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# Status of pyrethroid resistance and mechanisms in Brazilian populations of *Tuta absoluta*

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ABSTRACT

The tomato leafminer, *Tuta absoluta*, is a major pest of tomato crops worldwide. This study surveyed the resistance of *T. absoluta* populations from four regions in Brazil to pyrethroid insecticides, the frequencies of L1014F, T929I and M918T Na channel mutations, and the role of detoxification metabolism in the resistance. Resistance ratios varied from 1- to 11-times among populations and insecticides, but control failure likelihood assays showed that all pyrethroids assessed exhibited no efficacy at all (and thus, 98–100% control failure likelihood) against all *T. absoluta* populations. The activity of glutathione *S*-transferase and cytochrome P450-mediated N-demethylation in biochemical assays was significantly correlated with the level of resistance to deltamethrin and permethrin suggesting that these enzymes may play a role in resistance. TaqMan assays were used to screen for the presence of knockdown resistance (*kdr*) mutations and revealed that the L1014F *kdr* mutation was fixed in all populations and associated with two super-*kdr* mutations, M918T and particularly T929I, at high frequency. Altogether, results suggest that control failures are because of mutations in the domain II of the sodium channel, as a prevailing mechanism of resistance to pyrethroids in populations of *T. absoluta* in Brazil. But, enhanced cytochrome P450-dependent monooxygenases and GST activities also play an important role in the resistance of some populations, which reinforce that pyrethroids must not be used overall to control *T. absoluta*.



## 1. Introduction

The tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is one of the most economically important insect pests of tomato worldwide [1–3]. The larval stages of *T. absoluta* feed on tomato leaves but also damage the flowers, fruits and stems [2,4], resulting in yield losses between 40 and 100% [5,6]. This species is native to South America with its presence confirmed in southern Brazil, in the early 1980s [7]. Since then it has spread to all major tomato-producing regions in Brazil often seriously compromising productivity due to its destructive capacity [4]. This pest has also become a major concern for tomato cultivation in Europe, Africa and the Middle East [8,9]. The control of *T. absoluta* in most settings has relied heavily on the use of chemical insecticides; unfortunately, this has resulted in the development of resistance, with populations now described with resistance to a wide range of compounds [10–17]. Although, only recently, characterisation of resistance mechanisms have had attention, which may be used as tools to survey populations [15,16].

Pyrethroids are an important class of synthetic insecticide widely used to control many arthropod pests, including *T. absoluta*, as a result of their rapid action, high insecticidal activity and low mammalian toxicity [18]. Pyrethroids interact with the voltage-gated sodium channel and modify its kinetic function, leading to nervous system exhaustion and death [19–21]. One of the main mechanisms of pyrethroid resistance is reduction of neuronal sensitivity, known as knockdown resistance (*kdr*) [19,22,23]. This type of resistance was first documented in the housefly (*Musca domestica*) and was subsequently shown to be caused by two amino acid substitutions in domain II of the channel, a leucine to phenylalanine (L1014F) replacement in transmembrane segment IIS6, termed '*kdr*' and a threonine to methionine substitution upstream of segment IIS5, with the latter associated with an enhanced form of resistance termed super-*kdr* [24]. Subsequently, additional mutations associated with pyrethroid resistance, primarily in domains II or III of the channel, have been identified in a range of arthropod species [25].

Pyrethroid resistance in *T. absoluta* has recently been reported to be associated with the presence of the mutations L1014F, M918T and T929I in the sodium channel [16]. These authors found all three

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mutations at high frequency in field strains collected from Europe and South America, although no field populations from Brazil have been screened to date for the presence of those mutations. Furthermore, the role of metabolic detoxification in pyrethroid resistance in this species has not yet been investigated. In other insects metabolic resistance to pyrethroids has been associated with elevated levels of cytochrome P450 monooxygenases (P450s), carboxylesterases (CEs), and glutathione *S*-transferases (GSTs) [26–28].

Metabolism has been in particular assessed only in Chilean populations [15], and it is well known that such mechanism can confer broad resistance to insecticides, which impacts more the agriculture of developing countries. Full characterisation of Brazilian populations of *T. absoluta* regarding resistance has long been a necessity for improving the chemical management of this pest. Here, we provide a survey of resistance of *T. absoluta* populations from different geographical regions of Brazil to three representative pyrethroids using biochemical and molecular approaches as well as toxicological measures.

## 2. Materials and methods

### 2.1. Insecticides

Concentration–response curves were estimated for each population of *T. absoluta* through bioassays of larvae mortality using the following insecticides: Deltamethrin (Decis 25 CE, Bayer CorpScience S.A, recommended label rate, 7.5 mg AI/l of water), alpha-cypermethrin (Fastac 100 SC, BASF S.A., recommended label rate, 10 mg AI/l of water) and permethrin (Valon 384 CE, Dow Agrosciences Industrial LTDA, recommended label rate, 49 mg AI/l of water). The efficacy of each insecticide to control *T. absoluta* using the recommend label rate was also assessed.

### 2.2. Insects

Eight different populations of tomato leaf miner from commercial tomato crops in the Northeast, Midwest, Southeast and South of Brazil were collected in the period between 2010 and 2011 (Table 1). Individual larvae were obtained from various parts of plants, including stems, leaves and fruits. The populations were established and reared individually on leaves of tomato variety "Santa Clara" under ambient conditions as described in Campos et al. [29].

### 2.3. Bioassays

A toxicological bioassay was conducted using a completely randomised design with two replications, and the whole bioassay was repeated twice. For control failure likelihood [39], bioassays were conducted with the label rates stated above for each insecticide. Full dose–response bioassays were carried out using 7–8 concentrations of each insecticide that resulted in mortality of between 0 and 100%. Distilled water plus Triton X-100 at 0.01% was used as the control treatment. Leaflets of tomato cultivar "Santa Clara" were cleaned using a solution based on sodium hypochlorite 5%. After cleaning the leaflets in tap water, they were immersed horizontally for a minute in insecticide or control solution. The leaflets were kept on paper towels at room temperature until completely dry and then transferred to Petri dishes (80 × 15 mm) containing filter paper misted with distilled water. Each replicate comprised 10 second instar (L2) larvae of *T. absoluta* placed on a treated leaflet in a petri-dish. Petri dishes were sealed and maintained in a climate chamber (BOD) set at an average temperature of 25 ± 1 °C, 65 ± 5% relative humidity and photoperiod of 12 h. Mortality was evaluated after 48 hours with the aid of a light source and magnifying glass (Olympus SZ61, Olympus®, Center Valley, PA, USA). The larvae were considered dead if they could not move at least the extent of their length after touching [30]. Mortality caused by insecticide treatment were corrected for control mortality, using Abbott's formula [31].

### 2.4. Sample extractions for enzyme assays

For enzyme assays, 10 L2 larvae of each population were transferred to a microfuge tube with three replicates for each assay. For esterase and glutathione *S*–transferase assays, each sample was homogenised in 200 µl of sodium phosphate buffer (0.02 M, pH 7.2) or sodium phosphate buffer (0.1 M, pH 7.5), respectively using a Potter-Elvehjem homogeniser. Homogenates were centrifuged at 15,000 *g* and 4 °C for 15 min and supernatants harvested and stored at –20 °C. For cytochrome P450 assays, samples were homogenised in 500 µl sodium phosphate buffer (0.1M, pH 7.5) + glycerol at 20% and microsomes were prepared in the same buffer. Homogenates were centrifuged at 15,000 *g* and 4 °C for 15 min and the supernatant was ultra-centrifuged at 100,000 *g* for 60 min in an Optima™ L-80 XP ultracentrifuge (Beckman Coulter, Palo Alto, CA) with the resulting microsomal pellet resuspended in homogenisation buffer containing 20% glycerol. Quantitation of protein was determined by the bicinchoninic acid method using bovine serum albumin (BSA) as standard [32].

### 2.5. Esterase assays

Esterase activity was measured with a method adapted from van Asperen [33]. Stock solutions (250 mM) of α-naphthyl acetate and β-naphthyl acetate substrates were prepared in acetone. For each reaction, 2 µl α-naphthyl acetate at 25 mM, 10 µl of sample diluted to 1:100 and 188 µl of sodium phosphate buffer (0.02M, pH 7.2) were used. The same procedure was carried out for esterase analysis using β-naphthyl acetate as substrate; however, the samples were diluted to 1:10. Reactions were incubated at 30 °C for 15 minutes then stopped using 33.2 µl of 0.3% FAST Blue B. Absorbance was read at 595 ηm on a microtitre plate reader (ELx800, BioTek®, Winooski, VT, USA). Each sample was analysed in triplicate. A standard curve

**Table 1**
Sites of *Tuta absoluta* populations collected in Brazil.

| Populations | Geographic position | Collection date | History* |
|---|---|---|---|
| Anápolis – GO | 16° 29′ 46″ S, 49° 25′ 35″ W | Dec/2011 | Pyr, IGR, OPs, Avermectins |
| Guaraciaba do Norte – CE | 4° 10′ 01″ S, 40° 44′ 51″ W | Feb/2010 | Pyr, OPs, Cartap |
| Iraquara – BA | 12° 14′ 55″ S, 41° 37′ 10″ W | Nov/2011 | Pyr, IGR, OPs, Cartap, Spinosyns |
| Paulínia – SP | 22° 45′ 40″ S, 47° 09′ 15″ W | Aug/2010 | Pyr, IGR, OPs |
| Pelotas – RS | 31° 46′ 19″ S, 52° 20′ 33″ W | Nov/2011 | Pyr, IGR, OP, Cartap |
| Sumaré – SP | 22° 49′ 19″ S, 47° 16′ 01″ W | Sept/2011 | Pyr, IGR, OPs, |
| Tianguá – CE | 3° 43′ 56″ S, 40° 59′ 30″ W | Feb/2010 | Pyr, IGR, OP, Cartap |
| Venda Nova – ES | 20° 20′ 23″ S, 41° 08′ 05″ W | Aug/2011 | Pyr, IGR, OP, Cartap, Bt |

* Pyr – pyrethroids, IGR – insect growth regulator, OPs – organophosphates, Bt – *Bacillus thuringiensis*.