management. When larvae of *P. absoluta* feed on a tomato plant, the JA pathway is activated, which may lead to primed resistance against herbivores such as *P. absoluta* (Mouttet *et al.*, 2013). This is due to interactions between different plant defense pathways (Yan *et al.*, 2013). The induction of resistance could be implicated in constitutively produced defense allelochemicals which are toxic to pests, the release of volatile organic compounds (VOCs) that attract natural enemies, as well as the inhibition of the release of volatiles required by *P. absoluta* for host plant finding (De Backer *et al.*, 2015).

Aims and objectives

The aim of this study was to determine whether oviposition preference and larval preference of *P. absoluta* are influenced by different °Brix of tomato plant leaves. To achieve plants with different °Brix, different plant growth stimulants were applied to plants grown under different soil moisture conditions in a greenhouse.

The objectives of the study were to determine:

- the effect of soil water status and plant growth stimulants on °Brix of tomato plants
- the preference of *P. absoluta* moths for tomato plants grown under different soil moisture conditions and treated with plant growth stimulants
- the preference of *P. absoluta* larvae for tomato plants grown under different soil moisture conditions and treated with plant growth stimulants.

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Chapter 2

The effect of different plant growth stimulants and soil moisture conditions on °Brix of tomato leaves

Abstract

Growth stimulants are often applied to enhance symbiotic relationships between plants and microbes and to improve plant growth and defence mechanisms. Several studies have reported that the application of certain growth stimulants and optimal irrigation may result in higher °Brix of plants and subsequent reduced damage caused by insect pests, including the tomato leaf miner, *Phthorimaea absoluta* (Lepidoptera: Gelechiidae). In South Africa, plant growth- and biostimulants such as Seabrix, RealTrichoderma and NewSil, all marketed by RealIPM, are used in tomato production to benefit plant nutritional qualities. The aim of this study was to determine if these products, used either on their own or in combination and at different soil moisture levels, effectively increased the °Brix of plants. Two greenhouse experiments were conducted using potted plants. In one experiment, Seabrix, RealTrichoderma and NewSil, and combinations of these were evaluated. In the other experiment, which had a factorial design, there were two main treatments (Optimal or Excessive water conditions), each with three sub-treatments (control, Real Trichoderma, and a combination of RealTrichoderma and Seabrix). Sampling of leaves and determination of °Brix was done at weekly intervals, commencing 3 weeks after transplanting, and continued for six weeks. Results showed that the different treatment and soil water conditions did not have a pronounced effect on °Brix and that °Brix varied significantly over weeks. Growth stimulants had a small but significant effect, with the highest °Brix recorded after the SeaBrix treatment and the lowest with the SeaBrix + Trichoderma + NewSil treatment. The effect of growth stimulants on °Brix in this study was masked by the high variation recorded in °Brix at different sampling times. This study showed that treatment with plant growth stimulants did not result in pronounced differences in °Brix in this study. Further studies to determine the relationship between slight differences in °Brix between plants and pest incidence are needed before conclusions can be made regarding using these products in pest management.

Key words: °Brix, pests, plant health, Solanaceae

2.1 Introduction

Growth stimulants, also referred to as biostimulants, are used in agroecosystems to increase the number of symbiotic relationships between plants and microbes. The application of growth stimulants results in increased availability of nutrients to plants, which subsequently leads to improved plant health and crop yields (Baker, 1988; Sangha *et al.*, 2014).

The use of organic biostimulants is reported to improve plant growth and defence mechanisms (Yakhin *et al.*, 2017), which subsequently results in reduced pest attack on these healthier plants (Altieri & Nicholls, 2003). For example, the application of monosilicic acid as a growth stimulant of tomato plants increases various plant growth parameters (Bansode *et al.*, 2020). These include the number and weight of fruits per plant, as well as the quality of fruit in terms of °Brix and lycopene content.

°Brix is the amount of refraction that light undergoes when passing through a liquid, the higher the °Brix, the higher the total amount of dissolved sugars in the liquid and the denser the liquid (Harrill, 1998). A refractometer is used to determine the °Brix of a liquid which is faster, simpler and cheaper compared to laboratory analyses (Van Waes *et al.*, 1998). °Brix of plant sap therefore, involves the overall measurement of sucrose, fructose, vitamins, minerals, amino acids, proteins, hormones and other solids (Garcia & Barrett, 2006). Degree °Brix can be utilised to measure tomato fruit yield quality as it has been reported that a higher °Brix has a positive correlation with tomato yields (Garcia & Barrett, 2006).

The relationship between °Brix levels and the response of plants towards certain pests and diseases, has been reported by several authors. For example, reduced intensity of pest attacks by *Scirtothrips dorsalis* (Thysanoptera: Thripidae) and *Helicoverpa armigera* (Lepidoptera: Noctuidae) on tomato plants, have also been ascribed to application of silicic acid, which resulted in increased °Brix content (Bansode *et al.*, 2020).

The inoculation of plants with arbuscular mycorrhizal fungi and bioactive substances such as *Rhizophagus irregularis* (Glomerles: Glomeraceae) (Roussis *et al.*, 2022) and *Sargassum wightii* (Fucales: Sargassaceae) (Asimakis *et al.*, 2022), resulted in accumulation of defensive compounds within plant tissues, which may then result in microbe-induced (primed) resistance against pests. For example, Ivanov (2023) showed that application of *Trichoderma harzianum* (Hypocreales: Hypocreaceae), *Rhizophagus irregularis* and *Funneliformis mosseae* (Glomerales: Glomeraceae) resulted in reduced performance of *Phthorimaea absoluta* (Lepidoptera: Gelechiidae) on tomato plants. Infection by entomopathogenic fungi (EPFs) has been reported to effectively increase plant biomass and the mortality of *P. absoluta* larvae (Zheng *et al.*, 2023). Treatment of tomato plants with *Trichoderma asperellum* (Hypocreales: Hypocreaceae) resulted in a lower fruit °Brix (Ruiz-Cisneros *et al.*, 2018). Although, when *T.*

asperellum is utilized to control *Fusarium oxysporum* (Hypocreales: Nectriaceae), it was also reported to increase the °Brix of tomato fruit (Hasan *et al.*, 2021). The treatment of tomato plants with brown marine algae extracts such as SeaBrix (RealIPM) increases fruit weight and fruit Brix° (Hussain *et al.*, 2021). NewSil (RealIPM), which is a product that is applied as a foliar application, contains silicic acid and boron, and has been reported to result in increased Brix° and lycopene content in tomatoes (Bansode *et al.*, 2020).

The use of *Aspergillus niger* (Eurotiales: Aspergillaceae), which is a phosphate solubilizing microorganism, promotes plant health by increasing the chlorophyll content of leaves and nutrient content of fruit. For example, (Anwer & Khan, 2013) reported that lycopene and ascorbic content, total phenolic content, and the °Brix of fruit were higher in plants treated with *A. niger* and that salicylic acid accumulation was also higher in treated plants. °Brix can be seen as a measure of plant health as it is an indicator of the balance of nutrients and photosynthates within plants (Mann *et al.*, 2011). Brix° has a weak positive correlation with fruit quality, although there are various factors influencing the Brix° of leaves, making it a complex indicator of plant health (Cao *et al.*, 2024).

Different tomato genotypes can differ drastically regarding fruit °Brix (Luengwilai *et al.*, 2010). The Solara genotype produces an average °Brix of 9% while the Moneymaker genotype produced an average °Brix of 5% (Aldrich *et al.*, 2010; Luengwilai *et al.*, 2010). Different farming practices (organic vs conventional) has an effect on the °Brix of tomato fruit (Aldrich *et al.*, 2010; Barrett *et al.*, 2007).The amount and frequency of irrigation applied to tomato crops has an influence on the °Brix of fruits (Reid, 2002). The excessive application of water and high soil moisture levels have been reported to reduce the °Brix of tomato fruits while increasing the number of fruits per plant (Alordzinu *et al.*, 2021). A decreased irrigation frequency increased the °Brix of tomato fruit and marigold leaves in similar studies (Alvino *et al.*, 1979; Yari *et al.*, 2024). These trends suggest that °Brix is influenced by the soil moisture levels of plants and that it increases with a decrease in irrigation. Although the information provided above shows relationships between °Brix and soil moisture, no literature could be found that reported on the effects of plant growth stimulants on leaf °Brix of tomato plants, or interactions between these factors. Such information may be valuable regarding tomato pest management, since it may allow for the manipulation of °Brix to suppress pests. In order to utilize these bioactive substances to manipulate °Brix of plants as part of a pest management strategy, their ability to increase °Brix content and the possible influence of soil type and water conditions on this effect need to be elucidated.

In South Africa, various plant growth- and biostimulants are used in tomato production to improve plant health. These products include Seabrix (RealIPM, Thika, Kenya) and

RealTrichoderma (RealIPM) applied as soil treatments. These products are formulated from *Trichoderma asperellum* and are registered to control pathogenic fungi. Another such product is NewSil (RealIPM), a product applied as foliar fertilizer treatment that also increases plant resistance to biotic and abiotic stresses and improves nutrient uptake and crop quality. These products have also been reported to benefit plant nutritional qualities and adversely affect pests that attack treated tomato plants (Bansode *et al.*, 2020).

The aim of this study was to determine the effect of various plant growth stimulants and soil moisture levels on °Brix of tomato leaves in order to identify treatments that can be used to increase leaf °Brix and protect plants against *P. absoluta*.

2.2 Materials and methods

2.2.1 Cultivation of seedlings

Care was taken to cultivate a sufficient number of seedlings for all experiments. Tomato seedlings were cultivated in a greenhouse at the M-campus of the North-West University, Potchefstroom, South Africa. Seeds of the cultivar, Moneymaker were planted in Culterra seedling mix in plastic seedling trays (Figure 2.1). Each tray consisted of six cells, each with a size of 50 mm x 50 mm x 50 mm. Therefore, a single seedling plug had a volume of 0.125 dm^3. Two to three seeds were planted 20 mm deep to ensure good root establishment and availability of seedlings for use in greenhouse experiments. The seedling trays were placed on steel tables with a steel mesh packing surface. Seedlings were kept in a ventilated plant growth tunnel at ambient temperature and humidity. Seedlings received daily overhead mist irrigation as well as watering by hand.

Seventeen seedling trays were used for each treatment. The seed was planted on the 28th of February, 2023 and again on the 1st of March, 2023. Therefore, a total of 85 seedling trays were used, providing 510 cells containing at least one seed each. Seedlings were grown in seedling trays prior to transplanting them into 10 L pots for use in experiments. Five seedlings were transplanted into each pot and care was taken so as not to damage the root systems. This was done by carefully removing each seedling plug, placing it into the pot, and covering it with soil. Care was taken not to cover seedling stems with soil to prevent seedling rot. Transplanting took place on 5 April 2023, and the seedlings were thinned to three per pot on 18 April 2023.

Figure 2.1: Seedling trays filled with Culterra seedling mix containing one seedling per cell.

2.2.2 Trial one

2.2.2.1 Treatments

Trial 1 had five treatments. These were three different growth stimulants (Table 2.1), a combination of two treatments, and an untreated control. One treatment (NewSil) was administered as a foliar application, and the other three were applied as soil treatments (Table 2.2). Each of the five treatments had ten replicates. Each replicate consisted of one pot with three tomato plants.

Table 2.1: The commercial name, manufacturer, active ingredients and additional information of the treatments used in this study (RealIPM, 2024).

<table>
<tr><th>Commercial Name and Manufacturer</th><th colspan="10">Active Ingredients</th><th>About</th></tr>
<tr><td rowspan="7">SeaBrix™
Real IPM (SA) Ltd.
(Grabouw, 7160, South Africa)</td><td colspan="10">Nutrient Analyses</td><td rowspan="7">SeaBrix consists of a combination of macro and micro trace elements, plant stimulating hormones and enzymes which is formulated to increase plant growth, photosynthesis and °Brix. This leads to an increase of amino acids, gibberellins and auxin levels within the plant as well as stimulating the microbial activity in the soil.</td></tr>
<tr><td colspan="10">Macro elements</td></tr>
<tr><td>N</td><td>P</td><td>K</td><td>Ca</td><td>Mg</td><td>S</td><td>C</td><td></td><td></td><td></td></tr>
<tr><td>14</td><td>14</td><td>26</td><td>4</td><td>3</td><td>0</td><td>107</td><td></td><td></td><td></td></tr>
<tr><td colspan="10">Micro elements</td></tr>
<tr><td>B</td><td>Fe</td><td>Mn</td><td>Zn</td><td>Cu</td><td>Mo</td><td>Si</td><td>Co</td><td>Ni</td><td>Se</td></tr>
<tr><td>5</td><td>101</td><td>43</td><td>27</td><td><2</td><td>0</td><td>111</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td rowspan="5">NewSil™
Real IPM (SA) Ltd.
(Grabouw, 7160, South Africa)</td><td colspan="10">Nutrient Analyses</td><td rowspan="5">NewSil consists of 100% plant available silicon which is in a soluble and stable, bioavailable form. NewSil improves crop quality by increasing a plants resistance towards biotic and abiotic stress. Resistance is induced by forming an opal layer between the epidermis and cuticula when mono-silici enters the plants through the cuticle as a foliar application. Foliar sprays of NewSil consist of silicic acid at 2.5 % W/V and boron 0.20 % W/V.</td></tr>
<tr><td colspan="10">Macro Elements</td></tr>
<tr><td>Si</td><td>K</td><td>Cl</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>5.4</td><td>6.1</td><td>19</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td colspan="10">SG. 1.11 pH 2.0</td></tr>
<tr><td>RealTrichoderma™
Real IPM (SA) Ltd.
(Grabouw, 7160, South Africa)</td><td colspan="10"><i>Trichoderma asperellum</i>
Strain: TRC900
Concentration: 1 X10^{9} CFU/ml</td><td>RealTrichoderma has <i>Trichoderma asperellum</i> as the active ingredient with the main function of controlling various pathogenic fungi by the means of systemic acquired resistance, induced systemic resistance and competition by occupying the biological space. Therefore, classified as a biological pesticide. Furthermore, Real Trichoderma increases the growth of fine hair roots benefiting the nutrient uptake of the plant, classifying it as a biological fertiliser.</td></tr>
</table>

Table 2.2: Description of treatments, dosage rates and application intervals of treatments of trial one.

Treatments	Description	Dosage	Application intervals
Control (**C**)	No application. Optimal nutrient status of soil was maintained.	Irrigation water	Daily
SeaBrix (**SB**)	SeaBrix product applied to soil.	20 mℓ **SB** / 10 ℓ water	**SB**: 4 days prior to transplanting as a seedling drench. Once every week after transplanting.
SeaBrix + *Trichoderma asperellum* (**SBT**)	SeaBrix and *Trichoderma asperellum* applied to soil.	20 mℓ **SB** + 2 mℓ **T** / 10 ℓ water	**SB**+**T**: 4 days prior to transplanting as a seedling drench. Once every week after transplanting.
SeaBrix + *Trichoderma asperellum* + NewSil (**SBTN**)	SeaBrix and *Trichoderma asperellum* applied to soil.	20 mℓ **SB** + 2 mℓ **T** / 10 ℓ water	**SB**+**T**: 4 days prior to transplanting as a seedling drench. Once every week after transplanting.
	NewSil applied to the foliage of plants.	1 mℓ **N** / 500 mℓ water	**N**: Every two weeks after transplanting starting 7 days after transplanting.
NewSil (**N**)	NewSil applied to foliage of plants.	1 mℓ **N** / 500 mℓ water	**N**: Every two weeks after transplanting starting 7 days after transplanting.

2.2.2.2 Soil used

Soil with a sandy texture was used in this study. The soil was collected at the M-campus of the North-West University (26°67’37.13”S; 27°10’67.35”E). Three composite samples were analysed for the purpose of classifying the soil. The soil was operationally defined to have an optimal nutrient status (Appendix: A, Table 1A), and no nutrients were applied.

2.2.2.3 Treatment application

The first application of soil-applied products (SB, SBT and SBTN) (Table 2.2) was done as a drench into pots, four days before seedlings were transplanted into pots. Thereafter, application of all soil-applied treatments were repeated at weekly intervals for 8 weeks. The first application of treatments containing NewSil (SBTN and N) was done one week after transplanting seedlings into pots, followed by applications at two-week intervals. The amount of product applied to each seedling tray and pot is provided below (Table 2.3).

Table 2.3: Prescribed application rates of treatments (label information).

Product	Volume (mℓ) per 10 ℓ water	Volume (mℓ) per seedling tray	Volume (mℓ) per 10 ℓ pot
SeaBrix	20	0.0015	0.01
Real Trichoderma	2	0.0003	0.001
NewSil	20	-	Spray plants until droplets form on leaves (1 mℓ / 500 mℓ)
Water	1000	1.5	-

2.2.2.4 Sample collection

Sampling commenced 3 weeks after transplanting and was done at weekly intervals for 7 weeks. Leaves were collected from each plant to get sap used to determine °Brix. The three topmost fully developed leaves of each of the three plants per pot were removed on the day of sampling. Therefore, 30 leaf samples were collected for each treatment on each sampling day. Sampling commenced at the same time of day (09:00), to limit any possible effects environmental conditions could have on °Brix.

Sampling was done by cutting off leaflets from plants using handheld pruning scissors. The leaves of each sample were placed into a well-marked Ziplock bag. The bags were left open to prevent condensation within the bag. The bags were stored in a cooler box together with an ice brick in the correct order to simplify data collection. The cooler box with samples was placed at 5 °C until sample analysis was performed within 1 hour after sampling.

2.2.2.5 Sample analyses

The procedures used to analyse samples and determine the °Brix of the plant sap collected from the three leaves of each plant are described below. A digital refractometer (MSDR-P2-50 Professional Low Range 0~50% Digital Brix / RI Refractometer) was calibrated each week before readings were taken. This was done by pressing the calibration button for three seconds, followed by dripping a drop of distilled water on the measurement surface and pressing the calibration button again to complete the process. The refractometer was then cleaned by removing the droplet from the measurement surface with a paper towel.

The leaf samples were then removed from cooler box, after which the petioles were removed. Gloves were worn to crush leaves between fingers and drip sap onto the measurement surface of the refractometer. The reading was taken by pressing the “Read” button for three seconds to take 15 sequential readings of each sample, after which the temperature and °Brix were noted. The surface of the refractometer and the gloves were cleaned with water and dried with a paper towel before the following sample was handled. These steps were repeated for each sample.

2.2.2.6 Data collection

Samples were collected weekly for seven weeks, starting 26 April 2023, three weeks after transplanting. The °Brix data were recorded and entered into an Excel table.

2.2.2.7 Statistical analysis

The mean °Brix values of the different treatments were compared by means of Repeated Measures Analysis of Variance (ANOVA) followed by Tukey HSD post hoc test indicating significant differences. All statistical analyses were performed with TIBCO® Statistica™ software version 14.0.1.25 (TIBCO, 2020).

2.2.3 Trial two

2.2.3.1 Treatments and experimental layout

The experiment had six treatments in a factorial design with two main treatments, each with three sub-treatments. The main treatments were **Optimal** water conditions, and **Excessive** water conditions. The sub-treatments were 1) a control which did not receive any plant growth stimulants, 2) soil applications of Real Trichoderma, and 3) a combination of Real Trichoderma and Seabrix (Table 2.4). Each treatment had 12 replicates, consisting of one pot with two plants.

The pots which received the optimal amount of water, each received 250 ml, while those of the excessive treatment received 500 ml water immediately after transplanting. One of the two seedlings was marked with a tag to indicate the treatment and replicate and was not used for °Brix measurements. The latter seedlings were used in another study (Chapter 3) where the effect of these different growth stimulant treatments and varying °Brix contents on *P. absoluta* were determined.

Seedlings for this experiment were cultivated in 16 seedling trays for each treatment before they were transplanted into bigger plots. The seed was planted on the 8th of September 2023.

Therefore, a total of 96 seedling trays were used providing 576 cells containing at least one seed each.

Twelve 8 kg (25 cm) pots were used for each treatment (total=72 pots). Three seedlings were transplanted into each pot without damaging the root system. Each seedling plug was removed carefully and placed in the pot and covered with soil without covering the stems. Transplanting took place on 30 November 2023 and each pot received sufficient water for the seedlings to establish.

Table 2.4: Description of treatments, dosages of growth stimulants, and water application intervals for treatments of trial 2.

Treatment	Description	Growth stimulant dosage	Water dosage	Application intervals
Optimal water Control (**OC**)	No growth stimulant application with an optimal amount of water.	-	An optimal amount of water.	Every second day.
Optimal water + Trichoderma (**OT**)	Real Trichoderma with an optimal amount of water applied to soil.	2 mℓ **T** / 10 ℓ water.	An optimal amount of water.	**T**: Four days prior to transplanting as a seedling drench. Once every week after transplanting took place on the 6th of December 2023.
Optimal water + Seabrix + Trichoderma (**OSBT**)	Seabrix and Real Trichoderma products applied to soil.	20 mℓ **SB** + 2 mℓ **T** / 10 ℓ water	An optimal amount of water.	**SB**+**T**: Four days prior to transplanting as a seedling drench. Once every week after transplanting took place on the 6th of December 2023.
Excessive water + Control (**EC**)	No growth stimulant application with an optimal amount of water.	-	An excessive amount of water.	Every second day.
Excessive water + Trichoderma (**ET**)	Real Trichoderma with an optimal amount of water applied to soil.	2 mℓ **T** / 10 ℓ water.	An excessive amount of water.	**T**: Four days prior to transplanting as a seedling drench. Once every week after transplanting took place on the 6th of December 2023.
Excessive water + Seabrix + Trichoderma (**ESBT**)	Seabrix and Real Trichoderma products applied to soil.	20 mℓ **SB** + 2 mℓ **T** / 10 ℓ water	An excessive amount of water.	**SB**+**T**: Four days prior to transplanting as a seedling drench. Once every week after transplanting.

The pots were each filled with 8 kg of soil gathered from the premises of Mount Carmel farms (25°58'40.70"S, 28°20'56.01"E) near Babsfontein, Gauteng province (Appendix: A, Table 1B**.**).

One pot from each of the two different soil moisture treatments was fitted with a Chameleon soil moisture probe (Virtual Irrigation Academy Ltd., Pretoria, South Africa (VIA).

The probe consisted of three electrodes and a thermometer, which were inserted at different depths into the soil profile inside two of the pots. One electrode was buried at a depth of 6 cm, another, together with the thermometer, at 12 cm, and the third at 20 cm. Three lights on the probe indicated the amount of resistance the electrodes measured at the various depths. Readings between 0 and 4 kiloohms (kΩ) were indicated by a blue light, those between 4 and 40 kΩ by a green light, and those between 40 and 4000 kΩ by a red light. The probes were linked with the VIA website which facilitated easy collection of data. The resistance data collected through this system was retrieved and recorded in an Excel table for each treatment.

These probes were used to determine the optimal and excessive water application rates as well as to monitor the degree of water stress experienced by plants throughout the duration of the experiment. The Chameleon soil moisture probe indicates, by means of different coloured lights, whether the moisture content of soils is optimal or excessive (too wet). In this study, pots were weighed when the soil moisture probe indicated three green lights, which was an indication that moisture content was optimal. When the soil moisture probe indicated three blue lights, it was indicative of excessive moisture content. When probes indicated blue lights, pots were also weighed and information used to calculate how much water the pots should receive to maintain the specific watering conditions.

2.2.3.2 Determination of ‘excessive’ and ‘optimal’ soil moisture conditions

The amount of water needed to maintain pots at the required ‘excessive’ and ‘optimal’ moisture conditions was determined by weighing the two pots which were fitted with probes. These two pots were used as reference pots for the other pots. Pots were weighed on a scale with a maximum weight capacity of 15 kg.

To determine the amount of water needed for each pot in the experiment, the reference pots were weighed on two consecutive days to measure the reduction in weight over a 24-hour period. The reduction in weight over this period was considered as the loss of soil moisture content. An amount of water equal to the loss in weight was applied every day to each pot for one week. To account for increased moisture loss associated with plant growth over time, reference pots were weighed again after one week to calculate the new optimal and excessive water content. This process was repeated every week for 8 weeks until 26 January 2024.

2.2.3.3 Application of growth stimulants

The dosage of growth stimulants was calculated per pot surface area and was determined to be 0.6927 µℓ of the Trichoderma (**T**) solution per pot and 6.927 µℓ of Seabrix (**SB**) per pot. The growth stimulant products were suspended in water, and 5 ml of the suspension was applied per pot.

The Trichoderma treatment suspension was as follows: 0.69 µℓ **T** x 24 pots = 16.62 µℓ **T** with 120 (24 x 5) mℓ water. Since the Real Trichoderma (T) product is oil-based, a drop of commercial dishwashing liquid was added to the suspension to break the surface tension of the water. Five mℓ of the suspension was added to the irrigation water of each pot at weekly intervals. The SeaBrix / Trichoderma (**SBT**) treatment was as follows: 182.87 µℓ of a **SB** + **T** premixed concentrate with 120 (24 x 5) mℓ water was mixed uniformly. In this case, the **SB** acted as a binding liquid for the Trichoderma product; no dishwashing liquid was necessary. Five mℓ of the suspension was added to pots at weekly intervals.

2.2.3.4 Sample collection and °Brix determination

Because the °Brix of a plant is temperature sensitive, all measurements were made between 09:00 and 10:00 in a laboratory setting. Three leaves of each plant were used for the determination of °Brix at weekly intervals as described above. Leaves were removed from the bag seperately and the petioles were removed. The leaves were crushed and a droplet of leave sap dripped onto the reeding surface of a zero calibrated refractometer. The °Brix reedings were entered into the spreadsheet.

2.2.3.5 Pot maintenance

Pots were kept weed-free to limit the evaporation that could result due to their growth. Care was also taken not to remove soil with the roots of weeds since it would have influenced the weight of the pot. The flowers of the tomato plants were removed to encourage vegetative growth.

2.2.3.6 Data collection

The recording of °Brix commenced three weeks after transplanting (21 December 2023) and continued for six consecutive weeks until 25 January 2024.

2.2.3.7 Statistical analysis

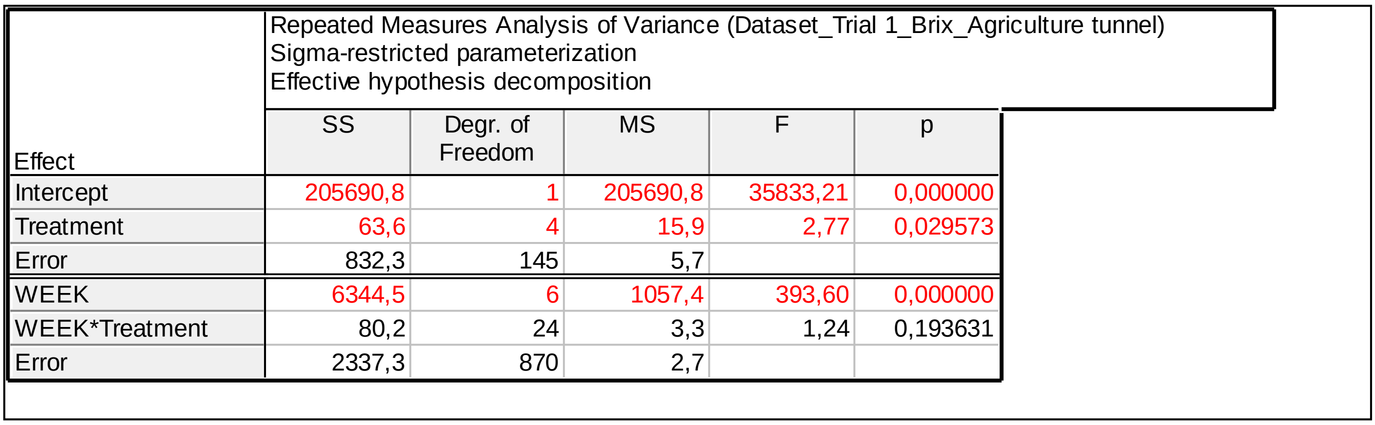
A factorial analysis with two main treatments (Optimal soil moisture conditions, and Excessive soil moisture conditions) and three sub-treatments (control, or soil applications of either RealTrichoderma, and SeaBrix and RealTrichoderma. The mean °Brix values recorded over six consecutive weeks for each soil moisture regime and each treatment, were compared by means of Univariate Tests of Significance followed by Tukey HSD tests. All statistical analyses were performed with TIBCO® Statistica™ software version 14.0.1.25 (TIBCO, 2020).

2.3 Results

2.3.1 Trial one

The results of the repeated measures ANOVA are presented in table 2.5. There was no significant interaction (p = 0.19) between the week in which °Brix was measured and the different growth stimulant treatments. The °Brix of plants of the different treatments for each week, as well as the overall weekly and treatment means, are provided in table 2.6.

Table 2.5: Results of the repeated measures ANOVA of the effects of week of sampling and plant growth stimulants treatment on °Brix of tomato plant leaves (trial 1).



Repeated Measures Analysis of Variance (Dataset_Trial 1_Brix_Agriculture tunnel)
Sigma-restricted parameterization
Effective hypothesis decomposition

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	205690,8	1	205690,8	35833,21	0,000000
Treatment	63,6	4	15,9	2,77	0,029573
Error	832,3	145	5,7		
WEEK	6344,5	6	1057,4	393,60	0,000000
WEEK*Treatment	80,2	24	3,3	1,24	0,193631
Error	2337,3	870	2,7		

Table 2.6: The mean (± standard error) °Brix of each treatment over a period of seven weeks.

	°Brix (mean ± SE)							
Treatment	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Mean**
Control	10.53 ± 0.38abcd*	12.27 ± 0.32efgh	12.6 ± 0.27fgh	16.48 ± 0.29i	17.32 ± 0.4i	13.25 ± 0.3h	16.65 ± 0.31i	14.16 ± 0.21AB***
SeaBrix	10.46 ± 0.41abc	12.33 ± 0.32efgh	13.25 ± 0.26h	15.68 ± 0.31i	17.32 ± 0.32i	13.59 ± 0.35h	17.38 ± 0.29i	14.29 ± 0.21B
Seabrix + Trichoderma	10.17 ± 0.39ab	10.99 ± 0.26abcdef	12.21 ± 0.34defgh	16.06 ± 0.32i	17.2 ± 0.27i	13.43 ± 0.34h	16.75 ± 0.34i	13.83 ± 0.22AB
SeaBrix Trichoderma + NewSil	9.86 ± 0.41a	11.28 ± 0.26abcdefg	11.86 ± 0.28bcdefgh	16.17 ± 0.26i	16.6 ± 0.33i	13.05 ± 0.35h	16.41 ± 0.38i	13.60 ± 0.22A
NewSil	10.71 ± 0.34abcde	12.15 ± 0.27cdefgh	12.91 ± 0.3gh	16.16 ± 0.27i	16.64 ± 0.28i	13.17 ± 0.35h	16.99 ± 0.31i	14.10 ± 0.19AB
Overall weekly mean	10.34 ± 0.17 B**	11.8 ± 0.16 C	12.57 ± 0.14 D	16.11 ± 0.13 F	17.01 ± 0.14 A	13.34 ± 0.15 E	16.83 ± 0.15 A	

*Means followed by the same lower-case letter do not differ significantly at P = 0.05 (Tukey HSD).
**Means followed by the same upper-case letter in this row do not differ significantly at P = 0.05 (Tukey HSD).
***Means followed by the same upper-case letter in this column do not differ significantly at P = 0.05 (Tukey HSD).

2.3.1.1 °Brix over the 7-week period

Mean overall leaf °Brix differed significantly ($p < 0.00001$) between weeks over the 7-week period (Table 2.6). The overall weekly mean °Brix of all growth stimulant treatments varied between 10.34 and 16.83 over the 7-week period (Table 2.6). There was a significant increase in °Brix from week 1 to week 5 with the highest °Brix readings recorded at weeks 5 and 7. °Brix readings taken at week 5 and week 7 did not differ significantly. The mean °Brix decreased by 3.7 °Brix to 13.3 from week 5 to week 6. Despite this decrease, the overall mean °Brix at week 6 was still significantly higher than °Brix in weeks 1 and 3 (Table 2.6).

2.3.1.2 °Brix of tomato plants subjected to different plant growth stimulant treatments

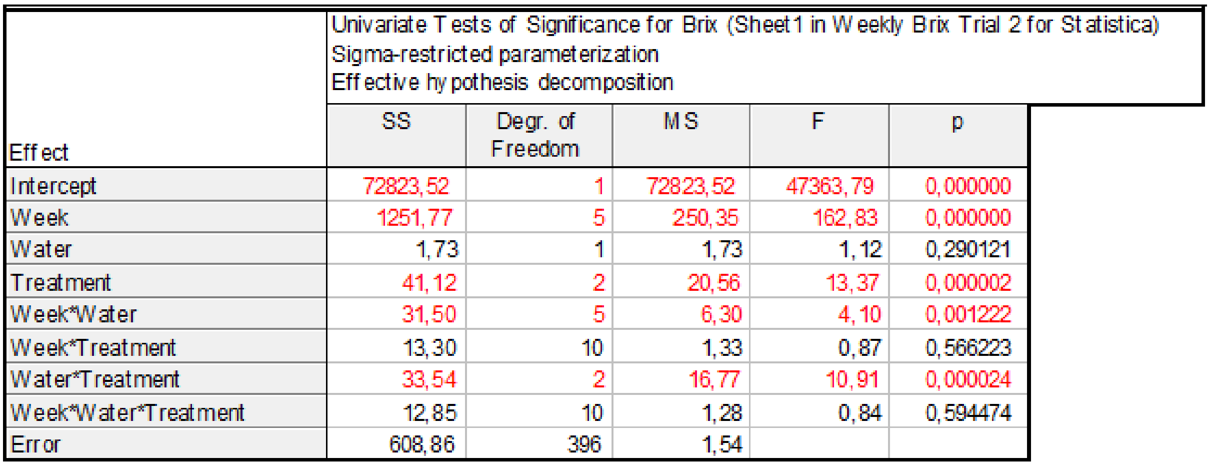
There were significant differences between the mean °Brix of leaves of the different growth stimulant treatments over the 7-week period (Table 2.6). The Seabrix treatment resulted in a significantly higher °Brix compared to the Seabrix-Trichoderma-NewSil treatment. The °Brix of the control treatment, Seabrix and Trichoderma treatment and the NewSil treatments did not differ significantly (Table 2.6).

The range of the difference between the lowest and highest °Brix reading of the different growth stimulant treatments was small (0.68), with only that of the SeaBrix treatment being significantly ($p = 0.029$) higher than that of the control treatment (Table 2.6).

2.3.2 Trial two

The results of the factorial analysis are presented in table 2.7. In general, the results of the factorial analysis show that, of the main effects, only week and growth stimulant treatment had significant effects on °Brix of leaves. The other main effect, soil moisture conditions, did not have a significant ($p = 0.29$) effect on °Brix. °Brix differed significantly between the different growth stimulant treatments ($p < 0.000002$). Significant interactions were observed between week (sampling interval) and soil moisture conditions ($p < 0.0012$), as well as the soil moisture and growth stimulant treatments ($p < 0.00002$). There was no significant interaction ($P = 0.566$) between the week the °Brix readings took place and the °Brix of the respective growth stimulant treatments (Table 2.7). There was no significant ($p = 0.594$) three-way interaction between the week, soil moisture condition and growth stimulant treatments.

Table 2.7: Results of the factorial analysis of the effects of soil moisture conditions, week of sampling and plant growth stimulants treatment on °Brix of tomato plant leaves from trial 2.



Univariate Tests of Significance for Brix (Sheet1 in Weekly Brix Trial 2 for Statistica)
Sigma-restricted parameterization
Effective hypothesis decomposition

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	72823,52	1	72823,52	47363,79	0,000000
Week	1251,77	5	250,35	162,83	0,000000
Water	1,73	1	1,73	1,12	0,290121
Treatment	41,12	2	20,56	13,37	0,000002
Week*Water	31,50	5	6,30	4,10	0,001222
Week*Treatment	13,30	10	1,33	0,87	0,566223
Water*Treatment	33,54	2	16,77	10,91	0,000024
Week*Water*Treatment	12,85	10	1,28	0,84	0,594474
Error	608,86	396	1,54		

The main effects and their interactions (Table 2.7) are discussed below.

2.3.2.1 Mean °Brix of tomato leaves over the six-week period

There were significant differences between the overall mean °Brix of plants at the different sampling times (Table 2.7). There was a large variation (range = 4.82) in the mean °Brix of leaves (10.10 - 14.92) over the 6-week period (Table 2.8). The highest and lowest °Brix was recorded at weeks 1 and 2, respectively. There was no consistent pattern of increase or decrease in °Brix over the trial period, with the overall average °Brix recorded at week 4 being significantly lower than those at weeks 1, 3, 5 and 6.

Table 2.8: The mean °Brix of tomato leaves of all treatments over a 6-week period.

Week	°Brix (mean SE)
1	14.92 ± 0.16a
2	10.10 ± 0.17d
3	13.86 ± 0.12b
4	11.27 ± 0.11c
5	13.86 ± 0.17b
6	13.90 ± 0.19b

*Means followed by the same lower-case letter do not differ significantly at P = 0.05 (Tukey HSD).

2.3.2.2 Mean °Brix of plants grown under optimal and excessive soil moisture conditions

The mean °Brix values did not differ significantly (p = 0.29) between plants grown under different soil moisture conditions (Table 2.7; Fig. 2.2). The mean °Brix of plants grown under excessive soil water conditions was 13.05 (S.E ± 0.16), while those grown under optimal conditions was 12.92 (S.E ± 0.13).

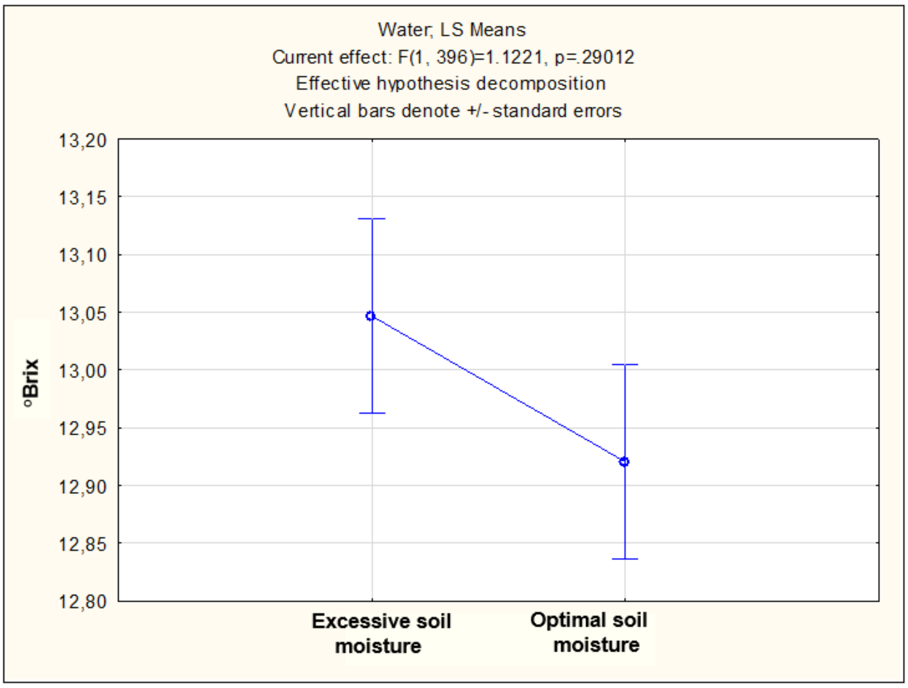


Figure 2.2: Interaction plot showing °Brix of tomato plant leaves subjected to excessive and optimal soil moisture regimes over a period of six weeks.

2.3.2.3 The effect of plant growth stimulant treatments on leaf °Brix

The results showing the effect of the different growth stimulant treatments on mean leaf °Brix over the 6-week period are provided in table 2.9. Mean leaf °Brix ranged from 12.38 to 13.73 (range = 0.71) and differed significantly between treatments. Leaf °Brix was the lowest for plants that received a combination of Trichoderma + SeaBrix treatments.

Table 2.9: Overall mean °Brix of leaves of tomato plants treated with different plant growth stimulants over a 6-week period.

Treatment	°Brix (mean ± SE)
Control	13.27 ± 0.19a
Trichoderma	13.12 ± 0.18a
Trichoderma + SeaBrix	12.56 ± 0.17b

*Means within column followed by the same letter do not differ significantly at P = 0.05 (Tukey HSD).

2.3.2.4 Soil moisture condition x week interaction

There was a significant (p = 0.001) interaction between soil moisture condition and week (Table 2.9). The mean °Brix of the two soil moisture treatments over the trial period are provided in table 2.10, and the interaction plot is in figure 2.3. The significant interaction between these main effects indicates that neither of the excessive nor optimal moisture condition treatments had consistently higher or lower °Brix levels than the other treatment over the 6-week period.

The mean °Brix was lowest at week 2 of sampling, with 9.77 and 14.93 for plants that received excessive and optimal watering, respectively (Table 2.10). There were no patterns of increase or decrease in °Brix values over time.

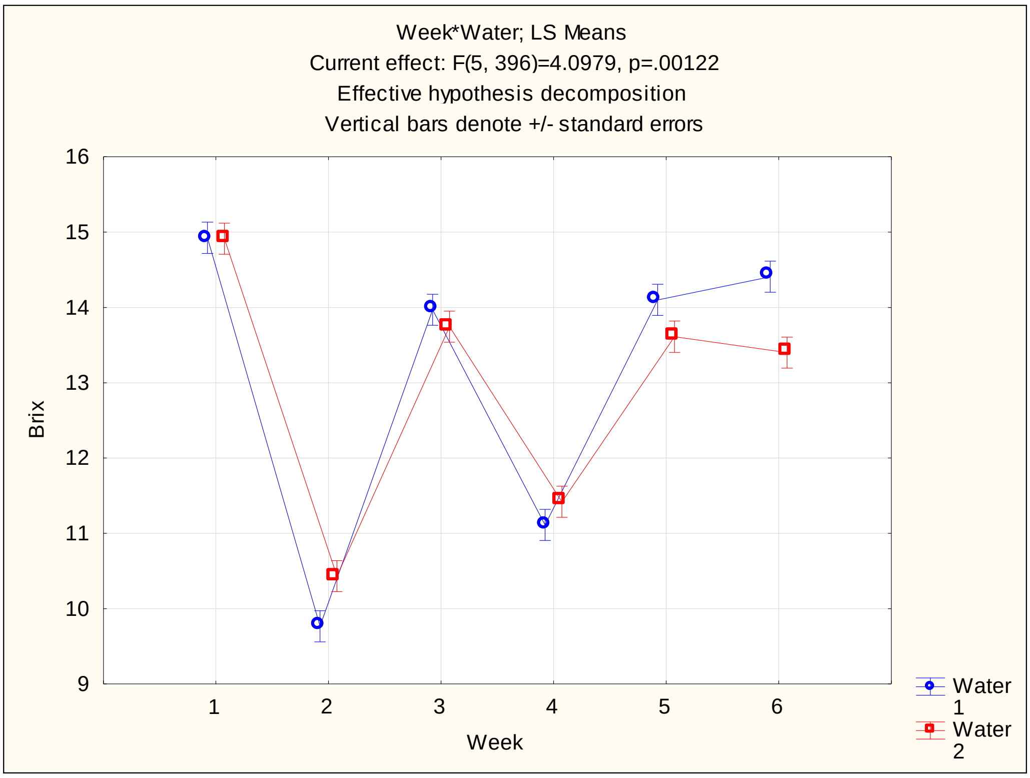


Figure 2.3: Interaction plot showing leaf °Brix of tomato plants grown under optimum (Water 1) and excessive soil moisture (Water 2) conditions, over a 6-week period.

Table 2.10: Mean °Brix of tomato plants grown under excessive or optimal soil moisture conditions over a 6-week period.

Week	**° Brix (mean ± SE)**	
	Water	
	Excessive	**Optimum**
1	14.93 ± 0.23a*	14.91 ± 0.24ab
2	9.77 ± 0.25g	10.43 ± 0.20fg
3	13.97 ± 0.17bcd	13.74 ± 0.13cd
4	11.11 ± 0.14ef	11.42 ± 0.19e
5	14.10 ± 0.21abcd	13.61 ± 0.26cd
6	14.41 ± 0.31abc	13.40 ± 0.20d
Mean	13.05 ± 0.16A**	12.92 ± 0.13A

*Means within a column followed by the same lower-case letter do not differ significantly at P = 0.05 (Tukey HSD).
**Means within row followed by the same letter do not differ significantly at P = 0.05 (Tukey HSD).

2.3.2.5 Soil moisture condition x growth stimulant treatment interaction

Results of the factorial analysis showing the significant ($p = 0.00002$) interactions between growth stimulant treatments x soil moisture conditions are provided in table 2.7.

The interaction plot (Figure. 2.4) shows that plants of the control treatment which did not receive growth stimulants, responded differently to the two soil water treatments than the treated plants.

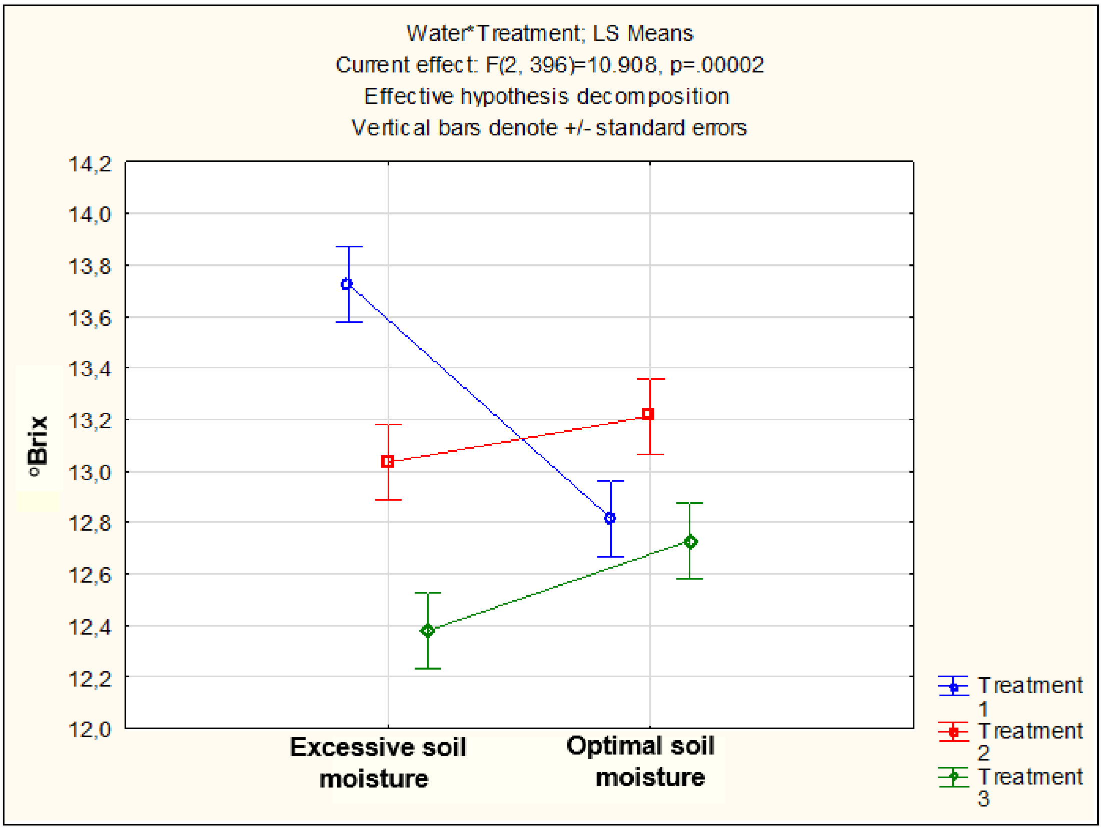


Figure 2.4: Interaction plot showing °Brix of tomato plants subjected to optimal and excessive soil moisture regimes and two growth stimulant treatments. Treatment 1: Control, Treatment 2: Trichoderma, Treatment 3: Seabrix and Trichoderma.

The overall mean °Brix of the different treatments over the 7-week period ranged from 12.38 to 13.73 (Table 2.13, Figure 2.4). The mean °Brix of the control treatment grown under excessive soil water conditions was significantly higher than those of the two plant growth stimulant treatments at the same moisture conditions (Table 2.11). However, under optimal soil moisture conditions, °Brix of plants of this control treatment did not differ significantly (p <

0.05) from those of plants grown under optimal conditions and which received the Trichoderma treatment (Table 2.11, Figure. 2.4).

Table 2.11: Interaction table showing the mean leaf °Brix of tomato plants treated with different plant growth stimulants and grown under excessive or optimal soil moisture conditions over a 6-week period (trial 2).

Water	**°Brix (mean ± SE)**			**°Brix (mean ± SE)**
	Treatment			
	Control	**Trichoderma**	**SeaBrix + Trichoderma**	
Excessive	13.73 ± 0.29a*	13.03 ± 0.26b	12.38 ± 0.25c	13.05 ± 0.16a**
Optimal	12.82 ± 0.23bc	13.21 ± 0.23ab	12.73 ± 0.22bc	12.92 ± 0.13a
Average	13.27 ± 0.19A***	13.12 ± 0.18A	12.56 ± 0.17B	

*Means within the first two rows followed by the same lower-case letter do not differ significantly at P = 0.05 (Tukey HSD).

**Means in this column followed by the same lower-case letter do not differ significantly at P = 0.05 (Tukey HSD).

***Means within the same row followed by the same upper-case letter do not differ significantly at P = 0.05 (Tukey HSD).

When the optimal soil moisture control treatment was compared to the excessive soil moisture control treatment, the °Brix of the optimal soil moisture control treatment was significantly lower (Table 2.11). The optimal Trichoderma treatment had a significantly higher °Brix than the excessive soil moisture Seabrix-and-Trichoderma treatment. The optimal Seabrix-and-Trichoderma treatment had a significantly lower °Brix than the control treatment from the optimal soil moisture regime (Table 2.11).

2.4 Discussion

The results of trial 1 showed that overall mean leaf °Brix varied significantly over weeks and between growth stimulant treatments. Over weeks, a pronounced range difference of °Brix

6.67 was recorded between the lowest and highest °Brix recorded at weeks one and six, respectively. Differences in plant °Brix at different sampling times have previously been reported by (Aldrich *et al.*, 2010). Although there were significant differences between the °Brix readings of some of the different plant growth stimulant treatments, the range of the difference between the treatments in this study was small (range = 0.56), and the °Brix of the growth stimulant-treated plants were all lower than that of the untreated control plants. This shows that none of the plant growth stimulants, or combinations thereof, had any pronounced effect on leaf °Brix.

In trial 2, the overall mean leaf °Brix varied significantly over weeks. While soil moisture conditions did not affect °Brix, pronounced differences in overall mean leaf °Brix were recorded over weeks. This is illustrated by the large range (°Brix 4.80) between the lowest and highest °Brix values recorded at weeks one and two, respectively. Other studies reported that, unlike the effect of soil water conditions on the fruit °Brix of tomatoes, leaf °Brix was not influenced by the soil moisture regime due to the inhibition of translocation due to moisture stress (Harrill, 1998). Luengwilai *et al.* (2010) also reported increased °Brix in tomato fruit when nutrients are translocated from leaves to fruit. Whereas an excessive amount of soil moisture leads to lower fruit °Brix (Alordzinu et al., 2021), the differences in soil moisture did not affect leaf °Brix in this study.

Treatment with the different growth stimulants also did not result in pronounced differences in °Brix between treatments (range = 0.71), with that of the Trichoderma treatment being the same as the control treatment.

The large differences in °Brix recorded at the different weekly sampling intervals in this study, indicated that there were significant short-term changes in leaf °Brix as plant developed, and that the general tendency was that °Brix increased over time. This can be ascribed to the gradual accumulation of sugars in leaves due to daily photosynthesis. The sudden decrease in °Brix at week six was most likely due to plants entering the fruiting stage (Osorio *et al.*, 2014; Wang *et al.*, 2020). During the fruiting stage, a process called fruit sinking takes place during which the sugars are translocated from the resource-producing- and storage organs of the plant to the fruits (Osorio *et al.*, 2014).

The range of variation in °Brix over the 6/7-week periods that trials were conducted was °Brix 6.67 and °Brix 4.80, for trials 1 and 2, respectively. These ranges are large compared to the range of differences in °Brix that resulted from treatment with the different growth stimulants in both trials. The wide range of variation in °Brix over weeks most likely masked the possible effects that the application of growth stimulants may have on °Brix.

Significant interactions were observed between the week and soil moisture condition treatments, as well as between the water and growth stimulant treatments. The interaction between week and soil moisture shows that plants under excessive and optimal soil moisture conditions responded differently in terms of leaf °Brix over time and that in some weeks, plants grown under optimal conditions had higher °Brix, while in other weeks, the same plants had lower °Brix. This interaction cannot be explained and is probably due to experimental variation and the possible physiological effects of plants entering the fruiting stage (Osorio *et al.*, 2014; Wang *et al.*, 2020).

Similarly, the interaction effect between water and growth stimulant treatments showed that plants of the control treatment, which did not receive growth stimulants, responded differently regarding leaf °Brix to the differences in soil moisture conditions than the treated plants. This interaction cannot be explained, and it is unclear why plants not treated with growth stimulants had higher °Brix when grown under excessive soil moisture conditions.

Plant °Brix is influenced by many environmental factors such as seasonal conditions, irrigation regime, rainfall, agronomic practices, cultivation system (open-field or greenhouse production), and plant age and sampling date (Bihon *et al.*, 2022; Colella *et al.*, 2014; Green *et al.*, 2023; Helyes *et al.*, 2008). However, despite the influences that these factors may have, the leaf °Brix of tomato plants reported from these and other studies were, nearly without exception, lower than those reported in this study.

In a paper by Helyes *et al.* (2008) the authors indicated that "outstanding differences could be detected in °Brix among varieties", and that the °Brix values of the different tomato varieties ranged from 5.2 to 8.7 (mean = 6.7). Since the °Brix readings of plant leaves in this study were mostly above 10 (the lowest was 9.86 – Table 2.6), it can be accepted that the leaf °Brix in this study was high, irrespective of treatment.

For example, Barrett *et al.* (2007) reported °Brix readings of between 4.66 and 5.96 for field-grown tomatoes. Harrill (1998) reported °Brix of field grown tomatoes to range between 4 and 8 and indicated that °Brix of 8 was considered good. °Brix readings above 12 was considered excellent. Luengwilai *et al.* (2010) reported that the °Brix of the fruit of the Solara tomato cultivar (°Brix 9-12) was considered to be high, compared to the °Brix of 5 of the Moneymaker cultivar, the latter which was also used in this study. This could imply that the °Brix of the

Moneymaker variety used in this study was such a high nature that the different soil moisture treatments and plant growth stimulants did not result in large scale changes in °Brix, which was initially envisaged for this study.

°Brix of different types of tomatoes was reported to range from 4 to 9 (Helyes *et al.*, 2008). Searle *et al.* (2004) and Tandon *et al.* (2003) reported tomato fruit °Brix to range between 4.05 and 7.90 for different tomato selections and varieties. °Brix readings reported by Colella *et al.* (2014) for tomatoes were between 5.1 and 6.6 for plants that received optimal irrigation and those that were water stressed, respectively. Bihon *et al.* (2022) reported °Brix of tomato fruit to range between 3.0 and 4.0 under wet cool conditions and 4.4 and 7.0 under dry cool conditions. This range in °Brix reported by Colella *et al.* (2014) and others are well below that reported in this study. Crisanto-Juárez *et al.* (2010) also reported much lower °Brix of tomato fruit (4.5 – 9.3) than the leaf °Brix reported in this study. Aldrich *et al.* (2010) also reported low fruit °Brix of tomatoes, only between 3.52 and 4.66 (mean = 4.25), compared to the leaf °Brix recorded in this study. The latter °Brix readings were taken from ten organically grown tomato cultivars. However, in a study by Maciel *et al.* (2017), conducted with mini tomato genotypes that were selected for their high acyl sugar content, °Brix ranged between 11.01 and 25.0 for the 15 genotypes, with most of them having °Brix readings of below 17. In a study by Costa *et al.* (2018), the range of °Brix of cherry tomatoes subjected to different fertilizer treatments was also lower (9.13 – 9.43) and varied less than those recorded for leaf °Brix in this study.

2.5 Conclusions

The different soil moisture conditions used in this study did not influence leaf °Brix of tomato plant leaves. The high °Brix readings and large differences recorded at the different weekly sampling intervals, irrespective of growth stimulant treatment, showed significant short-term changes in leaf °Brix that occur as plants grow. The lack of pronounced effects of plant growth stimulants on °Brix could be ascribed to the high inherently high °Brix readings recorded in this study, which masked the small changes in °Brix that could be ascribed to plant growth stimulants. While °Brix readings in this study, in which the Moneymaker cultivar was used, ranged between 9.86 and 16.65 for plants that did not receive any growth stimulant treatments, another study reported this cultivar to have a °Brix of 5. The application of growth stimulants that only result in minor changes in °Brix, will most likely not have a noticeable effect on a leaf in °Brix under field conditions. Since the resistance of plants to *P. absoluta* is also influenced by other chemical and physical plant properties, future studies should investigate moth and larval responses of tomato plants treated with these plant growth stimulants.

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Appendix A

Table 1A: The trial one soil analysis as received from Eco Analytica (11 Hoffmanstraat, Potchefstroom)

Macro elements (mg/l)

Sample no.	Ca	Mg	K	Na	PO_4	SO_4	NO_3	NH_4	Cl	HCO_3
1	29.66	16.89	30.11	4.00	23.74	23.05	114.71	0.53	3.90	42.71
2	28.05	15.80	30.11	3.85	19.94	21.74	112.85	0.18	3.63	42.71
3	28.05	16.77	31.10	3.65	23.74	21.13	111.61	0.01	3.70	42.71

Micro elements and other data

Sample no.	Fe	Mn	Cu	Zn	B	pH	EG	P-BRAY 1
			mg/l				*(mS/cm)*	*dpm*
1	1.39	0.01	0.08	0.00	0.09	7.13	0.39	505.5
2	1.23	0.02	0.08	0.00	0.08	7.05	0.37	436.5
3	1.26	0.02	0.08	0.00	0.10	7.01	0.38	600

Nutrient Status

Sample no.	Ca	Mg	K	Na	P	pH(H_2O)	pH(KCl)	Walkley Black
		(mg/kg)						%C
1	2065.0	351.0	226.0	0.5	505.5	7.34	6.83	1.8
2	2085.5	353.5	245.0	0.5	436.5	7.38	6.96	1.7
3	2312.0	395.0	264.0	0.5	600.0	7.44	6.99	1.8

Exchangeable cations

Sample no.	Ca	Mg	K	Na	S-value	pH(H_2O)	pH(KCl)
		(cmol(+)/kg)					
1	10.30	2.89	0.58	0.00	13.77	7.34	6.83
2	10.41	2.91	0.63	0.00	13.95	7.38	6.96
3	11.54	3.25	0.68	0.00	15.47	7.44	6.99

0,1N HCl Extract

Sample no.	Mn 55	Fe 57	Cu 63	Zn 66
		mg/kg		
1	98.72	48.97	3.56	9.41
2	86.53	47.10	3.05	8.36
3	110.14	55.74	3.19	9.61

Appendix A

Table 1B: Soil analysis results for trial 2, received from Eco Analytica (11 Hoffmanstraat, Potchefstroom).

Macro elements (mg/l)

Sample no.	Ca	Mg	K	Na	PO_4	SO_4	NO_3	NH_4	Cl	HCO_3
1	54.51	22.36	96.57	9.89	17.02	166.62	114.22	1.72	51.31	18.30
2	48.90	23.09	105.17	9.39	17.44	166.69	110.79	1.51	47.81	21.36
3	43.28	15.80	76.24	5.58	16.72	115.08	82.65	1.06	41.13	15.25

Micro elements and other data

Sample no.	Fe	Mn	Cu	Zn	B	pH	EG	P-BRAY 1
			mg/l				*(mS/cm)*	*dpm*
1	0.12	0.02	0.08	0.06	0.13	6.86	0.76	1.03
2	0.19	0.02	0.10	0.06	0.14	6.90	0.75	0.86
3	0.12	0.01	0.07	0.04	0.10	6.92	0.57	10.98

Nutrient Status

Sample no.	Ca	Mg	K	Na	P	pH(H_2O)	pH(KCl)
		(mg/kg)					
1	980.65	171.92	398.36	6.30		6.64	6.24
2	978.00	170.63	385.93	4.43		6.68	6.29
3	885.36	149.39	329.88	2.86		6.73	6.35

Exchangeable cations

Sample no.	Ca	Mg	K	Na	S-value	pH(H_2O)	pH(KCl)
		(cmol(+)/kg)					
1	4.89	1.41	1.02	0.03	7.36	6.64	6.24
2	4.88	1.40	0.99	0.02	7.29	6.68	6.29
3	4.42	1.23	0.85	0.01	6.51	6.73	6.35

0,1N HCl Extract

Sample no.	Mn 55	Fe 57	Cu 63	Zn 66
		mg/kg		
1	40.90	35.84	6.22	19.87
2	45.53	37.78	7.99	25.41
3	44.53	33.69	7.35	25.51

Chapter 3

The influence of differences in °Brix of tomato leaves on larval and oviposition preference of *Phthorimaea absoluta* (Lepidoptera: Gelechiidae)

Abstract

Plant growth stimulants can be used to improve plant health since application of such products are reported to increase nutrient availability and various plant growth parameters. °Brix of tomato plants have been reported to influence the preference of moths and third-instar larvae of the tomato leaf miner, *Phthorimaea absoluta* (Lepidoptera: Gelechiidae), for plants. The aim of this study was to determine the effects of °Brix of tomato plant leaves, on moth and larval preferences of *P. absoluta.* To accomplish this, tomato plants were grown in pots with different soil moisture conditions, and different treated with growth stimulants, which purportedly affects plant °Brix. Oviposition preference of moths as well as larval preferences for plants of different treatments were evaluated in bioassays. °Brix readings of plant leaves were taken on the day of the experiments with moths. This study showed that the different soil moisture conditions and treatments did not have a pronounced effect on °Brix levels, and that there was no significant correlation between the number of eggs laid on plants in no-choice ($r = -0.41$; $p = 0.086$) and two-choice ($r = 0.039$; $p = 0.711$) tests. Third-instar larvae only showed a preference for leaves of plants in two of the 15 combinations that they were provided with, and in each of these two cases, plants were subjected to a Trichoderma treatment. Results from this study not only illustrated the difficulty of manipulating plant °Brix by means of different growth stimulants but also showed no relationship between leaf °Brix of tomato plants and the preference of *P. absoluta* moths.

Key words: pests, pest management, plant growth stimulants, Solanaceae

3.1 Introduction

Plant health is key to pest management as weaker plants attract more pests (Altieri & Nicholls, 2003; Bezerra *et al.*, 2021). Plant growth stimulants are commonly used in vegetable production to improve plant health since the application of such products are reported to result in increased availability of nutrients and increases in various plant growth parameters (Baker, 1988; Bansode *et al.*, 2020; Sangha *et al.*, 2014). The benefits from plant growth stimulants include increased fruit weight and quality, especially in terms of plant °Brix. The °Brix of plants refer to the overall amount of total soluble sugars (of sucrose, fructose, vitamins, minerals, amino acids, proteins, hormones and other solids) within the plant sap (Garcia & Barrett, 2006; Harrill, 1998) and is also commonly used to assess the quality of fruit as it is a quick, easy and cost-effective manner of evaluation (Van Waes *et al.*, 1998).

The use of organic biostimulants is also reported to improve plant growth and defence mechanisms (Yakhin et al., 2017), which subsequently results in reduced pest attack on these healthier plants (Altieri & Nicholls, 2003). Growth stimulants which improve plant health and which may have pesticidal characteristics have been developed following research on cyanobacteria, seaweeds, macroalgae and microalgae (Asimakis *et al.*, 2022). The application of seaweed extracts has been shown to increase the °Brix and weight of tomato fruits (Hussain *et al.*, 2021). The °Brix of plants has also been reported to influence the development and performance of phytophagous insect pests of crops. For example, Yang et al. (2024) reported that the development of chewing and sucking insects was promoted by an increase in the sugar and protein content of host plants. An increase in the sugar and protein content within tomato plants was reported to favour the development of the tomato leaf miner, *Phthorimaea absoluta* (Lepidoptera: Gelechiidae) (Yang *et al.*, 2024), which is the most important pest of tomatoes in South Africa. This was ascribed to increased activity of digestive enzymes, which utilize nutrients and promote the growth of *P. absoluta* larvae that feed on nutrient rich host plants (Yang *et al.*, 2024; Zhi, 2021). *Phthorimaea absoluta* moths predominantly oviposit on leaflets of the upper third of tomato plants (Coelho *et al.*, 1984) but can also occur in stems and flowers. Larvae feed predominantly on leaf parenchyma tissue, on tender portions of the stems (especially axillary buds), and in both developing and mature fruit (Vargas & Vargas, 1970).

Raspberries with a higher °Brix were found to be more susceptible to *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) because, as the fruit ripens, the amount of sugar in the fruit increases and the skin of the fruit softens. This increase in °Brix subsequently results in oviposition preference of *D. suzukii* towards raspberries (Lee *et al.*, 2016). Many studies do, however, show no correlation between °Brix and insect behaviour (Syrovy & Prasad, 2010; Weißinger *et al.*, 2019). For example, Wang *et al.* (2019) showed that differences in °Brix did not have any effect on the development of *D. suzukii* from egg to adult on cherry fruits.

Knowledge of the indirect effect that application of plant growth stimulants has on herbivorous insects through increased or decreased °Brix of plants may have potential in terms of tomato pest management. If °Brix levels can be manipulated by means of abiotic and biotic factors, such as watering regimes and application of plant growth stimulants, these products could be used as part of a pest management system. The breeding of tomato cultivars with high °Brix content in the leaves and high levels of resistance to pests will contribute meaningfully to pest management strategies, which currently primarily rely on the application of chemical pesticides (Dias *et al*., 2013).

The potential of the use of watering regimes and plant growth stimulants to manipulate °Brix of crops as a pest management strategy has been investigated in potato (Yang *et al.*, 2024), vegetables (Zhi, 2021), raspberries (Lee *et al.*, 2016) and grapes (Entling *et al.*, 2019). Excessive moisture application to tomato plants has been reported to result in a decrease in the °Brix of tomato fruit (Alordzinu *et al.*, 2021), while induced water stress, on the other hand, increased the °Brix of tomatoes, without affecting the pH or colour of the fruit (Alvino *et al.*, 1979).

However, the full potential of these bioactive substances can only be realised when their effects on insect pests are understood and implemented within integrated pest management systems (Asimakis *et al.*, 2022). No investigation has, however, been done on the effect of different growth stimulants on °Brix of tomato plants and the possible effects that it could have on *P. absoluta* moth and larval preferences. Studies of insect behaviour can be used to determine the effects that certain plant characteristics, for example varying °Brix levels, may have on the preference of moths and larvae for plants. For example, the preferences of *P. absoluta* moths and larvae were studied on various host plants, including tomato, from the egg stage until larvae pupated and moths emerged (Fang *et al.*, 2019; Idriss *et al.*, 2020; Scott, 2021; Yang *et al.*, 2024).

The aim of this study was to determine the effects of the application of growth stimulants and watering status, which purportedly affect the °Brix of tomato plant leaves, on moth and larval preferences of *P. absoluta.*

3.2 Materials and methods

3.2.1 Insect rearing

A *P. absoluta* rearing colony was established and maintained throughout the study period on tomato plants in insect-rearing cages. Tomato seeds of the Moneymaker variety were planted into Culterra seedling mix (Culterra (PTY) Ltd., Nietgedacht) in plastic seedling trays. Each seedling tray consisted of six cells with a volume of 0.125 dm^3 per cell. The seedlings and plants were cultivated in a greenhouse with ambient temperature and irrigated with an overhead sprayer system. Once the seedlings formed their first true leaves, two plugs from the seedling trays, each containing one seedling, were transplanted into 25 cm diameter pots filled with sandy-loam soil. The potted plants were kept pest-free by keeping them in insect-rearing cages. Once the plants reached the end of the vegetative stage, approximately 30 days after transplanting, they were used in the insect-rearing colony.

Phthorimaea absoluta larvae were collected from infested tomato plant material collected at the M-campus of the North-West University, Potchefstroom, South Africa. The plant material was then placed into a plastic mesh cage (1.5 m x 0.4 m x 0.4 m x 0.4 m). Once the moths emerged, pots containing tomato plants were placed inside the cage for oviposition by the moths. The pots were removed after two nights and replaced with two other pots containing uninfested plants. The number of nights that the pots were placed inside the cage was guided by the number of eggs oviposited on the plants. This was done to limit the number of eggs per plant and to prevent severe larval damage to leaves before larvae entered the pre-pupal stage. The plants that were exposed to moths for oviposition were then placed into another similar mesh cage to allow for the pest to complete its life cycle. The moths were removed from the cage using a handheld aspirator and released into other oviposition cages to continue the rearing process. Third-instar larvae were collected from this rearing colony for larval choice tests and pupae for oviposition preference tests.

3.2.2 Larval preference assays

3.2.2.1 Cultivation of plants for larval preference assays

The six treatments that plants were subjected to prior to the larval preference assays are described in table 3.1. In this latter experiment, three plant growth stimulant treatments, applied under two watering regimes, were used. The leaf material used in the larval choice tests was collected from plants maintained in Trial 2 (Chapter 2). The latter plants were part of the study that assessed the effect of various plant growth stimulants and soil moisture regimes on the °Brix of tomato leaves. Larval preference assays were conducted four weeks after the last °Brix readings were taken.

To determine the °Brix of leaves, two leaves per plant were removed and placed into a small Ziplock bag (10 cm x 10 cm). The bags were placed into a polystyrene cooler box with ice bricks. Bags were left open to limit condensation. °Brix readings were taken using the methods described in Chapter 2.

Table 3.1: Description of treatments, application rates of growth stimulants, water status and application intervals of treatments used in the larval choice assays.

Soil moisture condition	Treatment	Description	Growth stimulant application rate	Application intervals for growth stimulants
Optimal	Control (**OC**)	No growth stimulant was applied. Optimal amount of water was applied.	-	Every second day.
Optimal	Trichoderma (**OT**)	Real Trichoderma with an optimal amount of water applied to soil.	2 mℓ **T** / 10 ℓ water	**T**: Four days prior to transplanting as a seedling drench. Once every week after transplanting took place on the 6th of December 2023.
Optimal	Seabrix + Trichoderma (**OSBT**)	Seabrix and Real Trichoderma applied to soil.	20 mℓ **SB** + 2 mℓ **T** / 10 ℓ water	**SB**+**T**: Four days prior to transplanting as a seedling drench. Once every week after transplanting took place on the 6th of December 2023.
Excessive	Control (**EC**)	No growth stimulant application with an optimal amount of water.	-	Every second day.
Excessive	Trichoderma (**ET**)	Real Trichoderma with an optimal amount of water applied to soil.	2 mℓ **T** / 10 ℓ water.	**T**: Four days prior to transplanting as a seedling drench. Once every week after transplanting took place on the 6th of December 2023.
Excessive	Seabrix + Trichoderma (**ESBT**)	Seabrix and Real Trichoderma applied to soil.	20 mℓ **SB** + 2 mℓ **T** / 10 ℓ water	**SB**+**T**: Four days prior to transplanting as a seedling drench. Once every week after transplanting.

3.2.2.2 Larval no-choice tests

No-choice tests were conducted to determine the preference of *P. absoluta* larvae for tomato leaves from plants subject to the six different treatments, watering regimes and plant growth stimulants. Each treatment had 12 replicates, and each replicate consisted of a single petri dish. Seventy-two Petri dishes (90 mm in diameter) were sterilized, prepared with agar-agar (3 mm thick) and allowed to cool off before the assay commenced. Leaves were collected from each treated plant and placed into marked plastic bags in a Styrofoam box to remain cool prior to use in the assays.

A total of 144 third-instar larvae were then collected from plants in the rearing colony. A cork borer was used to punch 72 leaf discs (20 mm diameter), 12 from each treatment, from the leaves, and a single disc was placed in the centre of an agar-agar prepared petri dish, with the adaxial side facing upwards. Two third-instar larvae were placed on either side of the leaf disc, after which the lid was replaced to prevent larvae from escaping. Each petri dish was placed inside a rearing room at 26 °C ± 1 °C, 60 ± 5% relative humidity, and a 16L:8D photoperiod. The positions of the larvae in each petri dish were recorded after four and 24 hours, using a stereo microscope. The position of larvae within the petri dish was either recorded as on or underneath the leaf, indicating that it was preferred, or wandering around in the dish, indicating that it preferred not to feed on the leaf.

3.2.2.3 Larval two-choice tests

Two-choice tests were conducted to evaluate the preference of *P. absoluta* larvae for leaves of plants subjected to different treatments. The six treatments were evaluated in fifteen different combinations (Table 3.2). Each treatment combination had 12 replicates, and each replicate consisted of a single petri dish with two larvae and two leaf discs. A total of 180 Petri dishes (90 mm in diameter) were sterilized, prepared with agar-agar (3 mm thick) and maintained as described above. Leaves were collected from each treatment and placed in plastic bags, which were kept in a Styrofoam box to remain cool. Three hundred and sixty third-instar larvae were collected from the rearing colony. Sixty leaf discs (20 mm diameter) were punched from leaves of each treatment. A leaf disc from the respective treatment was placed onto the agar with the adaxial side facing upwards. The distance between the discs was 20 mm.

Two third-instar larvae were placed between the leaf discs, and the lid of the petri dish was replaced to prevent the larva from escaping. Each petri dish was placed inside a rearing room at 26°c ± 1°c and 60 ± 5% relative humidity and 16L:8D photoperiod. The positions of the larvae in the petri dish were recorded after four and 24 hours, using a stereo microscope.

3.2.3 Moth preference assays

3.2.3.1 Determining the sex of *Phthorimaea absoluta* pupae

The sex of *P. absoluta* is easily recognizable whilst in the pupal stage. Leaves containing fourth-instar larvae were collected from the rearing colony to retrieve pupae for use in the experiments in which moth oviposition preferences were determined. Leaves were placed into containers with airable lids and placed within an insect rearing room at 26 ± 1°C, 60 ± 5% relative humidity, and a 16L:8D photoperiod. Pupae were retrieved from the containers and the sex was determined by examining the ventral side of the pupa for the location of the genitalia. The morphological features used to distinguish between male and female pupae are illustrated in figure 3.1. Pupae were picked up and moved around with a fine-tip paint brush, of which the tip was moistened to pick the delicate pupa. Male and female pupae were placed separately in containers in the rearing room until eclosion.

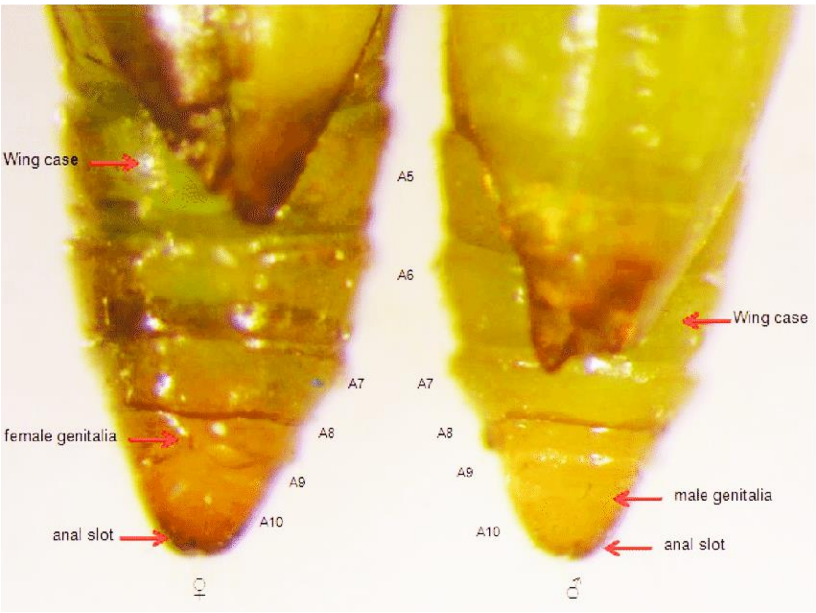

Figure 3.1: Location of male and female genitalia on pupae *Phthorimaea absoluta* (Genc, 2016).

3.2.3.2 Cultivation of tomato plants for moth oviposition preference assays

To produce tomato plants with varying °Brix levels, they were grown in pots in a greenhouse under different soil moisture conditions and treated with various plant growth stimulants. The treatments used in this trial are provided in table 3.1. Although these treatments were similar to those described in Chapter 2 (Trial 2), this experiment was conducted at a different time. Each week, for five consecutive weeks, 42 pots were filled with seedling mix and planted, making up seven pots (replicates) for each treatment. Staggering of plant dates was implemented to account for the possible varying availability of *P. absoluta* moths that were maintained in the insect rearing colony during the study period.

Three tomato seeds (Moneymaker cultivar) were planted in each of the 210 pots which were each filled with 800 g Culterra seedling mix (Culterra (PTY) Ltd., Nietgedacht). Pots were marked according to the treatment to be applied. Two of the pots from each moisture treatment regime were fitted with a Chameleon soil moisture probe from Virtual Irrigation Academy (VIA) to determine the amount of irrigation water needed to maintain optimum conditions, as described in Chapter 2 (section 2.2.3.4). Seedlings were thinned to one plant per pot, one week before the oviposition assays commenced.

3.2.3.3 No-choice oviposition preference assays

No-choice tests were conducted to determine the preference of *P. absoluta* moths for oviposition on tomato plants subjected to the six different treatments (Table 3.1). Each treatment had three replicates, and each replicate consisted of one pot with a single plant. These potted plants were put into a net cage (0.4 m x 0.4 m x 0.4 m) (Fig. 3.2) into which three male and three female moths were introduced. Moths were allowed to oviposit on plants for two nights, after which the number of eggs on each plant was counted and data entered into a spreadsheet with the correlating plant’s °Brix reading.

In this experiment, °Brix of plants were determined on the day that plants were used in the moth oviposition assays. Two leaves per plant were removed and placed into a small Ziplock bag (10 cm x 10 cm) on the morning of the implementation of each oviposition assay. The bags were placed into a polystyrene cooler box with ice bricks. Bags were left open to limit condensation. °Brix values were determined using the methods described in Chapter 2.

Figure 3.2: An example of a cage with one tomato plant used in the no-choice oviposition tests. 2: An example of a cage used in two-choice oviposition tests. Photos: Regardt Rademan.

3.2.3.4 Two-choice oviposition preference assays

Two-choice tests were conducted to determine the preferences of *P. absoluta* moths for tomato plants subjected to six different treatments and compared in 15 different combinations (Table 3.2). Each combination had three replicates, and each replicate consisted of two plants, each in its own pot (Fig. 3.2). Three male and three female moths were introduced into each cage. Moths were allowed to oviposit on plants for two nights, after which the number of eggs on each plant was counted and data entered into a spreadsheet with the correlating plant's °Brix reading.

To determine the leaf °Brix, two leaves per plant were removed and placed into a small Ziplock bag (10 cm x 10 cm), on the morning of the implementation of the oviposition preference assays. The bags were placed into a polystyrene cooler box with ice bricks, but left open to limit condensation. °Brix values were determined using the methods described in Chapter 2.

Table 3.2: The fifteen combinations in which the preference of *P. absoluta* larvae for leaves of tomato plants subjected to different plant growth and moisture treatments were evaluated. OC: Optimal soil moisture - control treatment, OT: Optimal soil moisture - Trichoderma treatment, OSBT: Optimal soil moisture - Seabrix and Trichoderma treatment, EC: Excessive soil moisture - control treatment, ET: Excessive soil moisture - Trichoderma treatment, ESBT: Excessive soil moisture - Seabrix and Trichoderma treatment.

Combination no.	Treatment 1	Treatment 2
1	Optimal soil moisture - Control	Optimal soil moisture - Trichoderma treatment
2	Optimal soil moisture - Control	Optimal soil moisture - Seabrix and Trichoderma treatment
3	Optimal soil moisture - Control	Excessive soil moisture - control treatment
4	Optimal soil moisture - Control	Excessive soil moisture - Trichoderma treatment
5	Optimal soil moisture - Control	Excessive soil moisture - Seabrix and Trichoderma treatment
6	Optimal soil moisture - Trichoderma treatment	Optimal soil moisture - Seabrix and Trichoderma treatment
7	Optimal soil moisture - Trichoderma treatment	Excessive soil moisture - control treatment
8	Optimal soil moisture - Trichoderma treatment	Excessive soil moisture - Trichoderma treatment
9	Optimal soil moisture - Trichoderma treatment	Excessive soil moisture - Seabrix and Trichoderma treatment
10	Optimal soil moisture - Seabrix and Trichoderma treatment	Excessive soil moisture - control treatment
11	Optimal soil moisture - Seabrix and Trichoderma treatment	Excessive soil moisture - Trichoderma treatment
12	Optimal soil moisture - Seabrix and Trichoderma treatment	Excessive soil moisture - Seabrix and Trichoderma treatment
13	Excessive soil moisture - control treatment	Excessive soil moisture - Trichoderma treatment
14	Excessive soil moisture - control treatment	Excessive soil moisture - Seabrix and Trichoderma treatment
15	Excessive soil moisture - Trichoderma treatment	Excessive soil moisture - Seabrix and Trichoderma treatment

3.2.4 Statistical analyses

The binomial distribution test was used to analyze the proportions of larvae that made a choice or not in no-choice assays. The order of preference of larvae that made a choice was determined by pooling the data and calculating the percentage preference of each treatment.

In two-choice assays, the binominal distribution test was used to analyze the proportions of larvae that made a choice between leaves of the different treatment combinations. The number of larvae which did not make a choice are indicated on the figures but were not included in the proportions. Bonferroni tests were implemented to adjust the data for means comparison of two-choice tests. Univariate Tests of Significance were used to determine the significance of the results obtained from the mean number of eggs per treatment as well as the mean °Brix per treatment.

Correlation analyses were conducted to determine if any relationship existed between the numbers of eggs per plant and the °Brix of plants of the different treatments, taken on the day of the experiment. Tukey HSD tests were used to indicate significance. All statistical analyses were performed with TIBCO® Statistica™ software version 14.0.1.25 (TIBCO, 2020).

3.3 Results

Larval preference assays

3.3.1 Larval no-choice assays

The results presented in figure 3.3 show that most larvae did not occur on the leaves provided in the petri dishes but were wandering around within the dishes. The number of larvae that were wandering around was significantly ($P<0.05$) higher than those that preferred to feed on leaves of all the treatments (Fig. 3.3).

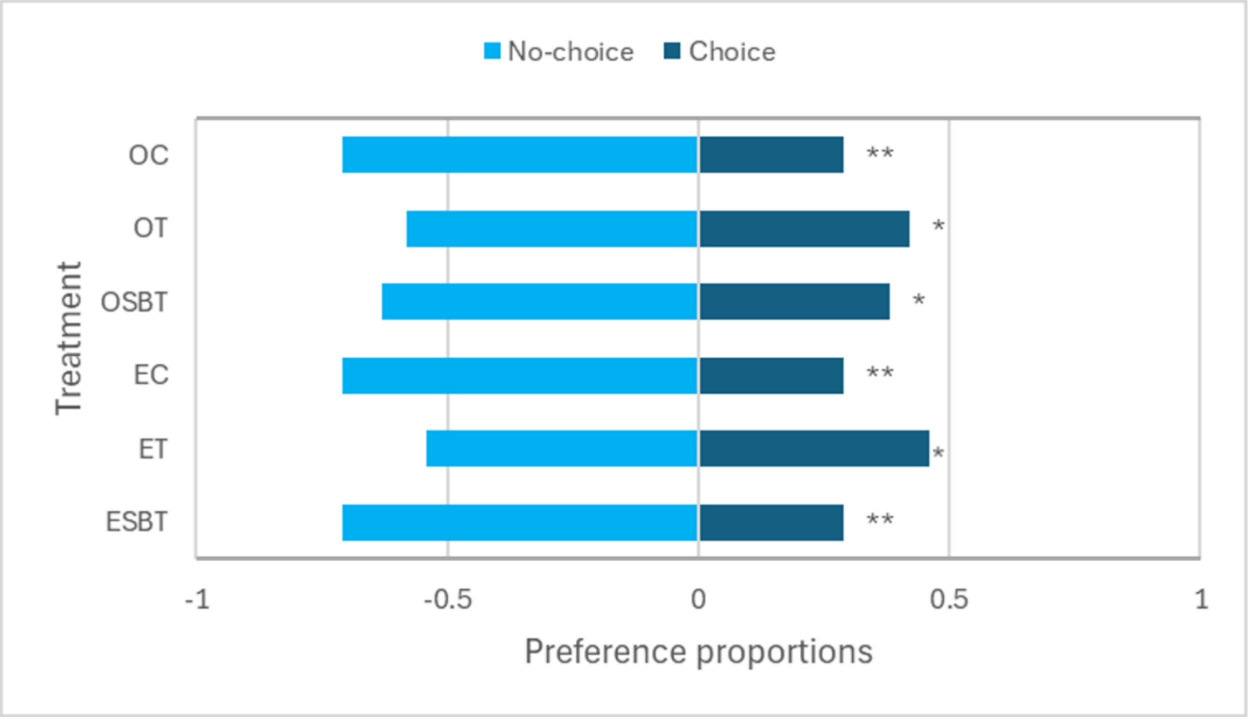

Figure 3.3: The preference responses of *Phthorimaea absoluta* larvae after 24 hours in no-choice tests with tomato leaves from plants receiving different plant growth stimulants and moisture treatments (**Table 3.1**). Significance indicated by * P<0.05; ** P<0.01. OC: Optimal soil moisture - control treatment, OT: Optimal soil moisture - Trichoderma treatment, OSBT: Optimal soil moisture - Seabrix and Trichoderma treatment, EC: Excessive soil moisture - control treatment, ET: Excessive soil moisture - Trichoderma treatment, ESBT: Excessive soil moisture - Seabrix and Trichoderma treatment.

The order of preferences of larvae that did make a choice to feed on the leaves that were provided ranked as follows: ET>OT>OSBT>(OC=EC=ESBT). Based on this comparative order of preference, larvae showed some positive response towards leaves of plants that received Trichoderma treatments, irrespective of the soil moisture condition.

3.3.2 Larval two-choice preference assays

Results from the two-choice tests indicate that larvae only exhibited significant preferences in two of the test-combinations (Fig. 3.4). When provided with a combination of leaves of plants grown under optimal soil moisture levels which received either no stimulants or Trichoderma treatment, larvae showed a significant preference ($P < 0.0033$) for leaves of the latter treatment. When larvae were provided with a choice between leaves of plants that were not treated and those that were treated with Trichoderma under excessive soil moisture conditions, they also exhibited significant (P < 0.0033) preference for the Trichoderma treatment (Fig. 3.4).

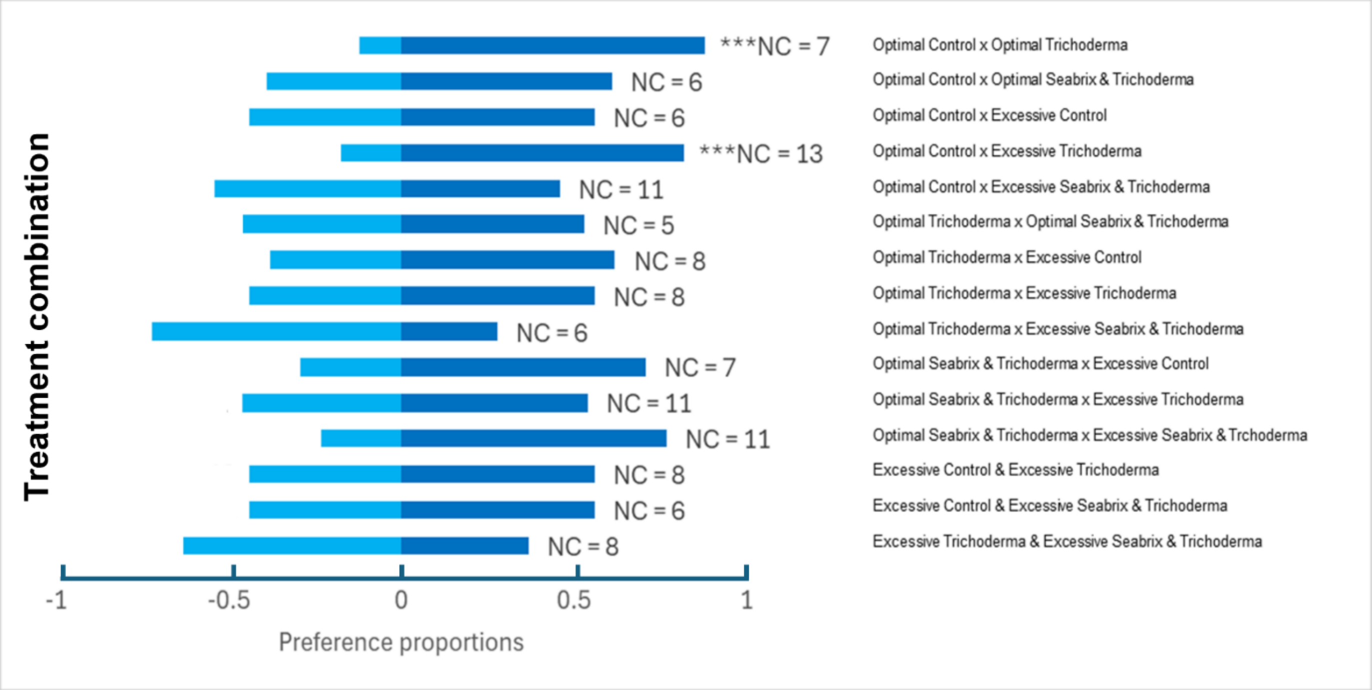

Figure 3.4: The response of *Phthorimaea absoluta* larvae in two-choice tests with leaves of tomato plants treated with different plant growth stimulants at different soil moisture levels (Table 3.2) after 24 hours. Significance indicated by ***P<0.0033 (Bonferroni adjustment). The number of larvae that did not exhibit any preference response (choice) is indicated by NC (no-choice) (out of 24 larvae per combination).

3.3.3 No-choice oviposition preference assay

There were no significant differences between the mean number of eggs laid by moths on tomato plants of the different treatments in the no-choice tests (Fig. 3.5). The mean number of eggs laid per plant ranged from 11.0 to 17.6 for the different treatments and varied largely between plants and treatments. The mean number of eggs per plant, laid by three moths over the 48-hour period, was 15.4, with the lowest and highest numbers being one and 41, respectively (data not shown).

The mean °Brix of plants ranged between 11.0 and 12.96 for the different plant growth stimulant treatments and did not differ significantly between treatments (Fig. 3.5). There was no significant correlation between leaf °Brix on the day of the experiment and the numbers of eggs per plant (r = -0.41; t-value = 1.828; df = 16; p = 0.086).

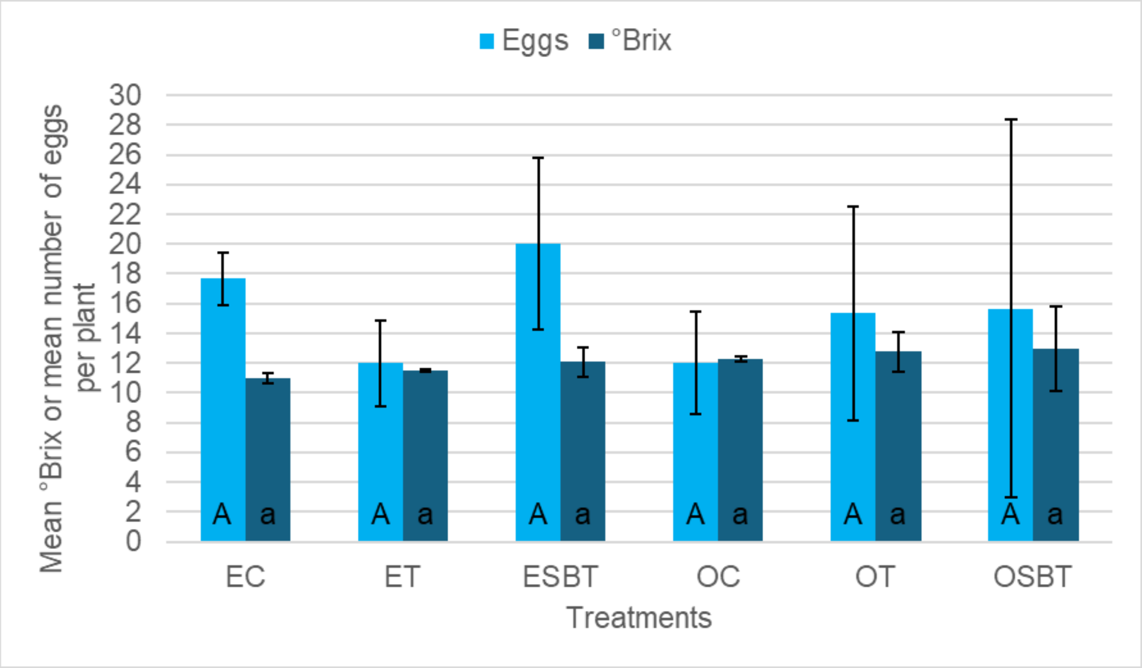

Figure 3.5: The average number of eggs laid on tomato plants and average °Brix of tomato leaves per treatment in no-choice tests. Treatments implemented (**Table 3.1.**) are as follows: **EC**: Excessive soil moisture - control treatment, **ET:** Excessive soil moisture - Trichoderma treatment, **ESBT**: Excessive soil moisture - Seabrix and Trichoderma treatment, **OC**: Optimal soil moisture - control treatment, **OT**: optimal moisture - Trichoderma treatment, **OSBT**: Optimal soil moisture - Seabrix and Trichoderma treatment. Bars indicate standard errors. All columns indicated with the same lower-case letter do not differ significantly at P = 0.5 (Tukey HSD). All columns indicated with the same uppercase letter do not differ significantly at P = 0.5 (Tukey HSD).

3.3.4 Two-choice oviposition preference assay

The number of eggs laid per plant varied largely between plants and treatments. There were no significant differences between the mean numbers of eggs laid by moths on tomato plants in the two-choice tests (Fig. 3.6). The mean number of eggs per plant, laid by three moths over the 48-hour period was 11.5, with the lowest and highest numbers being zero and 48 per plant, respectively (data not shown).

The °Brix of plants used in the two-choice assays ranged between 9.93 and 11.4 and did not differ significantly between treatments (Fig. 3.6).

No significant correlation existed between leaf °Brix on the day of the experiment and the numbers of eggs laid per plant in the two-choice bioassays (r = 0.039; t-value = 0.3705; df = 88; p = 0.711).

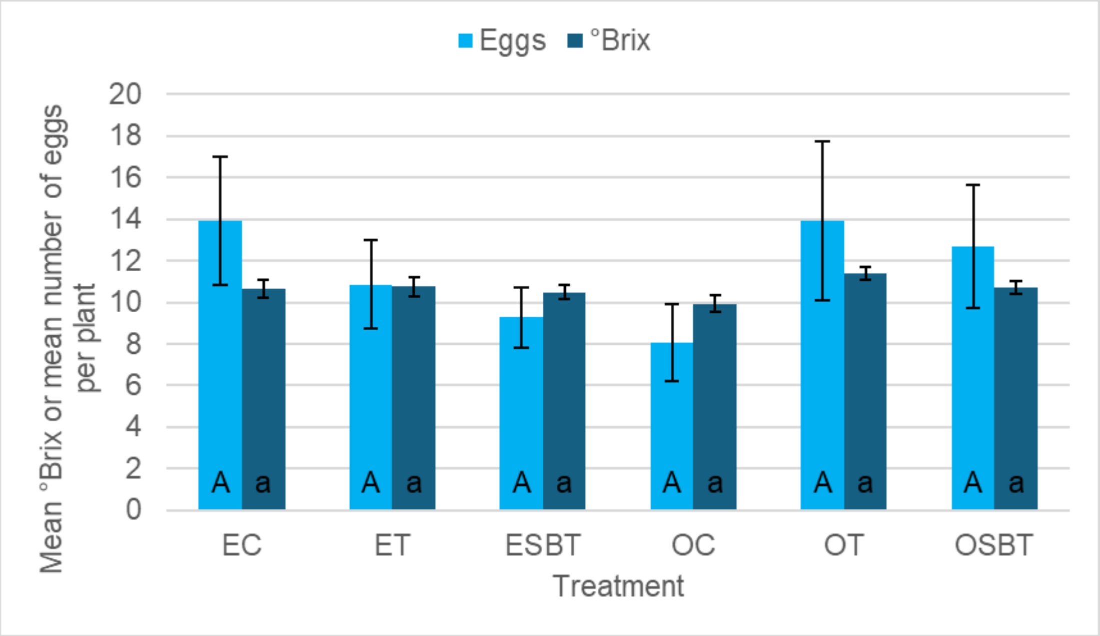

Figure 3.6: The average number of eggs laid on tomato plants and average °Brix of tomato plants per treatment in two-choice tests. All columns indicated with the same lower-case letter do not differ significantly at P = 0.5 (Tukey HSD). All columns indicated with the same uppercase letter do not differ significantly at P = 0.5 (Tukey HSD). Treatments (Table 3.1) were as follows: EC: Excessive soil moisture - control treatment, ET: Excessive soil moisture - Trichoderma treatment, ESBT: Excessive soil moisture - Seabrix and Trichoderma treatment, OC: Optimal soil moisture - control treatment, OT: optimal moisture - Trichoderma treatment, OSBT: Optimal soil moisture - Seabrix and Trichoderma treatment. Bars indicate standard errors.

3.4 Discussion

Significant relationships between °Brix levels and the response of plants towards certain pests and diseases, have been reported by several authors. The general tendency is that high sugar and protein content of host plants promotes the development of phytophagous insects, while lower sugar content leads to the reduction of fecundity and the increase of soluble sugar intake by increasing α-amylase activity. For example, high sugar and protein content within tomato plants was reported to favour the development of *P. absoluta* (Han *et al.*, 2022; Han *et al.*, 2019a; Yang *et al.*, 2024). Han *et al.* (2014) also showed that suboptimal nitrogen supply prolonged development time and caused high larval mortality of *P. absoluta*. Similarly, reduced intensity of attack by another lepidopteran pest, *Helicoverpa armigera* (Lepidoptera: Noctuidae) on tomato plants, has been ascribed to increased °Brix content following the application of silicic acid (Bansode *et al.*, 2020).

The effect of poor host status of tomato plants for *P. absoluta*, due to reduced fertilizer application, was reported to cause a reduction in pest densities and damage by *P. absoluta* (Konan *et al.*, 2024). Low fertilisation, especially with nitrogen) has a negative effect on the growth of many herbivorous insect species (Chen *et al.*, 2008). This is ascribed to the effects of changes in protein and/or specific amino acids, sugar deficiencies and plant defence activation (antixenosis), resulting in metabolic changes in insects during their critical growth period (Altesor *et al.*, 2014; Hosseini *et al.*, 2010; Le Bot *et al.*, 2009). Herbivorous insects would therefore prefer host plants with high fertilisation and therefore higher protein and sugar levels (Bentz *et al.*, 1995; Islam *et al.*, 2017). In terms of plant defence, nitrogen plays an important role in the emission and biosynthesis of secondary metabolites that affect insect behaviour (Altesor *et al.*, 2014; Bentz *et al.*, 1995; Han *et al.*, 2014).

In this study, *P. absoluta* larvae did not exhibit any pronounced preferences for leaves of any of the plant growth stimulants. Under no-choice conditions, most larvae did not exhibit any preference. However, the preferences of the larvae that did exhibit responses showed that most of them preferred the leaves of plants that were treated with Trichoderma, irrespective of whether these plants were grown under excessive or optimal soil moisture conditions. Results from the two-choice tests were largely inconclusive, with significant preferences only observed in two of the 15 test combinations. In the two-choice tests, *P. absoluta* larvae only showed significant preference for two of the Trichoderma treatments compared to leaves of the control treatments under optimal soil moisture conditions. The overall result of the larval choice tests therefore, largely indicated that there was no clear pattern of response to either the soil moisture regimes or any of the growth stimulant treatments.

The responses of *P. absoluta* moths to the plants grown under different soil moisture and plant growth stimulant treatments also did not yield clear patterns. This is similar to the findings of Mayse *et al.* (1997), who concluded that there was no correlation between western grape leafhopper, *Erasmoneura vulnerate* (Hemiptera: Cicadellidae) densities and °Brix levels of grape leaves, contradicting the theory that plants with increased °Brix suppress pest pressure. In sorghum, Triplett (2020) showed that leaf °Brix could not be used as an indicator of resistance or susceptibility to the sugar cane aphid, *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae).

This result differs from that of Dias *et al.* (2013) who studied the effect of acyl sugar content from different processing tomato genotypes on *P. absoluta*. Dias *et al.* (2013) found that there was a significant negative correlation between acyl sugar content of tomato leaves and oviposition preference of *P. absoluta* moths, and that larval performance was poor on leaves with high acyl sugar content, supporting the theory that increased sugar content of leaves leads to lower pest pressure. Resende *et al.* (2002) found that an increase of acyl sugar content of tomato leaves significantly decreased the mobility of spider mites, *Tetranychus evansi* (Trombidiformes: Tetranychidae), over a period of 60 minutes. Tomato cultivars, which were selected for their high acyl sugar content, could therefore be used in a pest management strategy for this pest due to the adverse effect they have on this mite species (Resende *et al.*, 2002). In both the studies of Dias *et al.* (2013) and Resende *et al.* (2002), specific plant genotypes with high and low acyl sugar contents were used, leading to the significant correlations they reported between °Brix and pest pressure. In the current study, no biologically significant increase °Brix could be induced by application of growth stimulants or different soil moisture conditions. The insignificant effect of °Brix on the larval- and oviposition preferences of *P. absoluta* could possibly be due to the very small variation in °Brix between treatments, or because the °Brix levels of plants were already of such a high nature that small variations between treatments did not influence *P. absoluta* responses.

Environmental factors such as seasonal conditions, irrigation regime, and plant age and sampling date have been reported to influence plant °Brix (Helyes et al., 2008; Colella et al., 2013; Bihon et al., 2022; Green et al., 2023). However, despite the influences that these factors may have, the leaf °Brix of tomato plants reported from these and other studies were, nearly without exception, lower than those reported in this study. °Brix of plants used in the two-choice oviposition assays in this study ranged from 9.93 to 11.4.

Helyes *et al.* (2008) indicated that differences in the range of °Brix 5.2 to 8.7 were typical of tomatoes. The °Brix readings of plant leaves in this study were mostly above 10 (the lowest was 9.86 – Table 2.6). It can therefore be accepted that the leaf °Brix in this study was high, irrespective of treatment. Furthermore, Luengwilai *et al.* (2010) reported that the fruit of the Moneymaker cultivar, which was also used in this study, had °Brix of 5. The latter study therefore supports the conclusion that the °Brix of the Moneymaker variety used in this study was most likely higher than what moths and larvae would encounter under field conditions. This could have affected the outcomes of this study and the little to no differences observed in oviposition and larval preferences for leaves of certain treatments.

Results from this study provided no support for either a positive or negative relationship between leaf Brix of tomato plants and the preference of *P. absoluta* larvae or moths for plants with higher or lower °Brix.

The relationships between plant health and resistance to pests are complex and involve more factors than °Brix levels alone. Many other factors also influence the preferences of moths and larvae for host plants. Agbessenou *et al*. (2020), showed that *T. asperellum*, which colonizes the whole tomato plant (roots, stem and leaves), is one of the most virulent endophytic fungal isolates that can be used in the management of *P. absoluta*. This fungus species has a negative effect on the oviposition, leaf mining, pupation and adult emergence of *P. absoluta*. However, the findings of this study contradict those of the latter authors.

In this study, third-instar larvae were used because of the extremely small size of first-instar larvae and difficulty using them in bioassays. Since first-instar larvae are the ones that make the first preference or non-preference for the host plant, a different outcome may occur if first-instar larvae are used in similar studies.

The discrepancies among studies reported in this chapter may be ascribed to the role that many other physical and chemical factors play as components of plant defences against herbivore attack. Plants are exposed to various biotic and abiotic stresses, which influence emissions of biogenic volatile organic compounds, which may have a larger effect on moth and larval choice than Brix levels alone (Dicke *et al*., 2014).

3.5 Conclusions

The relationships between plant health status, °Brix and pests are complex and involve more factors than °Brix levels alone. In this study, the small differences in leaf °Brix of tomato plants did not have a pronounced effect on *P. absoluta* larvae and moths. Furthermore, the °Brix levels determined in this study were high compared to other studies, which could possibly explain the lack of, or small differences in °Brix between treatments. A possible factor that could have influenced the outcomes of this study was the high leaf °Brix levels that were recorded. No information or guidelines exist on the general leaf °Brix of tomato plants or the relationship between leaf and fruit °Brix of tomato plants.

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Chapter 4

Conclusions and recommendations

The main factors influencing the successful cultivation of tomatoes are climatic factors, soil quality, soil fertility and pest pressure (Brévault *et al.*, 2014; Dam *et al.*, 2005; Gould, 2013). *Phthorimaea absoluta* (Lepidoptera: Gelechiidae) is the most serious pest of tomato production in the world and poses a major threat to tomato production (Biondi *et al.*, 2018; Brévault *et al.*, 2014; Soares & Campos, 2020). Management strategies for *P. absoluta* include mass trapping, mating disruption, changes in agronomic practices and the general management of plant health (Biondi *et al.*, 2018; Mansour *et al.*, 2018).

Weak plants attract more pests (Altieri & Nicholls, 2003; Bezerra *et al.*, 2021). Plant growth stimulants are commonly used in vegetable production to improve plant health since these products are reported to result in increased availability of nutrients and increases in various plant growth parameters (Baker, 1988; Bansode *et al.*, 2020; Sangha *et al.*, 2014). The benefits from plant growth stimulants include increased plant °Brix. °Brix is considered as a measure of plant health and is the indicator of total soluble solids in plants (Mann *et al.*, 2011).

The positive effects of biostimulants on plant growth and defence mechanisms against pests have been reported by several authors (Yakhin *et al*., 2017; Mirmajlessi & Radhakrishnan, 2020; Altieri & Nicholls, 2003). For example, the application of seaweed extracts and monosilicic acid increases the lycopene content and °Brix of tomato fruit (Bansode *et al*., 2020; Hussain *et al*., 2021). The application of *Trichoderma asperellum*, which is one of the effective potent endophytic fungi used for control of *P. absoluta*, on the other hand, may result in a decrease the °Brix of tomato fruits (Agbessenou *et al.*, 2020; Hasan *et al.*, 2021).

The potential of the use of watering regimes and plant growth stimulants to manipulate °Brix of crops as a pest management strategy have been investigated in potato (Yang *et al.*, 2024), vegetables (Zhi, 2021), raspberry (Lee *et al.*, 2016) and grapes (Entling *et al.*, 2019). Growing tomato plants under excessive soil moisture conditions has been reported to result in a decrease in fruit °Brix (Alordzinu *et al.*, 2021), while induced water stress, on the other hand, increased the °Brix of tomatoes (Alvino *et al.*, 1979).

In South Africa, various plant growth and biostimulants are used in tomato production to improve plant health. These products include Seabrix (RealIPM, Thika, Kenya) and RealTrichoderma (RealIPM), which are applied as soil treatments. These products are formulated from *Trichoderma asperellum* and are registered for the control of pathogenic fungi. Another such product is NewSil (RealIPM), a product applied as a foliar fertilizer treatment. These products have also been reported to benefit plant nutritional qualities and have adverse effects on pests that attack treated tomato plants (Bansode *et al.*, 2020).

However, the full potential of these bioactive substances can only be realised when their effects on insect pests are understood and implemented within integrated pest management systems (Asimakis *et al.*, 2022). If °Brix is a determining factor in terms of plant defence against herbivorous pests, the ability of growth stimulants to cause notable increases in °Brix will determine their value in terms of pest management.

No study has previously been done on the effect of different growth stimulants on °Brix of tomato plants and the possible effects that it could have on *P. absoluta* moth and larval preferences. The objective of the study was to determine whether plant Brix could be manipulated by the application of growth stimulants, and if these possible differences in °Brix influenced the oviposition and larval preferences of *P. absoluta*.

The results of the three experiments (Chapter 2, Trials 1 and 2; Chapter 3, moth oviposition experiment) during which °Brix readings were taken showed that neither soil water conditions nor growth stimulant treatments had pronounced effects on leaf °Brix. However, °Brix varied significantly over weeks and growth stimulants had only minor effects on °Brix. The highest °Brix was recorded after the SeaBrix treatment, and the lowest with the SeaBrix + Trichoderma + NewSil treatment. The differences in °Brix resulting from growth stimulant treatments in this study were, however, masked by the high variation recorded in °Brix at different sampling times. Results of this study therefore, show that treatment with plant growth stimulants did not result in pronounced differences in leaf °Brix.

Soil moisture condition and growth stimulant treatments were also shown to have limited effects on leaf °Brix levels, as well as moth and larval preferences for whole tomato plants and leaves, respectively. No significant correlation existed between the numbers of eggs laid on plants in no-choice ($r = -0.41$; $p = 0.086$) and two-choice ($r = 0.039$; $p = 0.711$) tests. Third-instar larvae only showed preference for leaves of plants in two of the 15 combinations that they were provided with, and in each of these two cases, plants were subjected to a Trichoderma treatment. Results from the study of moth and larval preferences further illustrate the difficulty to manipulate °Brix by means of the application of different growth stimulants, and showed no relationship between leaf °Brix of tomato plants and the preference of *P. absoluta* moths or larvae.

A possible factor that could have influenced the outcomes of this study was the high leaf °Brix levels that were recorded throughout the study. In the literature, °Brix of tomato fruit and not leaf °Brix is reported, and no information or guidelines exist on the relationship between leaf and fruit Brix of tomato plants. This lack of availability of data on the relationship between leaf and fruit °Brix of tomato plants hampered this study, since it seems that the high or low °Brix of tomato plants that were reported to influence the life history parameters of *P. absoluta* were based on fruit °Brix (Collella *et al*., 2014; Bihon *et al.,* 2022; Yang *et al.*, 2024). However, moths of this pest lay their eggs on plant leaves and larvae feed predominantly on leaf parenchyma tissue (Vargas & Vargas, 1970).

Furthermore, the fruit °Brix of tomato plants reported in other studies were much lower than the leaf °Brix reported in this study. The °Brix of the Moneymaker cultivar differed from what was recorded for leaf °Brix (> 9.96) in this study, compared to tomato fruit °Brix (5) reported by Luengwilai *et al.* (2010). This could imply that the leaf °Brix of the Moneymaker was of such a high nature in this study that the different soil moisture treatments and plant growth stimulants did not result in pronounced changes in °Brix.

Results from this study provided no support of either a positive or negative relationship between leaf °Brix of tomato plants and the preference of *P. absoluta* larvae or moths for plants with higher or lower °Brix. Based on the results of this study, in which potted tomato plants were used under the different soil moisture and growth stimulant treatments, °Brix levels alone cannot be used as an indicator of host plant suitability for this pest. It was also shown that leaf °Brix could not be manipulated by the application of growth stimulants under the experimental conditions used in this study.

A large body of research has been generated on the effects of other important factors that influence herbivore selection of host plants. Selection of plants for oviposition is strongly influenced by plant kairomones (Ruther *et al*., 2002; Carrasco *et al*., 2015) and it is generally accepted that increasing plant tissue nitrogen levels results in increased pest pressure (Cisneros & Godfrey, 2001; Jahn *et al.*, 2005; Jauset *et al.*, 2000). Furthermore, climatic conditions and nutrient availability can be important factors in determining the intensity and variability in the release of plant volatiles (Gouinguené & Turlings 2002).

Plant °Brix is therefore not the ideal parameter to utilize for the prevention of increased pest pressure.

Future recommendations

Based on results from this study, it is recommended that further research be conducted to elucidate the relationships between °Brix levels and the response of *P. absoluta*.

Research into the following aspects is recommended:

- Establish the relationship between leaf and fruit °Brix levels of tomato plants.
- Evaluate the effect of poor soils on the response of tomato plants to the application of plant growth stimulants.
- Evaluate the effects of acyl sugars or similar compounds, rather than comprehensive °Brix.
- Evaluate the effect of the application of plant growth stimulants under field conditions.

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DECLARATIONS

DECLARATION BY THE CANDIDATE

I, Regardt Rademan, declare that the work presented in this MSc thesis is my own work, that it has not been 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.

Signature: Date: 24/03/2025

DECLARATION AND APPROVAL BY SUPERVISORS

We declare that the work presented in this thesis was carried out by the candidate under our supervision and we approve this submission.

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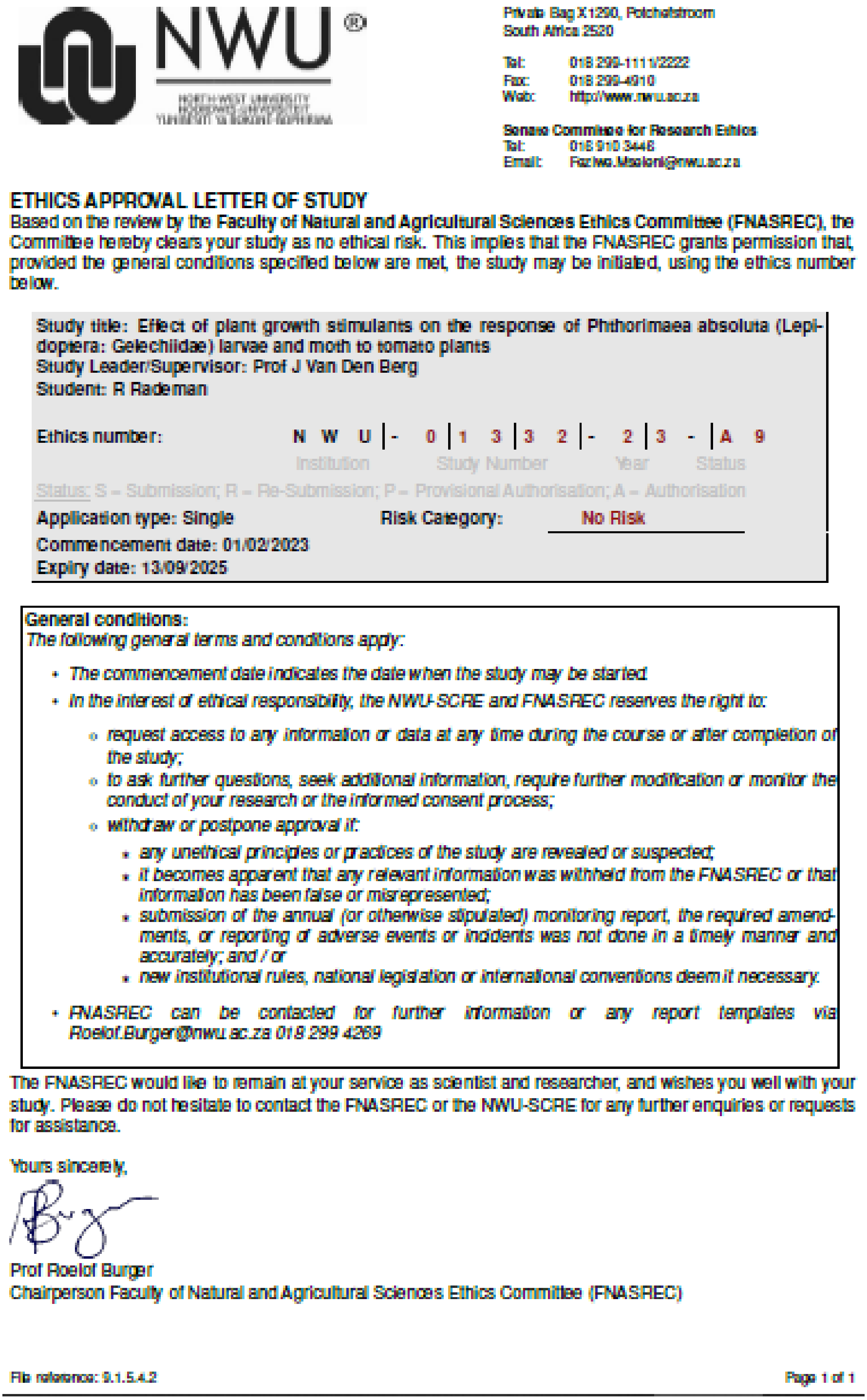
Signature: Date: 24/03/2025

Appendix B: Ethical clearance



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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: Effect of plant growth stimulants on the response of Phthorimaea absoluta (Lepidoptera: Gelechiidae) larvae and moth to tomato plants
Study Leader/Supervisor: Prof J Van Den Berg
Student: R Rademan

Ethics number:	NWU-	01332-	23-	A9
	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: 01/02/2023
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)

File reference: 9.1.5.4.2