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Azadirachtin avoidance by larvae and adult females of the tomato leafminer *Tuta absoluta*

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ABSTRACT

The tomato leafminer *Tuta absoluta* is a serious worldwide threat to tomato production and its control in open-field tomato has relied heavily on synthetic insecticides, which however are not allowed in organic tomato cultivation. Furthermore, insecticide resistance to synthetic insecticides is already a major concern in populations of the tomato leafminer. Azadirachtin is one of the main biorational pesticides in use today, particularly in organic farming, and has potential as an alternative to conventional insecticides for such use. However, the effects of neem-based products of high azadirachtin content on the tomato leafminer have been little studied and very little is known of their sublethal behavioral effects on this pest species. Here we assessed the insecticidal effect of a commercial neem-based formulation (as a source of azadirachtin) against two populations of the tomato leafminer and its behavioral effects on egg-laying preferences, walking by larvae and leaf-mining. Azadirachtin caused heavy mortality in insect larvae allowing only 2.5–3.5% survival at the Brazilian recommended field-concentration (i.e., 27 mg a.i./L) with negligible difference between the populations tested. Azadirachtin also caused egg-laying avoidance (under free-choice conditions, but not in no-choice conditions) and affected walking by larvae, but not leaf-mining. These results indicate the potential of azadirachtin not only as an insecticide potentially important for organic farming, but also as an egg-laying deterrent minimizing *T. absoluta* infestation although it may also favor escape by larvae to exposure since it sparks behavioral avoidance.

1. Introduction

The tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is currently recognized as a worldwide threat to tomato production after its introduction from South America to Europe, and then to North Africa and the Middle East becoming a major quarantine concern in Asia and North America (Desneux et al., 2010, 2011; Guedes and Picanço, 2012). The caterpillars of this species feed on the leaf mesophyll and also damage tomato flowers, fruits and stems (Miranda et al., 1998; Picanço et al., 1998; Desneux et al., 2010).

The tomato leafminer is difficult to control, particularly in open-field tomato cultivation where the use of conventional synthetic insecticides has been heavily relied upon for its management (Guedes and Picanço, 2012; Guedes and Siqueira, 2012; Tomé et al., 2012). This overreliance on the use of synthetic insecticides quickly led to problems of insecticide resistance, which are widespread in South America and also a quarantine concern because of the likely introduction of insecticide resistant insects in Europe and spread to other regions (Silva et al., 2011; Gontijo et al., 2013; Guedes and Siqueira, 2012; Haddi et al., 2012). Such problems of insecticide resistance and the increasing concerns and restrictions to pesticide use have been favoring the development and growing interest in bioinsecticides or biorational insecticides (Isman, 2006; Rosell et al., 2008), which are particularly useful in organic farming but have not yet been objects of much attention for leafminer control in tomatoes.

Open-field cultivation of organic tomato is also subjected to severe losses by the tomato leafminer without the benefit of the range of insecticides available for use in conventional farming (Guedes and Siqueira, 2012). Even one of the main natural compounds in use, the tetraterpenoid azadirachtin obtained from the neem plant (*Azadirachta indica* A. Juss (Meliaceae)), has received little attention despite its reported insecticidal and behavioral effects on some agriculture pest species (Naumann and Isman, 1995; Liang et al., 2003; Riba et al., 2003; Seljansen and Meadow, 2006; Pineda et al., 2009). In addition, azadirachtin has also been reported as safer for non-target organisms than synthetic insecticides (Medina et al., 2004; Charleston et al., 2006; Mordue et al., 2010). The perception of the general safety of azadirachtin against natural

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enemies has been challenged (Gordon and Gimme, 2001; Medina et al., 2004; Cordeiro et al., 2010; Arnó and Gabarra, 2011; Biondi et al., 2012), but the development of insect populations resistant to this compound seems less likely (Schmutterer, 1995; Feng and Isman, 1995). Therefore, neem-based insecticides remain a recognizable alternative to conventional synthetic insecticides.

The impairment of larval development with reduced pupation is one of the most frequently reported effects of azadirachtin in Lepidoptera larvae (Liang et al., 2003; Bruce et al., 2004; Seljansen and Meadow, 2006; Pineda et al., 2009). However, the behavioral effects of azadirachtin are more controversial and seem to vary with insect species and reports vary even in the same species (Qiu et al., 1998; Liang et al., 2003; Charleston et al., 2006; Seljansen and Meadow, 2006; Hasan and Ansari, 2011). Here we assessed the potential effect of azadirachtin as a management tool in organic tomato farms against the leafminer exploring its insecticidal and behavioral effects. The broad insecticide activity of azadirachtin is likely to extend to the leafminer as well, but the potential behavioral effects of this compound are hard to predict and may either minimize or reinforce its insecticidal activity. As the study of this compound has been neglected in leafminers, we focused our behavioral studies on the relevant and characteristic behaviors of female egg-laying, walking by larvae and leaf-mining.

2. Material and methods

2.1. Insects and insecticides

Two populations of the tomato leafminer were used in the study. They were collected in commercial tomato fields in the counties of Viçosa (20° 45′ 14″ S and 42° 52′ 53″ W) and Araguari (18° 38′ 56″ S and 48° 11′ 13″ W), both in the State of Minas Gerais, Brazil. These two population sampling sites were selected because they are representative of the two major biomes and their respective cultivation systems in Brazil. The Viçosa population is from the Atlantic Forest biome collected in an open-field tomato cultivation system for fresh market. In contrast, the Araguari population is from the Brazilian Savannah collected in an open-field cultivation system for industrial processing. Both populations were established and maintained in laboratory using leaves of commercial tomato (*Solanum lycopersicon* L. var. Santa Clara). Each populations was maintained using a four wooden-cage system (50 × 50 × 50 cm) covered with organza, where each cage was used for each insect developmental phase as described by Silva et al. (2011). The insects were maintained and the bioassays were carried out under controlled conditions of temperature (25 ± 2 °C), relative humidity (75 ± 5%) and photoperiod (12:12 L:D).

The azadirachtin-based insecticide formulation used was Azamax® EC (12 g a.i./L; emulsifiable concentrate; DVA Brasil, Campinas, SP, Brazil), which is registered in the Brazilian Ministry of Agriculture for use in tomato crops (Ministério da Agricultura, 2012). The insecticide was diluted in water (double distilled) and the concentrations used were established from the registered label rate (i.e., 27 mg a.i./L) for tomato fields (Ministério da Agricultura, 2012).

2.2. Survival bioassays

The survival bioassays were carried out using 2nd instar larvae in tomato leaves treated with four insecticide concentrations (0.0 (i.e., control), 1.7, 6.8 and 27 mg a.i./L). Concentrations higher than the label rate (i.e., 27 mg a.i./L) were not considered here since they are unlikely to occur in the field. The tomato leaves were immersed in the insecticide solution for 5 s, air dried and placed in 2 L polyethylene terephthalate (PET) bottles. The bottles had side-openings covered with organza for ventilation. Each PET-bottle contained one insecticide-impregnated leaf with the petiole inserted into a 100 mL glass vial containing water to maintain leaf turgescence as described by Silva et al. (2011). Twenty larvae were placed in each PET-bottle and monitored every other day for nine days recording not only mortality, but also the insect development. Four replicates were used for each combination of concentration and population, where each replicate (i.e., experimental unit) encompassed a single PET-bottle with a tomato leaf and 20 larvae.

2.3. Concentration-mortality bioassays

The concentration-mortality bioassays were carried out using the same experimental units described above and detailed by Silva et al. (2011). Seven insecticide concentrations were used in the bioassays (0.84, 1.68, 3.37, 6.75, 13.5, 27, 54 mg a.i./L), in addition to a control treatment where only water was used. Four replicates with 20 insects each were used for each combination of azadirachtin concentration and insect population. Larval mortality was assessed after 144 h exposure by prodding the larva with a fine hair brush. They were considered as dead if they were unable to move more than their body length.

2.4. Azadirachtin egg-laying avoidance (with and without choice)

Twenty non-sexed adults of the tomato leafminer were released in wooden cages covered with organza (50 × 50 × 50 cm) and containing four tomato leaves with their petioles in 100 mL glass vials (one leaf in each vial). Each leaf was placed in a different corner of the wooden cage, two of them treated with azadirachtin and two untreated ones placed in opposite corners. The azadirachtin concentrations used were the LC_{10}, LC_{50} and LC_{90} values estimated for each insect population, in addition to a control without insecticide (see 2.3. Concentration-mortality bioassays). These concentrations were not lethal with the short exposure time used. The egg-laying preference was assessed for two consecutive days by counting the number of eggs laid in each leaf. Six replicates were used for each population and insecticide concentration; a control with untreated leaves placed in each cage corner was used to test the methodology.

A similar experimental set-up was also used to assess azadirachtin egg-laying avoidance with a no-choice test. In this case, all of the leaves within the wooden cage were subjected to the same treatment – either without azadirachtin (water-treated only), or with azadirachtin concentrations corresponding to the LC_{10}, LC_{50} and LC_{90} values estimated for each insect population, as previously described. Egg-laying was assessed as described for the free-choice test indicated above and six replicates were used for each population and insecticide treatment (including the control).

2.5. Azadirachtin walking avoidance (on inert substrate)

Second and 4th instar larvae were used to assess the potential azadirachtin-mediated walking response in the two leafminer populations following methods adapted from Guedes et al. (2009) and Cordeiro et al. (2010) where the insecticide solution was pipetted on filter paper and let dry at room temperature. Filter papers (Whatman no. 1) containing dried insecticide residue (applied as 1 mL of the LC_{90} solution for the population and air dried for 20 min) were placed on Petri dishes (9.0 cm diameter) with their inner walls coated with Teflon PTFE® (DuPont, Wilmington, DE, USA) to prevent insect escape. Half of each arena was covered with an untreated half of filter paper with half remaining treated with azadirachtin (no mortality was observed with the exposure time used). The treated and untreated zones were