Research Article

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Residual activity of spinosad applied as a soil drench to tomato seedlings for control of *Tuta absoluta*

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Abstract

BACKGROUND: *Tuta absoluta* (Lepidoptera: Gelechiidae) is difficult to control by means of foliar insecticides, partly because of the endophytic feeding behavior of its larvae. The biopesticide spinosad is applied as a foliar spray for control of *T. absoluta* and has systemic properties when applied as a soil drench to the growing medium of tomato plants. The aims of this study were to determine the: (i) instar-dependent tolerance of larvae to spinosad; (ii) efficacy of spinosad drench application for the control of larvae; (iii) residual period of systemic activity of spinosad in leaves and fruit after drenching; and (iv) effect of spinosad drenching on tomato plant growth parameters.

RESULTS: The estimated LC_{50} value (Lethal Concentration at which 50% of the larvae died) differed between instars. The LC_{50} for second-instar larvae (0.41 ppm) to spinosad was significantly lower than that for third- (0.64 ppm) and fourth-instar (0.63 ppm) larvae. The LC_{80} value (Concentration at which 80% of the larvae died) for fourth-instar larvae (2.48 ppm) was 2.6- and 1.7-fold higher than that for the second- and third-instar larvae, respectively. The spinosad concentration recorded in leaves at 25 days after treatment (DAT; 0.26 μg g^{-1}) was significantly lower than that in leaves sampled at 3, 10 and 15 DAT. High larval mortalities were, however, recorded for the duration of the experiment, which lasted 25 days (equivalent to one *T. absoluta* generation).

CONCLUSION: Systemic spinosad effectively controlled *T. absoluta* larvae over a prolonged period. However, drenching this insecticide violates the recommendation of the Insecticide Resistance Action Committee to avoid treating consecutive insect generations with the same mode of action and can therefore result in the evolution of insecticide resistance.

Keywords: insecticide drench; insect resistance; residual period; systemic; toxicity

1 INTRODUCTION

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) is one of the main threats to global tomato production.[1–8] It is established in both northern and sub-Saharan Africa.[9–11] Application of synthetic chemical insecticides is the most commonly used practice to control this pest. However, selection pressure caused by an overreliance on insecticides has led to resistance evolution and the subsequent reduced efficacy of insecticides.[12–17]

Biopesticides are increasingly used in the management of food crop pests. Among the biopesticides, spinosad is considered as one of the most promising options, especially with its acceptance in organic farming.[18–20] Spinosad is a mixture of spinosyns A and D and is a product of the fermentation of a naturally occurring actinomycete, *Saccharopolyspora spinosa* (Pseudonocardiales: Pseudonocardiaceae).[21] With two modes of action (MoA), namely depolarizing nicotinic acetylcholine and γ-aminobutyric acid receptor neurons, the target spectrum of spinosad includes species from several insect orders (Coleoptera, Diptera, Hymenoptera, Isoptera, Lepidoptera and Thysanoptera).[22,23] The tomato pinworm, *T. absoluta*, is among the lepidopterans controlled by spinosad.

Field-evolved resistance to spinosad has been reported in *T. absoluta* populations from Brazil, Chile and the United Kingdom.[24–26] Spinosad acts primarily on nicotinic acetylcholine receptors, with Grant et al.[26] and Silva et al.[27] reporting a mutation in the alpha 6 subunit, leading to high levels of resistance. Resistance management strategies for this pest largely involve rotation of pesticides with different MoA. Larval instar-dependent insecticide tolerance has been reported for several lepidopteran species, with a decrease in susceptibility to insecticides in later larval instars.[28–30] The implication is that later-instar larvae, being more tolerant, could survive dosages that would kill earlier-instar larvae.

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To date, instar-dependent tolerance of *T. absoluta* to spinosad has not been investigated.

Systemic insecticides currently registered for control of *T. absoluta* in South Africa hold registration for foliar application only. However, off-label application through drip irrigation systems is currently a common practice and a major concern in South Africa (H. du Plessis, pers. obs.). The systemic properties of spinosad applied as a drench to the roots of tomato plants were demonstrated by Van Leeuwen et al.[31] against *Tetranychus urticae* (Koch) (Acari: Tetranychidae) and both *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) and *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae).[32]

The concentration of product within the leaves of drenched tomato plants and the period of persistence inside plant tissue are, however, unknown and could pose a serious threat in terms of resistance evolution. The objectives of this study were therefore to investigate the larval instar-dependent tolerance of *T. absoluta* to spinosad and to determine the efficacy of control of drench applications at different concentrations. The concentration of spinosad inside leaves and fruit, as well as the persistence of systemic toxicity in the leaves and the effects on tomato plant growth parameters were also determined.

2 MATERIALS AND METHODS

2.1 Insect rearing

A tomato pinworm rearing colony was established from a population collected from an infested tomato field at Marble Hall in the Mpumalanga province, South Africa (25°01′47.1″S 29°13′38.1″E). Infested leaves were placed on a plastic mesh (hole diameter = 2 cm) suspended from the roof of an insect-rearing cage. Non-infested potted tomato plants (cv. Moneymaker) were placed underneath the suspended leaves to enable larvae to migrate to the tomato plants and complete their life cycle. Adults were collected daily from these rearing cages and transferred to oviposition cages with potted tomato plants for oviposition. Cotton swabs soaked in 10% sugar solution were provided in Petri dishes at the bottom of each cage, as an energy source. Plants were replaced every second day to ensure that the laid eggs would hatch at approximately the same time. Rearing and oviposition cages were maintained at 26 ± 1°C, 65% relative humidity (RH) and a 14:10 h light/dark photoperiod. Larvae of the F1 and F2 generations of this population were used in experiments.

2.2 Insecticide

A commercial formulation of spinosad, Tracer™ 480 SC (Dow AgroSciences), registered as a foliar spray for control of *T. absoluta* in South Africa, was used. The recommended label dosage rate (mid-range) for spinosad was 30 ml per 100 L (144 ppm).

2.3 Leaf-dip bioassay

Bioassays were conducted according to the leaf-dip bioassay method (Insecticide Resistance Action Committee [IRAC], susceptibility test method No. 022), with second-, third- and fourth-instar larvae. Morphological differences, as specified by Sannino and Espinosa,[33] were used to distinguish between the four larval instars of the pest. Preliminary range-finding experiments were conducted using serial dilutions of spinosad prepared from a stock solution. Based on the results of the preliminary experiment, a range of concentrations for each instar was selected to provide at least six points between LC_{20} (Lethal Concentration at which 20% of larvae died) and LC_{80} of the dose–response curve. In total, ten concentrations per larval instar were prepared for the susceptibility bioassays, including the control and a concentration expected to result in 100% larval mortality. All dilutions were prepared with deionized water.

Tomato leaflets were immersed for 5 s in different insecticide dilutions containing 0.2% Triton-X-100 and allowed to air dry for 2 h on a plastic mesh with the abaxial leaf surface facing upwards. Each leaflet was transferred into a well of a 32-well bioassay tray (Frontier Scientific) with the adaxial leaf surface facing upward. Before leaflet transfer, the bottom of each bioassay well was lined with a thin layer of agar (28 g L^{-1}) to maintain leaflet turgidity throughout the bioassay period. Tomato plants were exposed daily for 1 h to *T. absoluta* moths for oviposition. The number of days taken to reach the desired instar were first determined. Larvae of each of the second, third or fourth instar were carefully removed from the galleries of infested tomato leaves in the rearing cages on the day that they molted into the required instar. A single larva was transferred to each well. All wells were sealed with transparent ventilated adhesive lids (Frontier Scientific). Thirty-two larvae were tested at each concentration and the experiment was repeated three times. Bioassay trays were maintained in incubators under a controlled environment of 26 ± 2° C, 60–65% RH and a 16:8 h light/dark photoperiod. Larval mortality was recorded after 72 h. Larvae were considered dead if they were unable to make coordinated movements in response to an external stimulus (gentle probing with a fine paintbrush).

2.4 Systemic toxicity of spinosad as drench treatments

2.4.1 Application at different concentrations

The systemic toxicity of spinosad applied to tomato as a drench to control *T. absoluta* larvae was evaluated in a commercial glasshouse. Tomato seedlings (cv. Moneymaker) were grown from seed and transplanted to 2-L pots (diameter: 16 cm) filled with a 4:1 soil/compost mixture to 2 cm from the brim. Commercially available compost was used (Culterra). The soil contained 1.3% clay, 78% sand, 1.2% silt, and the pH was 6.49 at the time of planting. Soil pH was not determined after watering and application of nutrient media. The experiment consisted of 11 treatments, which were serial dilutions ranging between 1.2 and 48.0 ppm prepared from a stock solution. These concentrations were 1.2, 1.7, 2.5, 3.6, 5.2, 7.5, 10.9, 15.8, 22.9, 33.2 and 48.0 ppm, based on preliminary experiments to determine a high concentration that will provide 100% mortality of second-instar larvae. Deionized water was applied as the control treatment. Each treatment was repeated nine times and each potted plant served as one replicate.

The experiment commenced 2 weeks after the seedlings were transplanted into pots. Exactly 200 ml of each spinosad dilution was applied evenly to the soil surface in the pots to obtain a homogeneous distribution of the compound in the substrate. This translated into the application of 0.24–9.6 mg of active ingredient (a.i.) per plant. After the initial spinosad application, the plants were watered (200 ml) every fourth day.

Second-instar larvae were used, similar to the leaf-dipping method as specified by the IRAC for susceptibility testing of *T. absoluta*. Twelve larvae were inoculated onto the leaves of each plant, 10 days after treatment (DAT). These larvae were confined individually with clip-on cages, to a leaf area of approximately 3.14 cm^2 per larva. Each clip-on cage consisted of a 5 mm high plastic tube with a diameter of 15 mm. The top of the tube was covered with a fine mesh (50 µm) to allow for air movement and to contain the larvae. A strip of felt was glued to the bottom brim

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of the tube to ensure that it sealed the tube onto the leaf, without causing damage to the leaf surface. The clip-on cages were kept in place on the leaves with hairpins. Larval mortality was recorded 5 days after larval inoculation.

Temperature was recorded at 30-min intervals by means of IButton® wireless data loggers (ColdChain ThermoDynamics) for the duration of the experiment. The mean minimum and maximum temperatures during the experiment were 23.11and 31.61°C respectively, with an overall mean (± SD) of 27.24 (± 1.42)°C.

2.4.2 *Persistence and efficacy of the active ingredient*

The persistence and efficacy of the drench treatments were determined by assessing larval mortality after feeding on leaf tissue from treated plants at different time intervals after drenching.

The experimental design was similar to that described above. Forty potted tomato seedlings were drenched with spinosad at a concentration of 48 ppm (10 ml per 100 L), the highest concentration applied in the soil drench experiment (Section 2.4.1), with 96.5% mortality recorded 25 days after application. The application volume was 200 ml, which translated into an application of 9.6 mg a.i. per plant. Plants (40) of the control treatment were drenched with 200 ml of deionized water. Second-instar *T. absoluta* larvae were inoculated onto the treated and untreated plants at different time intervals after drenching. These time intervals were 3, 10, 15, 20 and 25 DAT. Each treatment consisted of 12 larvae per plant and was repeated eight times. Larval mortality was recorded 5 days after inoculation of each treatment.

The concentration of spinosad inside the leaves over time was determined by means of liquid chromatography–tandem mass spectrometry (LC–MS/MS). Leaf samples (± 1 g) were collected from ten treated and untreated plants at each larval inoculation interval. Plants were used only once for leaf sampling. Leaves were randomly collected per plant and were representative of both old and new growth. Tomato fruit were sampled at the breaker fruit stage from both treated and untreated plants, approximately 80 DAT. Leaf and fruit samples were frozen and stored at −80°C until extraction. All plants were watered (200 ml) every fourth day, and fertilized fortnightly with 100 ml of nutrient solution at a rate of 1 g L^{-1} (Nutrifeed; Stark Ayres (Pty) Ltd).

2.5 Quantification of spinosad concentrations

2.5.1 *Reagents and chemicals*

Spectrometry-grade acetonitrile and water from Honeywell were used. The formic acid and ammonium formate that were used were LC–MS eluent additive grade (Sigma-Aldrich). Spinosad analytical standards were obtained from Sigma-Aldrich (33706-50MG) and Agilent Technologies (PST-3735A1000) as 100 µg ml^{-1} solutions in acetonitrile. They were mixed and diluted in acetonitrile to the appropriate concentrations and stored in a fridge at 4°C.

2.5.2 *Spinosad extraction from plant tissue*

A QuEChERS protocol was followed using Agilent Bond Elut QuEChERS kits (p/n 5982-5550) to provide fast and easy extraction. Dispersive solid phase extraction (SPE) clean-up was performed using Agilent Bond Elut QuEChERS dispersive kits, 15-ml dispersive SPE tubes (p/n 5982–5056). Sample filtration was performed by means of a Captiva Premium Syringe Filter, with a nylon membrane, 15 mm, 0.2 µm (p/n 5190-5088).[34]

2.5.3 *Analytical equipment*

Separation was carried out using an Agilent 1290 Infinity binary pump, Agilent 1290 Infinity High Performance autosampler and an Agilent 1290 Infinity thermostat column compartment. The liquid chromatograph was coupled to an Agilent Technologies 6470 triple quadrupole LC–MS/MS with an Agilent Technologies Jet Stream electrospray ionization source. Agilent Technologies MassHunter Workstation Software – Qualitative Analysis (version B.03.01) was used for data acquisition and data analysis. Analysis was carried out in positive ionization in dynamic MRM mode using two transitions per compound.

2.6 Effect of spinosad drench application on plant growth parameters

The possible effects of a spinosad drench treatment on plant growth were investigated in a glasshouse experiment. The experiment consisted of 20 spinosad-treated plants and 20 untreated plants in a randomized complete block design. Tomato seedlings were planted and maintained in conditions similar to those mentioned above. Potted seedlings were drenched with spinosad at a rate 48 ppm, with 200 ml of solution added per plant. Deionized water was used for control plants.

Plant height, stem diameter and the fresh mass of whole plants, aerial parts and roots were recorded 30 DAT. Plant height was recorded as the distance from soil level to the shoot tip along the stem, using a non-stretchable rope to accommodate stem curvature. Stem diameter was recorded for all plants at a height of 2 cm above soil level. Each plant was carefully removed from the substrate, the root system rinsed to remove all debris, and the plant allowed to air dry for 5 min before recording the mass of the whole plant. The root system was cut from the stem and the mass of the aerial and root systems determined separately.

2.7 Data analyses

Abbott's formula was used to correct for larval mortality in the control treatment[35] before estimation of the instar-dependent tolerance of *T. absoluta* larvae to spinosad, and evaluation of the systemic toxicity of spinosad. Corrected mortality data for leaf-dip bioassays and systemic toxicity were subjected to probit analysis and the relative potency ratio among responses was calculated using PoloSuite® software (LeOra Software LLC, version 1.8). Responses were considered significantly different when the 95% confidence interval of the relative potency ratio did not include the value 1.[36]

Data on the residual persistence of spinosad at different concentrations, as well as mortality data over time, were tested for normality (Shapiro–Wilk's tests) and homogeneity of variance (Levene's test). Data on the concentration of residual spinosad in the leaves met these assumptions and were subsequently subjected to one-way analyses of variance. Treatment means were separated using the Unequal N Tukey's HSD test at $p = 0.05$. Corrected percentage mortality data were neither normally distributed nor homoscedastic, and were therefore analyzed by means of the non-parametric Kruskal–Wallis test, followed by Dunn's multiple comparison post hoc test. Student's *t*-tests were used to compare the means of the respective plant growth parameters. Analyses were done using TIBCO Statistica version 13.3.[37]

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3 RESULTS

3.1 Leaf-dip bioassays

Responses of *T. absoluta* larvae from the respective instars fitted the log (dose)/probit (mortality) model at $p < 0.05$ (Table 1). The slope coefficients were similar for both second- (2.28) and third-instar (2.23) larvae, suggesting a homogenous response to spinosad for these instars. The slope coefficient for fourth-instar larvae (1.42) was considerably lower.

The estimated LC_{50} values of the respective larval instars differed significantly according to the 95% confidence intervals of the relative potency ratio ($p < 0.05$) (Table 1). The estimated LC_{50} value for second-instar larvae (0.41 ppm) was significantly lower than that for both third- (0.64 ppm) and fourth-instar (0.63 ppm) larvae, with no differences in LC_{50} values for larvae from the latter two instars. However, the LC_{80} values of second-, third- and fourth-instar larvae differed significantly between instars (Table 1). The LC_{80} value for fourth-instar (2.48 ppm) larvae was 2.6- and 1.7-fold higher than that for the second- and third-instar larvae, respectively.

3.2 Systemic toxicity of spinosad at different concentrations

The mortality of *T. absoluta* larvae that fed on spinosad drenched plants fitted the log (dose)/probit (mortality) model at $p < 0.05$, with a slope coefficient of 2.54 (Table 2). The application volume was 200 ml per plant. Therefore, the estimated LC_{50} and LC_{80} values, converted to dosage a.i. per plant, were 9.35 ppm (1.87 mg a.i. per plant) and 20.10 ppm (4.02 mg a.i. per plant) respectively (Table 1), with 100% larval mortality at 9.60 mg a.i. per plant.

3.3 Residual persistence toxicity of spinosad applied systemically

Good linearity was recorded for spinosyn A (0.01–5.0 μg g^{-1}) and spinosyn D (0.01–5.0 0 μg g^{-1}) in pure solvent with high

TABLE 1. Log-dose probit mortality data for different *Tuta absoluta* larval instars treated with spinosad

Instar	*n** (df)	LC_{50} (ppm)	FL (95%)	LC_{80} (ppm)	FL (95%)	Slope	SE	$X^{2\dagger}$
Second	672 (5)	0.41	0.354–0.463 A	0.97	0.842–1.163 A	2.23	1.78	3.51
Third	794 (6)	0.64	0.570–0.705 B	1.49	1.335–1.701 B	2.28	1.17	3.54
Fourth	818 (6)	0.63	0.467–0.801 B	2.48	1.956–3.406 C	1.42	0.12	7.25

Abbreviations: FL = Fudicial Limits; LC_{50} = Lethal Concentration; Concentration at which 50% of larvae died; LC_{80} = Concentration at which 80% of larvae died.
*n, number of larvae tested.
† Chi-square test for linearity of the dose–mortality response.

TABLE 2. Log-dose probit mortality for *Tuta absoluta* that fed on tomato plants with systemic spinosad toxicity

Treatment	*n**	LC_{50} (ppm)	FL (95%)	LC_{80} (ppm)	FL (95%)	Slope	SE	$X^{2\dagger}$
Spinosad	640	9.35	8–11.05	20.1	16.45–25.9	2.54	0.17	7.30

Abbreviations: FL = Fudicial Limits; LC_{50} = Lethal Concentration; Concentration at which 50% of the larvae died; LC_{80} = Concentration at which 80% of larvae died.
*n, number of larvae tested.
† Chi-square test for linearity of the dose–mortality response.

TABLE 3. Mean spinosad concentrations (μg g^{-1} ± SE) in tomato leaves over time after drenching with 48 ppm spinosad per seedling and the corrected percentage larval mortality (±SE) on treated plants

Days after treatment	Number of plants sampled*	Mean concentration spinosad (μg g^{-1})†	Number of larvae inoculated‡	Mean percentage mortality§
3	9	0.50 ± 0.06 A	96	98.85 ± 1.15 A
10	9	0.46 ± 0.05 A	96	98.85 ± 1.15 A
15	10	0.46 ± 0.03 A	96	100 ± 0.00 A
20	10	0.37 ± 0.03 AB	96	100 ± 0.00 A
25	10	0.26 ± 0.02 B	96	96.55 ± 1.68 A
		$F(4, 42) = 6.64$; $p < 0.05$		$H(4) = 6.69$, $p = 0.15$

*n = Number of plants sampled.
† Mean residual spinosad concentration in the leaves at the respective time intervals. Values within the column followed by the same upper case letter are not significantly different at $p < 0.05$ (unequal HSD).
‡ n = Number of larvae inoculated.
§ Values within the column, followed by the same upper case letter are not significantly different at $p < 0.05$ (Kruskal–Wallis followed by Dunn's multiple comparison test).

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TABLE 4. Comparison of plant growth parameters of plants treated with spinosad

Treatment	Number of plants*	Plant height (cm ± SE)	Stem diameter (cm ± SE)	Root mass (g ± SE)	Aerial mass (g ± SE)	Whole plant mass (g ± SE)
Spinosad	20	40.29 ± 1.36 A	1.34 ± 0.03 A	4.90 ± 0.34 A	15.98 ± 0.87 A	20.88 ± 0.95 A
Control	20	35.49 ± 1.09 B	1.32 ± 0.02 A	4.49 ± 0.2 A	16.19 ± 0.70 A	20.68 ± 0.82 A
		t (38) = 2.75	t (38) = 0.41	t (38) = 1.04	t (38) = −0.19	t (38) = 0.16
		$p < 0.05$	$p = 0.69$	$p = 0.31$	$p = 0.85$	$p = 0.87$

*Means within the same column followed by the same upper case letter are not significantly different at $p < 0.05$ (Student's t-test).

correlation coefficients (r^2) > 0.99. Matrix effects were negligible with slope ratios of 0.99 for spinosyn A and 1.01 for spinosyn D. The concentration of spinosad present in leaf tissue started to decrease at 20 DAT. (Table 3). The spinosad concentration recorded in leaves at 25 DAT (0.26 μg g^{-1}) was significantly lower than that in leaves sampled at 3, 10 and 15 DAT ($F = 6.64$; df = 4, 42; $p < 0.05$) (Table 3). However, although the spinosad concentration decreased, high larval mortalities (96.55%–100%) were recorded during the entire experimental period (H (4) = 6.69; $p = 0.15$). Larval feeding on leaves ceased within 24 h. The spinosad concentrations in fruit sampled from treated plants at 80 DAT were below the detection limit (0.003 μg g^{-1}) of the equipment used for the LC–MS/MS method.

3.4 Plant growth parameters

Plants drenched with spinosad were significantly taller than untreated plants (Table 4) (t (38) = 2.75, $p < 0.05$). The other plant growth parameters (stem diameter, mass of fresh, whole plants, aerial parts and roots) did not differ significantly between the spinosad-treated and untreated control plants.

4 DISCUSSION

Results from the leaf-dip bioassay indicated instar-dependent tolerance of *T. absoluta* larvae to spinosad. Later-instar larvae (third and fourth instar) were more tolerant to spinosad compared with second instars. Several aspects of an insect's biology could provide explanations for increased tolerance at later larval instars. Weight increase plays an important role in lethal dose stage dependency.[38] However, the assumption of a linear relationship between lethal dose and weight could result in an overestimation of lethal dosage in high-weight individuals, and an underestimation in low-weight individuals.[39] For example, Robertson et al.[40] reported that the lethal doses of insecticides were not directly proportional to different weight groups of fourth-instar *Choristoneura freemani* (Razowski) (Lepidoptera: Tortricidae) larvae.

In addition to weight increase, other factors such as degradative metabolism[38] also contribute. Kim et al.[28] attributed the larval instar-dependent tolerance in *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) to enhanced detoxification enzymes and reduced acetylcholinesterase activity in later larval instars. Although no significant correlations were found between the reduced susceptibility of spinosad-resistant *T. absoluta* larvae in Chile and the activity of glutathione *S*-transferases, esterases and mixed-function oxidases (MFO), enhanced MFO activity was reported as the possible resistance mechanism.[24] This resistance mechanism has already been associated with resistance to spinosad in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) and *S. exigua*.[41,42]

Results on the susceptibility of *T. absoluta* from leaf-dip bioassays suggest that all larval instars should be effectively controlled at the recommended label rate in South Africa, of 144 ppm (mid-range). This may explain the current effective control of *T. absoluta* with leaf applications of spinosad in South Africa. The estimated LC_{50} value for second-instar larvae (0.41 ppm) from this study was similar to that reported for a Brazilian population of this pest.[25] The Brazilian population was able to rapidly develop spinosad resistance under laboratory conditions, reaching resistance levels of 180 000-fold after only seven generations of selection for resistance.[25] This emphasizes the importance of proper insect resistance managment by means of rotation of active ingredients with different mode of actions. However, to date, only three cases of field-evolved resistance against spinosad in *T. absoluta* have been reported.[24–26]

High mortality of second-instar *T. absoluta* larvae was caused by the systemic toxicity of spinosad administered as a drench. A mortality rate of 80% (LC_{80}) was estimated with drenching at approximately 4 mg a.i. per plant (20.1 ppm).

The two most important properties that regulate translocation of pesticides are lipophilicity (octanol–water partition coefficient [logP_{oct}]) and acidity (acid dissociation constant [pK_a]).[43,44] A study by Inoue et al.[43] on the physiochemical factors that affect the systemicity of compounds in barley, concluded that compounds with intermediate lipophilicity (logP_{oct} = 2–3) and acidity (pK_a = 7.5 to 8.5) are particularly effective in systemic uptake. The logP_{oct} of spinosyn A, the main component of spinosad, ranges between 2.8 and 5.2 with a pK_a of 8.10, resulting in effective take-up of spinosyn A by plants.[31]

The systemic persistence of spinosad at levels toxic to second-instar larvae of *T. absoluta* lasted at least 25 days, causing 96.5% mortality when drenched at an application rate of 48 ppm (9.6 mg a.i.) per plant. This contrasts with foliar applications of spinosad, where spinosyn A and D rapidly dissipate from foliage predominantly through photolysis.[21] Spinosad has a half-life of 1.6–16 days on plant foliage,[21] which agrees with the decrease of up to 97% in spinosad residues 10 DAT (spray) reported on cabbage and cauliflower leaves.[45]

The long systemic persistence of spinosad when applied as a drench is most likely due to continuous uptake through the root system. A study in which spinosyn A and D were applied to a layer of silt soil reported quick degradation in the initial stages with half-lives of 17 and 7 days for spinosyn A and D, respectively.[46] However, subsequent degradation occurred at a much slower rate with half-lives estimated to exceed 100 days, indicating that some of the residues are absorbed into the soil before ultraviolet light (UV) exposure can take place.[46]

The root uptake of spinosad applied as a soil drench to tomato was reported to be much higher in a rockwool substrate,

compared with other substrates such as black earth, sand, sand–clay and peat–clay mixtures.[31,32] A difference in efficacy of control of *T. urticae* of approximately 40% was reported when spinosad was systemically applied to rockwool compared with an application to a sandy soil. This can be explained by the higher rate of bacterial breakdown of spinosyns by microbial communities in living soils.[31] Lower spinosad lipophilicity correlates with lower pH levels and therefore lower uptake by partitioning onto root solids. The sandy soil–compost mixture in the current study had a pH of 6.49, lower than the high pH of rockwool, which ranged between 7 and 8.[31] Because strong sorption of spinosad also occurs in fine-textured soil,[21] sandy soils may also be suited to induce systemic toxicity when spinosad is applied as a drench.

The recommended foliar application rates of spinosad onto tomato in South Africa range between 72 and 180 g ha^{-1}. With an estimated plant density of 20 000 plants ha^{-1} in greenhouses, when applied directly to these plants the current registered foliar application rates range between 3.6 and 9.0 mg a.i. per plant. The estimated LC_{80} for *T. absoluta* when spinosad is administered by means of a drench application is 4.02 mg a.i. per plant. This indicates that a drench application rate of 9 mg a.i. per plant, similar to the current registered foliar application rate, will provide effective control of *T. absoluta* larvae for at least 25 DAT.

The levels of spinosad detected in tomato fruit (0.003 μg g^{-1}) 80 days after drenching with spinosad were well below the maximum residual limits set for South Africa (0.2 μg g^{-1}) and the European Union (0.5–1.0 μg g^{-1}).[47] In this study, spinosad was drenched during the seedling stage. Considering the systemic persistence of spinosad at high concentrations in leaves 25 DAT, further studies are needed regarding residual limits associated with drenching of spinosad at later plant growth stages, closer to harvest.

Stem diameter, root mass, aerial mass and whole plant mass of tomato plants were not affected by spinosad drench treatments during the seedling stage. Spinosad-treated plants did, however, grow taller than control plants. Thus, spinosad applied as a drench to tomato seedlings had no negative effect on tomato plant growth.

Insecticides exert a selection pressure that favors the survival of resistant genotypes, eventually leading to reduced efficacy as a result of resistance evolution.[12–15] Repeated application of a single insecticidal MoA[48] or application of persistent insecticides, for example systemically applied spinosad, would increase the selection pressure due to a prolonged period of exposure to the active ingredient in the plants.[31,32]

However, insecticide resistance not only evolves because of the repeated application of a lethal concentration that eliminates all susceptible individuals, but also favors the survival and reproduction of heterozygous individuals exposed to sublethal concentrations.[49] Sublethal insecticide exposure might delay the selection of major single-gene resistant alleles while favoring polygenic resistance, which results in small increases in the magnitude of insecticide resistance over time.[50] Exposure to sublethal insecticide dosages could also influence resistance beyond the selection of resistant individuals through the induction of detoxifying enzymes.[49] Inducing detoxification enzymes through sublethal insecticide exposure of a population to an insecticide may prime the target insect against the same or other compounds.[51,52] The continuous decrease in the concentration of spinosad within tomato plants coupled with generational overlap and larval instar-dependent tolerance could, therefore, also result in inadequate control and resistance evolution as a result of sublethal exposure.

It is, however, not only *T. absoluta*, but the entire species complex feeding on these tomato plants that is subjected to selection for insecticide resistance. Effective control of more than one pest species could, therefore, be jeopardized in future by drenching tomato seedlings with spinosad for control of *T. absoluta*. Krechemer and Foerster[53] reported that *T. absoluta* has an average developmental time of approximately 24 days at 25°C. Spinosad applied systemically at a rate of 9.6 mg a.i. per plant could effectively control *T. absoluta* for at least 25 DAT, thus providing sufficient control for the duration of an entire life cycle of the target pest. An insecticide with a different MoA should therefore be applied at approximately 20–25 DAT. This will ensure that larvae of the follow-up generation are exposed to another MoA, for effective insect resistance management.

Systemic insecticides are present in plant tissues[54,55] including the pollen and nectar of flowers and could have negative effects on pollinators.[56] However, van Leeuwen et al.[32] report that the non-target parasitic wasp, *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae), was not affected when exposed to tomato plants drenched with spinosad. Parasitoids could, however, be affected by feeding on hosts that consumed plant material containing the active ingredient (secondary poisoning).[57]

5 CONCLUSION

This study reports on the larval instar-dependent tolerance of *T. absoluta* to spinosad, the systemic properties of spinosad in tomato as well as the effect of systemically applied spinosad on mortality of *T. absoluta* up to 4 weeks after treatment. The levels of susceptibility of second- to fourth-instar larvae estimated in this study indicated that spinosad applied as a foliar treatment at the recommended dose effectively controls *T. absoluta* larvae, regardless of their developmental stage. Because of the long residual activity of spinosad in tomato plants when applied as a drench, further extensive studies need to be performed, focusing on the effect of continuous exposure of pest complexes to residual spinosad present in plant tissue.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Hannalene du Plessis and Johnnie van den Berg conceived research. Reynardt Erasmus and Peet Jansen van Rensburg conducted experiments. Reynardt Erasmus and Hannalene du Plessis analyzed data and wrote the manuscript. All authors revised and approved the manuscript.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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