ELIZEU DE SÁ FARIAS

PHTHALIDES AS PROMISING INSECTICIDES AGAINST *Tuta absoluta* (LEPIDOPTERA: GELECHIIDAE)

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de *Magister Scientiae*.

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ABSTRACT

FARIAS, Elizeu de Sá, M.Sc., Universidade Federal de Viçosa, February, 2017. **Phthalides as promising insecticides against *Tuta absoluta* (Lepidoptera: Gelechiidae)**. Adviser: Marcelo Coutinho Picanço. Co-adviser: Elson Santiago de Alvarenga.

The tomato leafminer *Tuta absoluta* (Meyrick) is an important pest of Solanaceae crops. This pest is mainly controlled with insecticides. However, few active ingredients are currently efficient against *T. absoluta* due to the high level of resistance of populations of this pest to the recommended insecticides. Thus, the demand for new molecules with potential use in the control of the tomato leafminer is constant. In this work, we evaluated the insecticide potential of eight phthalides derived from furan-2(5*H*)-one against *T. absoluta* larvae. We determined the potency of the most active phthalides and the susceptibility of six different *T. absoluta* populations to these compounds. We also evaluated their toxicity to two non-target species (*Solenopsis saevissima* Smith and *Tetragonisca angustula* Latreille). Two phthalides (3 and 4) presented insecticide potential against *T. absoluta*. Phthalide 4 was as toxic as piperine (positive control) and both phthalides exhibited rapid action (LT_{50} < two hours). The variation in the susceptibility of *T. absoluta* populations to the phthalides 3 and 4 was low. Both phthalides did not present physiological selectivity in favor of the non-target species. Phthalides 3 and 4 are promising insecticides against the tomato leafminer. However, their use must follow the principles of ecological selectivity.

RESUMO

FARIAS, Elizeu de Sá, M.Sc., Universidade Federal de Viçosa, fevereiro de 2017. **Ftalidas como inseticidas promissores contra *Tuta absoluta* (Lepidoptera: Gelechiidae)**. Orientador: Marcelo Coutinho Picanço. Coorientador: Elson Santiago de Alvarenga.

A traça do tomateiro *Tuta absoluta* (Meyrick) é uma praga importante de solanáceas. Seu controle é realizado principalmente através da aplicação de inseticidas. Entretanto, poucos ingredientes ativos são atualmente eficientes, uma vez que muitas populações desta praga têm apresentado resistência aos inseticidas recomendados. Assim, há uma demanda constante por novas moléculas com potencial de uso no controle da traça do tomateiro. Nesse trabalho, o potencial inseticida de oito ftalidas derivadas de furan-2(5*H*)-ona a *T. absoluta* foi avaliado. Nós determinamos a potência das ftalidas mais ativas e a susceptibilidade de seis populações brasileiras de *T. absoluta* a esses compostos. A toxicidade das ftalidas selecionadas a duas espécies não-alvo (*Solenopsis saevissima* Smith e *Tetragonisca angustula* Latreille) também foi avaliada. Duas ftalidas (3 e 4) apresentaram potencial inseticida sobre a praga. A ftalida 4 foi tão tóxica quanto a piperina (controle positivo) e ambas ftalidas exibiram ação rápida (LT_{50} < duas horas). A variação na susceptibilidade das populações de *T. absoluta* às ftalidas 3 e 4 foi baixa. Os dois compostos não apresentaram seletividade fisiológica em favor das espécies não-alvo. As ftalidas 3 e 4 são promissoras como inseticidas contra a traça do tomateiro. Entretanto, o uso dessas moléculas deve seguir os princípios da seletividade ecológica, uma vez que não apresentaram seletividade fisiológica em favor das espécies não-alvo testadas.

1. INTRODUCTION

The tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), also known as tomato moth, is an important pest of Solanaceae. In regions where it occurs, this pest preferentially attacks the tomato. Native to South America, this lepidopteran was introduced in Europe in 2006.[1] Currently, this pest is found throughout Europe, North Africa and the Middle East.[2] *T. absoluta* attacks the tomato at all stages of development. Their larvae consume the leaf mesophyll and can damage the stem, flowers, and fruits.[3–5]

The main control method for *T. absoluta* is the application of insecticides. In Brazil, there are 32 active ingredients belonging to 11 chemical groups registered for the control of the tomato leafminer.[6] However, few of these molecules are currently efficient since many *T. absoluta* populations are resistant to recommended insecticides.[7,8] This situation has created a demand for the discovery of new molecules with potential use in the control of the tomato moth.

The process of developing new insecticides comprises four phases. The first phase involves laboratory studies, which aim to determine for the candidate compounds the efficacy against the pest and the toxicity to humans and non-target organisms.[9–10] The second phase includes performing small-scale field trials in order to determine suitable formulations and their persistence in the environment. In the third phase, large-scale field trials are conducted to access the cost-benefit ratio and the impact of the formulations on human health. The last phase includes testing the formulations by independent laboratories, aiming to decide whether to register or not the new insecticide.[9,10]

The cuticle is the largest organ of insects. Thus, the greater penetration of the insecticidal molecules occurs through the exoskeleton.[12] The main methods involving cutaneous input of insecticides are topical application and spraying on surfaces (glass, filter paper, and leaves) where insects walk or rest.[11,13] By comparing these methods, topical application has the advantage of requiring small amounts of tested compounds.[13] This feature is particularly important in bioassays involving organic synthesis of candidate molecules, where the quantities of compounds are limited. Such bioassays are often only feasible by topical application.

Natural products extracted from plants have been constantly used as models for the synthesis of new insecticides.[14] The focus on the use of these compounds occurs due to the production of secondary metabolites by plants for protection against herbivores.[15] A class of compounds that have been studied are the phthalides or isobenzofurenones, volatile lactones present in plants of the family Apiaceae.[16] Recent studies have demonstrated that derivatives of these compounds present insecticidal, nematicidal and acaricidal activity.[17–20]

In this work, we aimed to select by the method of topical application, synthetic phthalides with insecticidal potential against the tomato leafminer. Besides determining the potency of the most active phthalides, we determined the susceptibility of different *T. absoluta* populations to these compounds. We also verified the toxicity of the selected phthalides to the predatory ant *Solenopsis saevissima* Smith (Hymenoptera: Formicidae) and to the stingless bee *Tetragonisca angustula* Latreille (Hymenoptera: Apidae), beneficial organisms that occur in tomato crops. The information generated in this study is of great importance because it provides implications on the practical efficacy against the pest and possible limitations of the tested phthalides.

2. MATERIAL AND METHODS

2.1. Chemicals

Eight phthalides derived from furan-2(5*H*)-one were used in this work. Chemical structures of the evaluated compounds can be found in Figure 1. The complete synthesis of these lactones has been described previously.[21,22]

2.2. Insecticidal bioassays

2.2.1. Insects

Bioassays were performed with second instar larvae of *T. absoluta*. Six populations of the tomato leafminer were used in this work (Figure 2). The populations were maintained in the laboratory.[7] Larvae were fed leaves of commercial tomato (*Solanum lycopersicon* L. var. Santa Clara).

We also used adults of *S. saevissima* and *T. angustula*. These insects were collected from nests located at the Universidade Federal de Viçosa, Viçosa, Minas Gerais State, Brazil.

2.2.2. General procedures

It was performed four bioassays with the pest. Initially, a screening bioassay was held to identify the bioactive phthalides. The second part consisted in the determination of the potency of the selected compounds against the pest. In a third bioassay, we evaluated the susceptibility of different populations of the tomato leafminer to the selected phthalides. The fourth bioassay was performed to determine the speed of action of the phthalides against the pest. We also carried out a bioassay aiming to determine the selectivity of the bioactive phthalides in favor of the non-target species *S. saevissima* and *T. angustula*.

All the bioassays were held by topical application. The doses were prepared in μg of the compound mg^{-1} of insect. In order to dilute the compounds, the average weight of the insect was obtained by measuring the mass of 20 insects on an analytical scale before each bioassay.

Each insect was treated on the abdominal tergum with solutions of the compounds diluted in acetone PA (99.5%, Vetec, Rio de Janeiro, RJ, Brazil). It was used a micro-syringe (model 701N, Hamilton Co., Reno, NV, USA) to deliver 0.5 μL of a test solution in each individual. For negative control, the insects were treated with an equal volume of acetone. We used piperine (≥97%, Sigma-Aldrich, St. Louis, MO, USA) as positive control. The insecticidal activity of this compound has largely been described.[23–26] Extracts from *Piper amalago* L. (Piperaceae), plant rich in piperine-like amides, have demonstrated insecticidal activity against the tomato leafminer.[27]

The experimental design was completely randomized for all bioassays. After the application, the insects were supplied with appropriate food as follows: a tomato leaflet (cv. Santa Clara) for *T. absoluta* while *S. saevissima* and *T. angustula* both received small pieces of cotton moistened with pure water and honey mixture (honey and water in a proportion of 1:1) placed in plastic containers (1.5 cm diameter × 1.0 cm height).

Mortality counts were made after 24 and 48 hours of exposure to the treatments. Individuals were considered dead when they did not respond to stimulus. All data submitted to statistical tests were first checked for normality (Shapiro–Wilk test) and for homoscedasticity of residuals (Bartlett test). All analyses were performed using SAS 9.0 (SAS Institute, Cary, NC, USA) and SigmaPlot 12.5 (Systat Software, Chicago, IL, USA).

2.2.3. Screening bioassay

In this part of the study, we used larvae of *T. absoluta* from the Viçosa population. This population was selected because it has presented high susceptibility to several insecticide active ingredients.[7,8,28] The treatments were the eight phthalides, piperine, and the negative control. The dose used in this bioassay was 30 µg of the compound per mg of larva. We carried out at least three replications per treatment. Each experimental unit consisted of ten larvae kept on a round plastic container (6 cm diameter × 5 cm height) with lid. The containers were kept in an acclimatized room at 25 ± 0.5 °C and 75 ± 5% relative humidity with a photoperiod of 12 hours.

The larvae mortality data were submitted to one-way Analysis of Variance (ANOVA) and the means were compared using Tukey HSD test at the 5% level.

2.2.4. Potency of the selected phthalides

The treatments were the phthalides selected in the screening bioassay, piperine, and the negative control. We used the same procedure described in the screening. Initially, three doses of each compound were tested to identify the range of doses that would provide mortality greater than zero and less than 100%. Once the range of dose was defined, we tested five to six concentrations in six replications for each compound.

Mortality data were submitted to probit analysis using the PROC PROBIT procedure of SAS to estimate dose-mortality curves.[29,30] The lethal doses that caused 50 and 90% mortality (LD_{50} and LD_{90}) were also estimated.

2.2.5. Susceptibility of T. absoluta populations to the selected phthalides

We carried out dose-mortality bioassays with five populations of the pest collected from different locations. The populations were collected at Uberlândia (Minas Gerais State), Paulínia (São Paulo State), Caçador (Santa Catarina State), Ibicoara (Bahia State) and Ouro Verde de Goiás (Goiás State). These populations sampling sites were selected because they represent different biomes and important cultivation systems in Brazil (Figure 2).

The treatments were the selected phthalides, piperine, and the negative control. We used the same procedure described in the previous bioassay. We also used in this comparison the dose-mortality curves calculated from the

Viçosa population in the bioassay of determination of the potency of the selected phthalides. We compared the susceptibility of these populations using the Susceptibility Index (SI). This index was calculated as follows: SI = LD_{50} of the less susceptible population ÷ LD_{50} of the most susceptible population. SI indexes were considered different when their mean did not overlap the fiducial interval of the SI of another population.

2.2.6. Time-mortality curves of the selected phthalides

T. absoluta larvae from the Viçosa population were subjected to time-mortality bioassays when submitted to the LD_{90} of the selected phthalides. Each treatment was set up with 100 larvae of *T. absoluta*. The death of the larvae was monitored for 48 hours by noting the time at which each insect died. The intervals between assessments for each treatment (ranging from 30 min to 2 h) were determined previously. From the experimental data, survival curves were estimated by the Kaplan-Meier product-limit method using the LIFETEST procedure.[30,31] Survival curves were compared by log-rank test ($P < 0.05$), and the median lethal times (LT_{50}) of the larvae were estimated.

2.2.7. Risk assessment to non-target organisms

In this bioassay, the LD_{90} for the Viçosa population of the most active phthalides were applied to the beneficial insects. We carried out five replications per treatment. For the predator *S. saevissima*, each experimental unit comprised a round plastic container (6 cm diameter × 5 cm height, with lid) containing ten insects. For *T. angustula*, each experimental unit comprised a Petri dish (9 cm diameter × 2 cm height, covered with organza) containing ten insects.

After the application, the insects were kept in an acclimatized room at 25 ± 0.5 °C and 75 ± 5% relative humidity. *T. angustula* was subjected to a photoperiod of 12 h while *S. saevissima* was kept in the dark during the bioassay. We compared the mortality of the non-target species with the mortality of the pest exposed to the same dose (LD_{90}) by the Student's t test ($P < 0.05$).

3. RESULTS

3.1. Screening bioassay

There was a significant effect of the treatments in the mortality of *T. absoluta* larvae ($F_{9, 29}$ = 158.17, P < 0.001). The phthalides 3 and 4 and piperine (positive control) caused 100% of mortality of *T. absoluta* larvae (Fig. 3). Mortality of larvae submitted to the other treatments ranged from 62.23% (phthalide 7) to 0.00% (negative control). Therefore, the compounds 3 and 4 were selected for the next bioassays.

3.2. Potency of the selected phthalides

The compound 4 (LD_{50}=3.13 μg mg^{-1}, LD_{90}=13.87 μg mg^{-1}) presented similar toxicity to the positive control piperine (LD_{50}=3.01 μg mg^{-1}, LD_{90}=11.93 μg mg^{-1}) and superior toxicity to the compound 3 (LD_{50}=11.25 μg mg^{-1}, LD_{90}=23.42 μg mg^{-1}) (Table 1).

3.3. Susceptibility of *T. absoluta* populations to the selected phthalides

The LD_{50} values of the phthalide 4 were in the range of 1.90-6.99 μg mg^{-1} for the populations of *T. absoluta*. The most susceptible population was that from Paulínia. The population from Ouro Verde de Goiás was the less susceptible (SI=3.68). The SI for the populations from Uberlândia, Viçosa, Caçador and Ibicoara were 1.58, 1.65, 2.87, and 3.07, respectively (Table 2).

The LD_{50} values of the phthalide 3 were ranged from 1.99-16.44 μg mg^{-1}. The most susceptible population was that from Paulínia. The susceptibility indices of Uberlândia, Caçador, Ibicoara, Viçosa, and Ouro Verde de Goiás were 1.46, 2.22, 2.67, 5.65 and 8.26, respectively.

3.4. Time-mortality curves of the selected phthalides

Survival analysis of *T. absoluta* larvae exposed to the control and to the selected phthalides indicated significant difference among the treatments (log-rank test, χ^2=315.30, df = 2, P < 0.001) (Fig. 4A). Two hours after the application of the treatments, survival of the larvae was below 50% for both phthalides. After six hours of exposure, mortality of larvae was 100 and 70% for the compounds 4 and 3, respectively.

Median lethal time (LT_{50}, lethal time to 50% of the treated larvae) was 0.130 (eight minutes) and 1.65 hours to the phthalides 4 and 3, respectively (Fig. 4B).

3.5. Risk assessment to non-target organisms

The selected compounds caused mortality above 71% to the bee *T. angustula* and to the ant *S. saevissima*. These mortalities were not significantly different from the mortality caused to the pest (Figure 5).

4. DISCUSSION

From the eight tested phthalides, two (3 and 4) showed high insecticidal activity against *T. absoluta*. These compounds caused mortalities higher than 90% when applied at the selection dose. This implies that these molecules are potential insecticides against the pest. [32]

When tested against the Viçosa population, phthalide 4 presented an LD_{50} similar to that of piperine and superior to that of phthalide 3. As the potency of insecticides is inversely proportional to their lethal doses,[33] phthalide 4 was the most potent against the moth. Since phthalide 4 was more potent than the phthalide 3, it could be assumed that the former is more promising than the latter. However, this situation is more complex considering that factors other than the potency against the pest are evaluated in candidate compounds. The speed of action, cost-effectiveness and toxicity to terrestrial (mammals, birds, plants and invertebrates such as earthworms, springtails, woodlice and bees) and aquatic organisms (fish, algae and chironomids larvae) are also assessed.[34] In the specific case of insecticides used against the tomato leafminer, some products are until 100-fold less potent than others,[6] and even so, are used by farmers to control the pest.

Both phthalides 3 and 4 showed fast action (LT_{50} lower than two hours) against *T. absoluta*. This characteristic is desirable for insecticides used to control pests that cause severe economic damage.[11] *T. absoluta* larvae migrate from the leaves to the fruits where they cause great economic losses.[35] Thus, a rapid control is necessary to avoid serious economic damage in crops attacked by the tomato leafminer. Therefore, the fast-acting characteristic of the phthalides 3 and 4 is important in the control of this pest.

We could verify difference in the susceptibility of the *T. absoluta* populations to the selected phthalides. However, this variation (3.68 and 8.26-fold for the phthalides 4 and 3, respectively) may be considered low when compared with studies conducted with other insecticides. Variations in susceptibility of the tomato leafminer populations have been found to be greater than 22-fold to indoxacarb and cartap.[7,28] For chlorantraniliprole and flubendiamide, some populations may be up to 1700-fold more susceptible than others.[36] This fact indicates that the phthalides selected in this work can present good control efficiency of *T. absoluta* when used in tomato crops from different regions.

Since the tomato leafminer is native to South America,[37] it is expected to be found in this region greater genetic variability between its populations.[38–40] The tomato crops of South America, especially in Brazil, are also those that suffer major damage from *T. absoluta*.[37] Thus, even when applied to populations from the region where the pest exhibits greater genetic variability and causes higher damage, the phthalides 3 and 4 showed high toxicity. These facts reinforce the potential insecticide use of these substances against the moth.

We did not study the mode of action of the compounds used in this work. However, the rapid action of the selected phthalides against the pest is an indicative of neurotoxic activity.[41] Some studies have suggested that natural phthalides act in the neurotransmitter acetylcholine and in the octopamine and γ-aminobutyric acid receptors.[19,20,42] We were able to observe some behaviors of treated insects that corroborate the neurological activity of phthalides. Such behaviors included paralysis and inhibition of feeding.

Although the *T. absoluta* populations presented low variation in susceptibility to the phthalides 3 and 4, the use of these compounds should rely on the principles of insecticide resistance management programs.[5,43,44] The application of these principles aims to avoid the phenomenon of resistance and maintain the efficiency of insecticides. A reduced reliance on chemical control, through the adoption of action threshold levels for the pest and application of other tactics of control (cultural, biological, behavioral and plant resistance) can benefit agroecosystems and constrain the development of insecticide resistance. The alternation of insecticides with different modes of action is also a recommended measure to prevent the resistance to these chemicals.[5,7,8,44]

We verified that the promising phthalides are not selective in favor of the tested non-target species. Thus, the use of these compounds should be performed obeying the principles of ecological selectivity. According to these principles, the exposure of natural enemies and pollinators to insecticides can be reduced by improving both location and timing of applications of these chemicals.[45] Predatory ants, including *S. saevissima*, have the soil as their main foraging and nesting site. By targeting the sprays to the pest attack site and by using smaller volumes of insecticide solution, insecticide residues in the soil can be reduced. Thus, the negative impacts of insecticides on these important predators can be mitigated.[46] The flower visits by the bee *T. angustula* start at seven o'clock in the morning, with a peak at noon and gradual reduction from that time.[47] Hence, insecticide applications in the late afternoon are ideal, since these bees are not active during this time.

5. CONCLUSIONS

We selected two phthalides (3 and 4) with insecticidal potential against *T. absoluta*. Phthalide 4 is as toxic as piperine (positive control) and both phthalides exhibit rapid action. The variation in the susceptibility of *T. absoluta* populations to the phthalides 3 and 4 is low. These two compounds do not present physiological selectivity in favor of the non-target species *S. saevissima* and *T. angustula*. Therefore, the phthalides 3 and 4 are promising insecticides against the tomato leafminer, but their application is conditioned to the principles of ecological selectivity.

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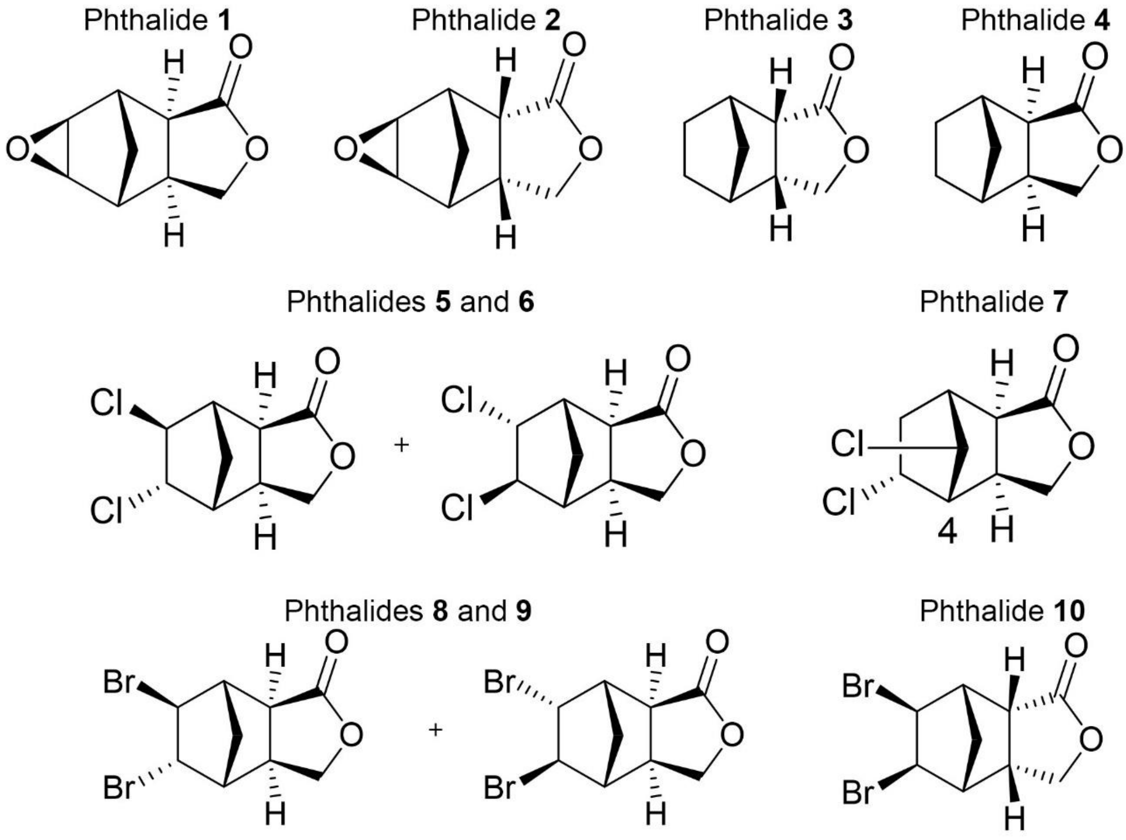
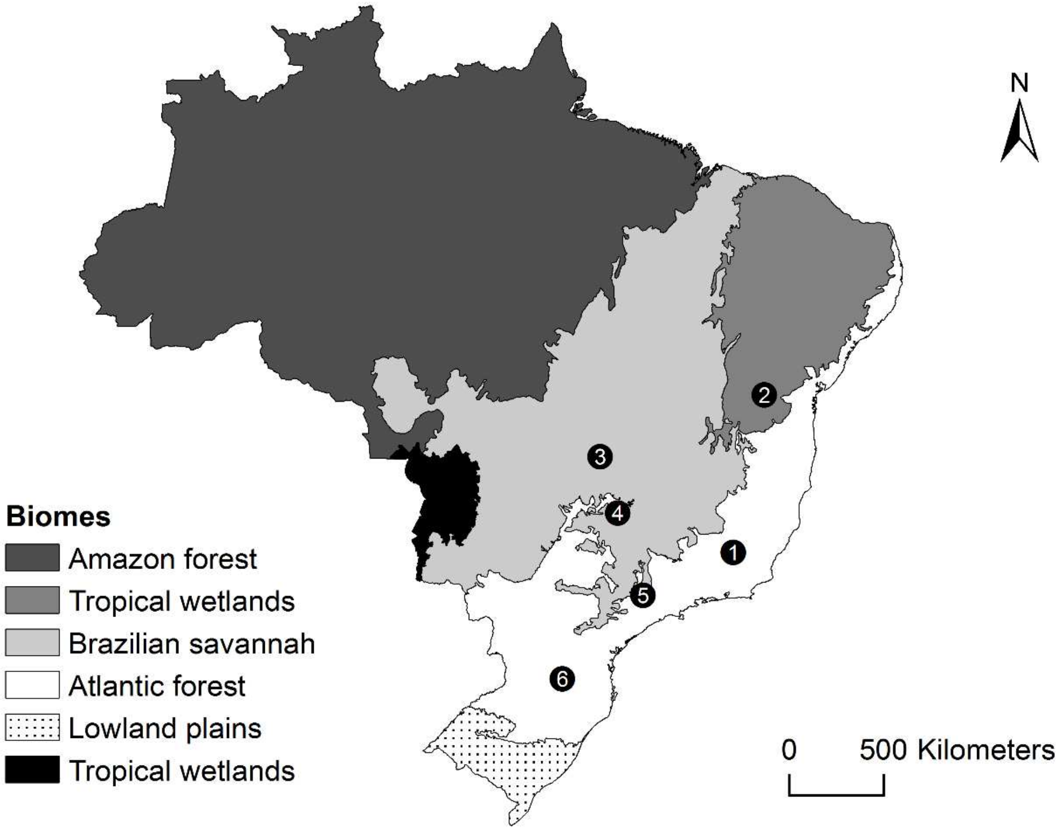


Figure 1. Chemical structures of the tested phthalides



	Sampling site	Month and year collected
1.	Viçosa, Minas Gerais State	January 1999
2.	Ibicoara, Bahia State	December 2014
3.	Ouro Verde de Goiás, Goiás State	November 2014
4.	Uberlândia, Minas Gerais State	November 2014
5.	Paulínia, São Paulo State	January 2015
6.	Caçador, Santa Catarina State	October 2014

Figure 2. Sampling sites and dates of collection of the field populations of the tomato leafminer *Tuta absoluta*

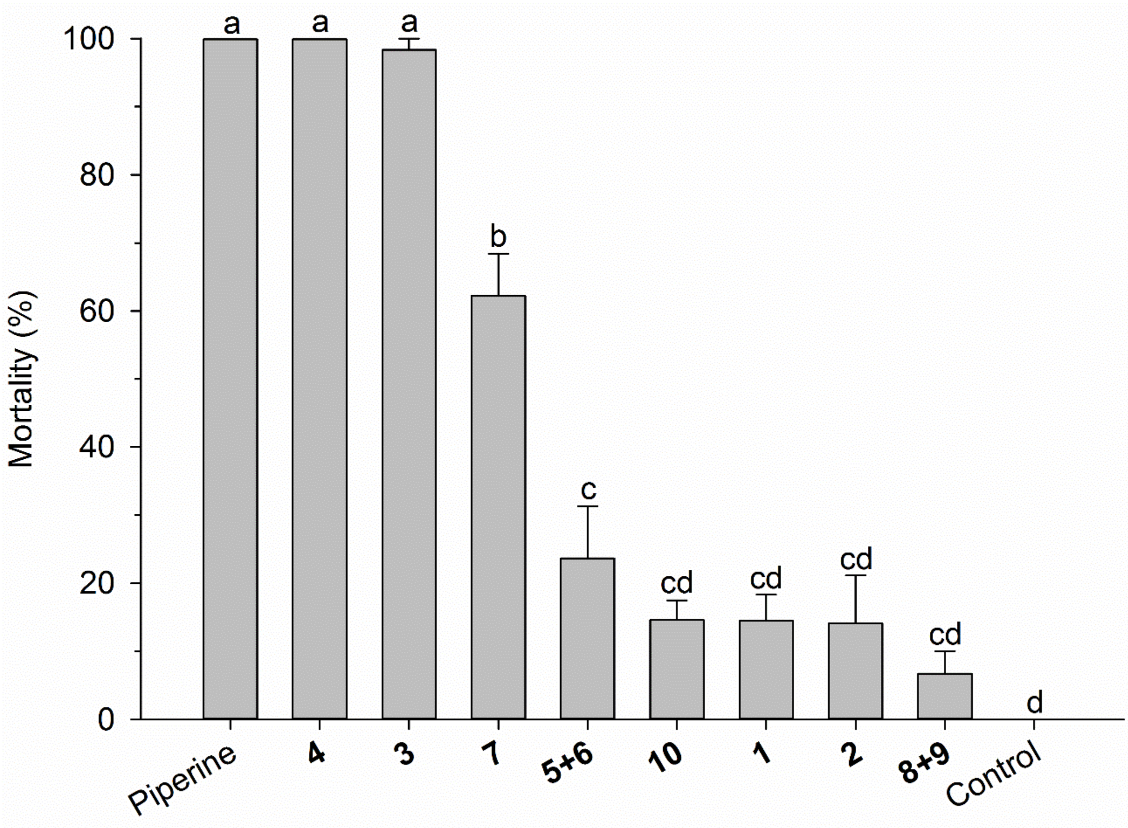


Figure 3. Mortality (mean ± standard error) of second-instar larvae of *Tuta absoluta* 48 hours after topical application of the treatments. Eight phthalides and piperine (positive control) were applied at the dose of 30 µg mg^{-1}. Control consisted in the application of acetone. Different letters indicate significant differences by Tukey's test at $P < 0.05$

Table 1. Dose-mortality curves of the selected phthalides and of the positive control to second instar larvae of *Tuta absoluta* from the population of Viçosa 48 hours after topical application

Compound	N^a	Slope ± SEM	LD_{50} (μg mg^{-1}) (95% FL)[b]	LD_{90} (μg mg^{-1}) (95% FL)[b]	χ^2	*P*-value
Phthalide **4**	300	1.98 ± 0.24	3.13 (2.38 - 3.99)	13.87 (10.07 - 22.00)	4.87	0.18
Phthalide **3**	360	4.03 ± 0.48	11.25 (9.91 - 12.67)	23.42 (19.86 - 29.78)	4.27	0.37
Piperine[c]	300	2.14 ± 0.25	3.01 (2.36 - 3.75)	11.93 (8.90 - 18.06)	3.04	0.38

[a] Number of insects, [b] Lethal dose with 95% fiducial limits (FL). [c] Positive control.

Table 2. Relative toxicity of the selected phthalides to populations of *Tuta absoluta*. The phthalides were applied topically and the mortality was accessed after 48 hours. Asterisks following the susceptibility indices indicate significant difference in comparison to the most susceptible population

Compound	Population	N^a	Slope ± SEM	LD_{50} (μg mg^{-1}) (95% FL)[b]	SI (95% FL)[c]	χ^2	*P*-value
Phthalide **4**	Paulínia	360	3.64 ± 0.49	1.90 (1.62 - 2.23)	1.00 (0.85 - 1.17)	5.89	0.21
	Uberlândia	300	2.21 ± 0.29	3.01 (2.35 - 3.73)	1.58 (1.24 - 1.96)*	5.67	0.13
	Viçosa	300	1.98 ± 0.26	3.13 (2.38 - 3.99)	1.65 (1.25 - 2.10)*	4.87	0.18
	Caçador	300	3.31 ± 0.42	5.46 (4.55 - 6.47)	2.87 (2.40 - 3.40)*	2.55	0.47
	Ibicoara	240	2.46 ± 0.50	5.84 (4.05 - 7.42)	3.07 (2.37 - 3.90)*	3.10	0.21
	Ouro Verde de Goiás	420	3.27 ± 0.47	6.99 (5.71 - 8.27)	3.68 (3.00 - 3.45)*	8.44	0.13
Phthalide **3**	Paulínia	240	3.58 ± 0.58	1.99 (1.52 - 2.44)	1.00 (0.76 - 1.23)	1.27	0.53
	Uberlândia	480	2.29 ± 0.26	2.91 (2.33 - 3.49)	1.46 (1.17 - 1.75)*	9.61	0.14
	Caçador	300	2.46 ± 0.31	4.41 (3.40 - 5.43)	2.22 (1.71 - 2.73)*	1.38	0.71
	Ibicoara	300	2.91 ± 0.41	5.32 (4.07 - 6.48)	2.67 (2.36 - 3.26)*	0.22	0.98
	Viçosa	360	4.03 ± 0.48	11.25 (9.91 - 12.67)	5.65 (4.98 - 6.37)*	4.27	0.37
	Ouro Verde de Goiás	300	2.35 ± 0.52	16.44 (14.20 - 18.33)	8.26 (7.14 - 9.21)*	4.89	0.18

[a] Number of insects, [b] Lethal dose with 95% fiducial limits (FL), [c] Susceptibility index (LD_{50} of the less susceptible population ÷ LD_{50} of the most susceptible population)

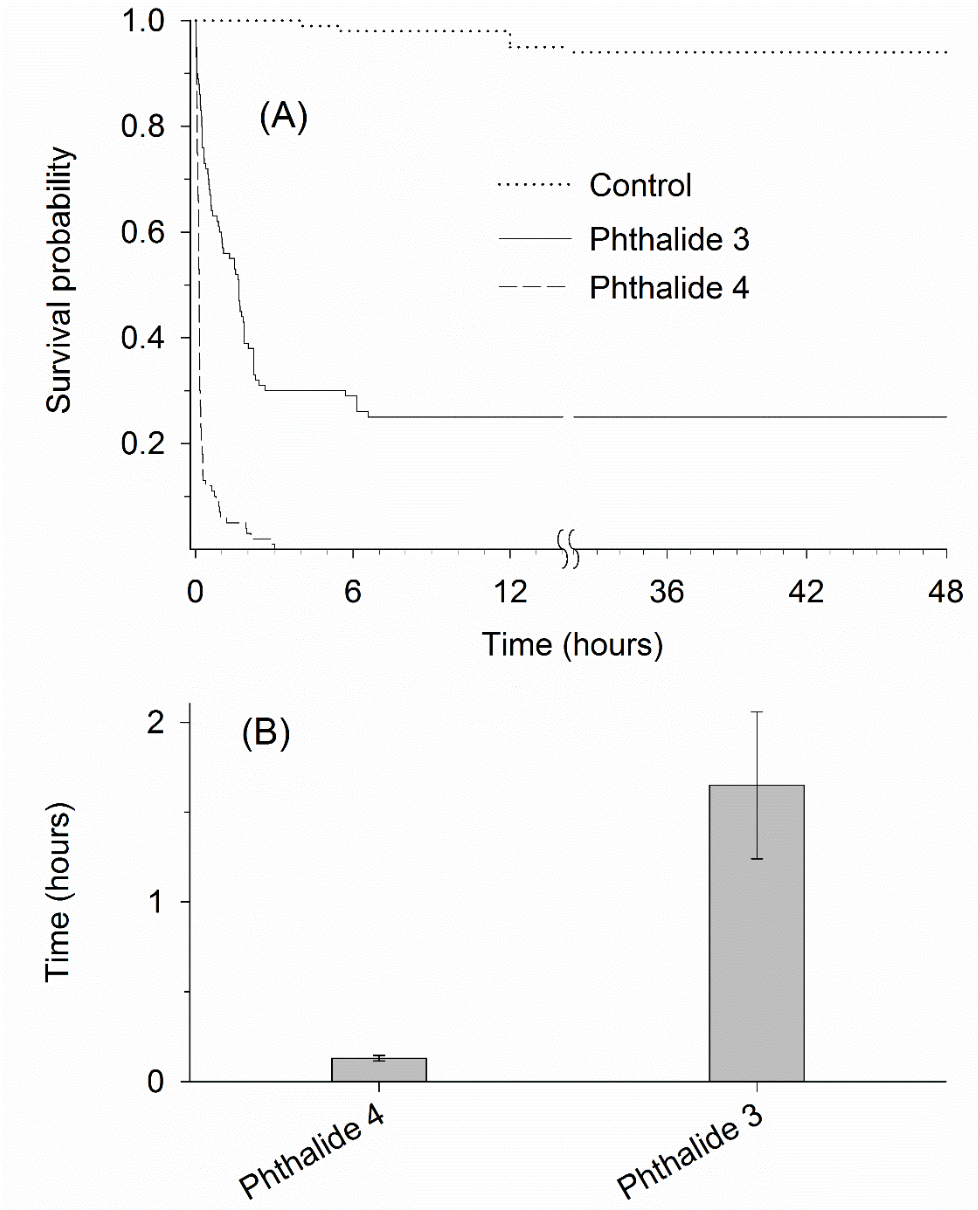


Figure 4. (A) Kaplan-Meier survival curves and (B) median lethal time with 95% fiducial limits of *Tuta absoluta* larvae exposed to the LD_{90} of the selected phthalides. Control consisted in the application of acetone

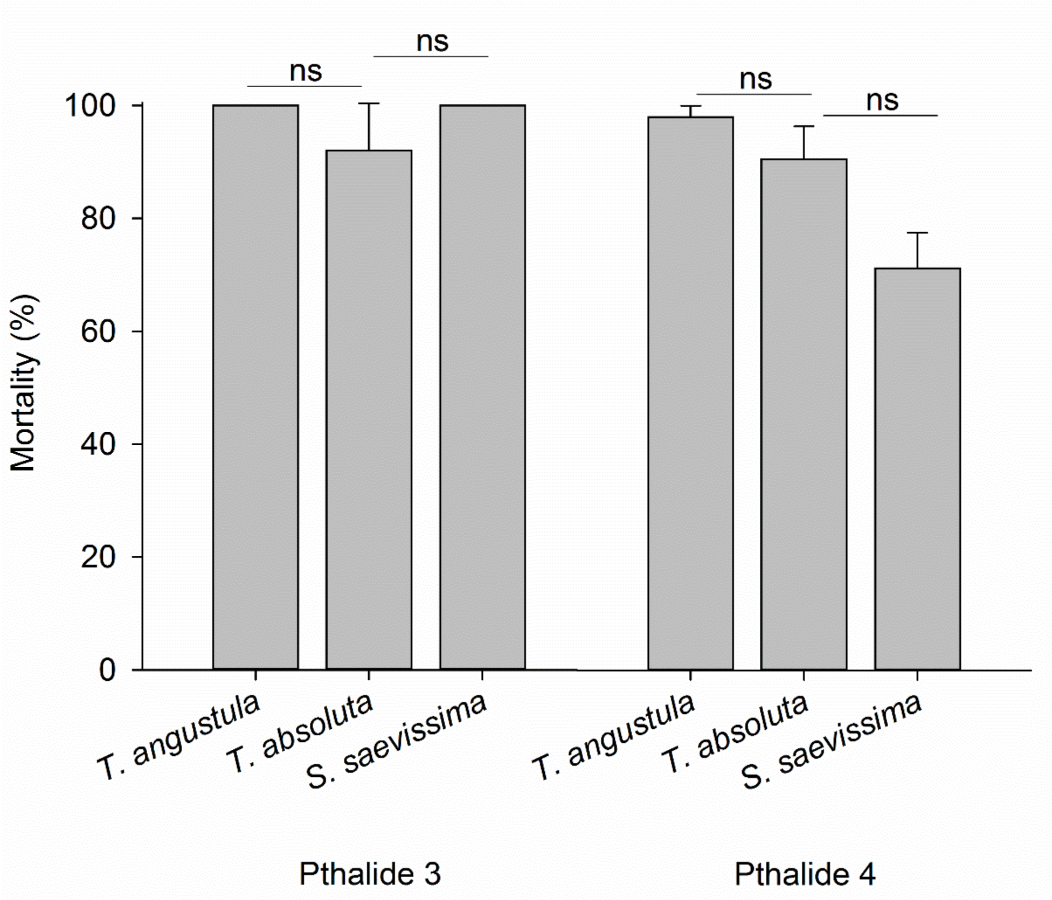


Figure 5. Mortalities of larvae of *Tuta absoluta*, adults of the predator ant *Solenopsis saevissima* and of the bee *Tetragonisca angustula* 48 hours after application of the LD_{90} for *Tuta absoluta* of the selected phthalides (3 and 4). ns means no significant differences by t test (P > 0,05)