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EVALUATION OF THE SYNTHETIC MAJOR COMPONENT OF THE SEX PHEROMONE OF *Tuta absoluta* (MEYRICK) (LEPIDOPTERA: GELECHIIDAE)

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Abstract—In wind-tunnel bioassays, dispensers loaded with 1 μg of the synthetic major component (3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrienyl acetate (TDTA) of the sex pheromone emitted by *Tuta absoluta* (Meyrick) females were found to be highly attractive to conspecific males. Field experiments were conducted to evaluate the efficacy of five trap designs. The best trap, baited with 100 μg of the synthetic sex pheromone caught on average 1200 males per trap per night, while those baited with virgin females caught only 201 males. The male response to this pheromone is restricted to the same early-morning time window during which females exhibit calling behavior. The high biological activity of the synthetic pheromone suggests that it could be useful for pest monitoring and in mating disruption.

Key Words—*Tuta absoluta*, sex pheromone, (3*E*,8*Z*,11*Z*)-3, 8,11-tetradecatrienyl acetate, field tests, monitoring.

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INTRODUCTION

Tuta (=Srobipalpuloides) absoluta (Meyrick) (Lepidoptera:Gelechiidae) is a key pest of tomato *(Lycopersicon esculentum* Mill.) due to the damage it causes and the lack of ecologically acceptable methods for its control. It has been considered to be the major limiting factor for tomato production in South America (Bahamondes and Mallea, 1969; Quiroz, 1976).

IPM programs for *T. absoluta* may be achieved through the use of sex pheromone. Populations of other Gelechiidae, such as *Keiferia lycopersicella* (Walsingham) have been efficiently monitored with traps baited with sex pheromone (Jenkins et al., 1990). Trumble and Alvarado-Rodriguez (1993) reported a successful management strategy (sex pheromone for monitoring the population as well as for mating disruption) for the control of *K. lycopersicella* in industrial tomato crops. For *Phthorimae operculella* (Zeller), pheromone traps have been used as part of IPM strategies (Kennedy, 1975; Raman, 1984; Salas et al., 1985).

Using *T. absoluta* virgin females, Quiroz (1978) captured more than 100 males/trap/day, and Uchôa-Fernandes and Vilela (1994), using the same technique, compared different trap designs, heights, and displacement in tomato fields for capturing *T. absoluta* males. They reported high specificity and sensitivity of traps baited with natural pheromone, besides being more economical and convenient than the light traps.

In this work we report laboratory and field evaluations of the major synthetic component of the sex pheromone of *T. absoluta*, (3*E*, 8*Z*, 11*Z*)-3,8,11-tetradecatrienyl acetate (TDTA), which was identified and synthesized by our group (Attygalle et al., 1995, 1996). Its structure was confirmed by Griepink et al. (1996).

METHODS AND MATERIALS

Insect Rearing. Larvae of *T. absoluta* were collected on tomato in fields around Viçosa, MG, Brazil and reared on artificial diet (Pratissoli, 1995) at 23 $\pm$ 2°C, 75 $\pm$ 5% relative humidity and a 14L:10D photoregime. After separation of females and males (Coelho and França, 1987), pupae were maintained in separate rooms to avoid exposure of males to female sex pheromone and consequent development of habituation (Bartell, 1977).

Wind-Tunnel Bioassays. Bioassays were conducted in a wind tunnel (1.5 m long and 0.5 m height) at 24 $\pm$ 1.5°C, 83 $\pm$ 3%, relative humidity, and air flux of 30 mm/sec. The TDTA source was placed at a height of 0.3 m and 1.0 m from the point of insect release. After each bioassay, the tunnel and its accessories were thoroughly cleaned with neutral detergent and water. The synthetic pheromone was dissolved in doubly distilled hexane and appropriate volumes were impregnated on a rubber septum and allowed to air dry for 24 hr. For field trials, treated septa were sealed in aluminum foil and transported in an ice box. All septa used for impregnating the pheromone were previously extracted for 24 hr with distilled CH_2Cl_2 in a Soxhlet extractor.

The attractiveness of the synthetic acetate (0.01, 0.1, 1 and 10 μg/septum) was compared to that of nine virgin females during their calling period (Hickel and Vilela, 1991; Hickel et al., 1991; Uchôa-Fernandes et al., 1995a). Hexane (100 μl) was placed on a rubber septum, air dried, and used as control. The experimental design was entirely randomized with 10 replications, each consisting of three males (1 to 3 days old), released from glass tubes open on both sides. Each observation lasted 5 min and the response of males was computed once, after which they were eliminated.

Field Experiments. Field experiments were conducted in two tomato crops (staked and industrial) to evaluate the efficiency of five types of traps as well as to determine the attractiveness of TDTA. In addition, the variation of insect capture during the day and during the tomato plant phases was studied.

Staked Tomatoes. These experiments were conducted in a 20-ha staked (commercial) tomato crop (Santa Clara variety), in Araguari, MG, Brazil during April 1–11, 1994. The following five trap designs were evaluated: (1) commercial Delta sticky trap (AgriSense Ltd.) with a vertical height of about 7 cm at the triangular opening and a base of 29 $\times$ 30 cm; (2) CICA-Q trap (home made), which consisted of a white square plastic tray (24 $\times$ 37 $\times$ 6 cm) covered with a thin plate (V shaped) and held together with four strong metal wires; (3) CICA-R trap (home made) consisted of two black round trays (commonly used for house gardening in Brazil), with the larger one (32 cm in diameter) serving as the base and the smaller one (20 cm in diameter) serving as the top. The two plates were held together by three strong metal wires equidistant from each other. Both traps contained water and detergent to trap the insects; (4) home made PVA 200 trap (Uchôa-Fernandes and Vilela, 1994) made up of a white PVC tube (200 mm diameter) and an adhesive cardboard inside, similar to the Delta trap, and (5) home made PVC-M 200 trap, which was a modification of PVA 200 trap with two lateral longitudinal openings.

Each trap was baited with 1 μg of TDTA and placed at 30-m intervals and at 1.2 m above ground (Uchôa-Fernandes and Vilela, 1994) during three days. The experimental design was a Latin square (Perry et al., 1980), and the number of insects caught in each trap, with five replicates (25 traps) was counted daily. The highest number of insects caught was with CICA-R traps and, hence, some of the subsequent tests were conducted with this type of trap.

The periodicity of male capture over a 24-hr period was evaluated by using Delta traps containing 1 μg of the synthetic TDTA impregnated on a rubber septa, with four replicates, for four 24-hr periods.

Industrial Tomatoes. These experiments were conducted in Petrolina, PE, Brazil during June 25–July 5, 1994 on a commercial 20-ha industrial tomato crop (cultivar IPA). The five traps described in the previous experiment were evaluated at 0.4 m above the ground over a five-day period. Each trap was baited with a rubber septum containing 1 μg of the synthetic TDTA and its position remained unchanged during the experiment. The experiment was arranged in a randomized block with five replications.

In order to evaluate the variation of insect capture during the day, traps containing 1 μg of the TDTA (five replications) were spaced 30 m apart. To evaluate the effect of trap height, CICA-R traps were placed in three different crop phases. CICA-R traps containing 1 μg of the TDTA were placed at 0.2, 0.4, 0.6, 0.8, and 1.0 m above ground in the first crop phase, i.e., just before planting. CICA-R traps containing 1 μg of the TDTA were placed at 0.2, 0.4, 0.6, 0.8, and 1.0 m above ground in second crop phase (0.2-m high plant). CICA-R traps containing 1 μg of the TDTA were placed at 0.2, 0.4, 0.6, 0.8, and 1.0 m above ground in the third crop phase, which was crop with flowers. Five replications were used.

Statistical Analyses. The data obtained in wind-tunnel bioassays were transformed into arcsine (X). After that they started to present normal distribution and were statistically analyzed at 5% significance (Scott and Knott, 1974). The following statistical analyses were utilized for three parameters evaluated: in field experiments: The attraction data were transformed into log ($X + 1$). After that, they started to present normal distribution and they were statistically analyzed at 5% significance (Scott and Knott, 1974). The data of periodicity of male capture were transformed into $\sqrt{(X + 0.5)}$. After that, they started to present normal distribution and were statistically analyzed at 5% significance (Duncan). The trap design data with staked tomatoes presented normal distribution and were analyzed without transformation. The trap design data obtained in the field experiments with industrial tomatoes were transformed into $\sqrt{X}$. After that they started to present normal distribution and were statistically analyzed at 5% significance (Scott and Knott, 1974).

The data for height of traps for the first phase (before planting) as well as for the second phase, (20-cm-high plants), presented normal distribution and were analyzed without transformation, whereas the data for the third phase (plants with flowers) were transformed into $\sqrt{X}$. All data were subjected to ANOVA analysis and the averages were compared by Scott-Knott test (Scott and Knott, 1974) or the Duncan test at 5% significance.

RESULTS

Wind-Tunnel Bioassays. The response of *T. absoluta* males to the synthetic TDTA was immediate (within 1–5 min), with males landing on the pheromone source, indicating a possible application in insect trapping. The highest response was obtained with 0.1, 1.0 and 10.0 μg (Figure 1). Male wing fanning was significantly higher with 1 μg than that obtained with other doses of the synthetic pheromone (Figure 1).

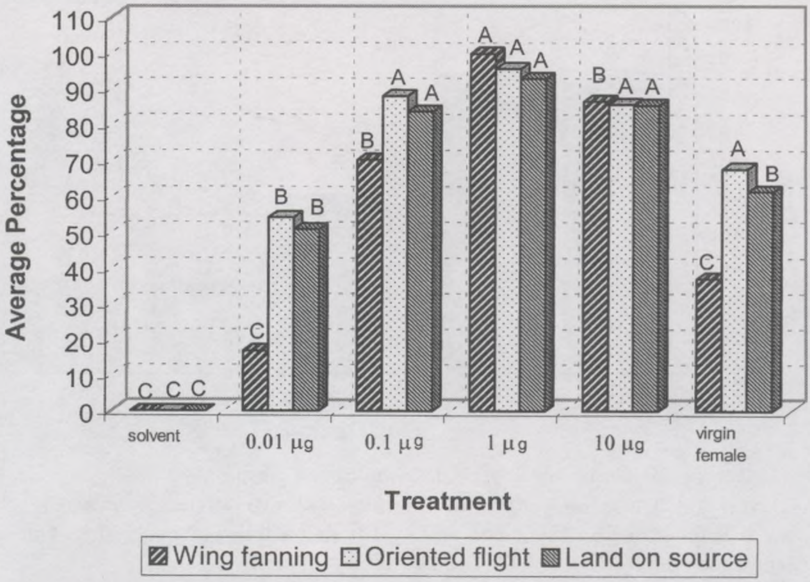

FIG. 1. Average percentage of *T. absoluta* males that respond to different amounts of TDTA, to virgin females, and to the solvent in a wind tunnel. Bars followed by the same letter are not significantly different ($\alpha = 0.05$, Scott-Knott test, $N = 10$).

Response to Trap Design in the Field. The results of the experiments with staked and industrial tomatoes are shown in Figures 2 and 3, respectively. In staked tomato plantations, both CICA-R and CICA-Q traps provided the best insect captures (Figure 2) while in the industrial tomato crops, at initial insect infestation (low), the CICA-R and PVC traps captured the highest number of males, but the captures were not significantly different from the CICA-Q and Delta traps (Figure 3). The adhesive cardboard of the PVC traps presented the disadvantage of not retaining newly attracted insects due to surface saturation. This was not observed with traps that used water and detergent to trap the insects in highly infested fields. Consequently, a greater number of insects was trapped with both CICA-Q and CICA-R traps. However, in a low infestation situation, CICA-Q traps did not present significant difference in the number of insect trapped. The CICA-R trap, which was black and totally open, was more efficient probably due to its format.

Concentrations of TDTA in the Field. The highest response of *T. absoluta* males was obtained at a concentration of 100 μg of the main component of the sex pheromone TDTA per trap (Figure 4).

Periodicity of Male Captures. In the staked and industrial tomato crops, the capture of the males occurred between 5 and 9 AM, with a peak around 7 AM

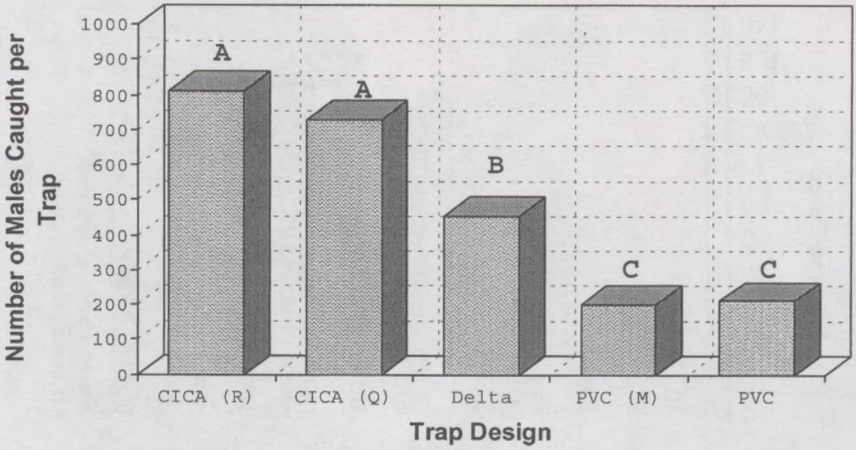

FIG. 2. Average number of males of *T. absoluta* caught per morning per trap in a staked tomato crop in Araguari, MG, Brazil, during 1994, with five different trap designs. Each trap was baited with 1 μg of synthetic TDTA. Histograms followed by the same letter are not significantly different ($\alpha = 0.05$, Scott-Knott test, $N = 5$).

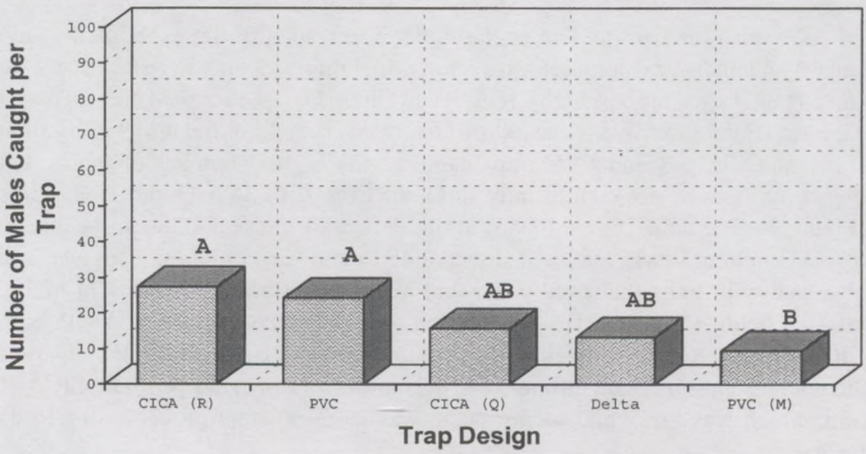

FIG. 3. Average number of males of *T. absoluta* caught per morning per trap in an industrial tomato crop in Petrolina, PE, Brazil, during 1994, with five different trap designs. Each trap was baited with 1 μg of synthetic TDTA. Histograms followed by the same letter are not significantly different ($\alpha = 0.05$, Duncan test, $N = 5$).

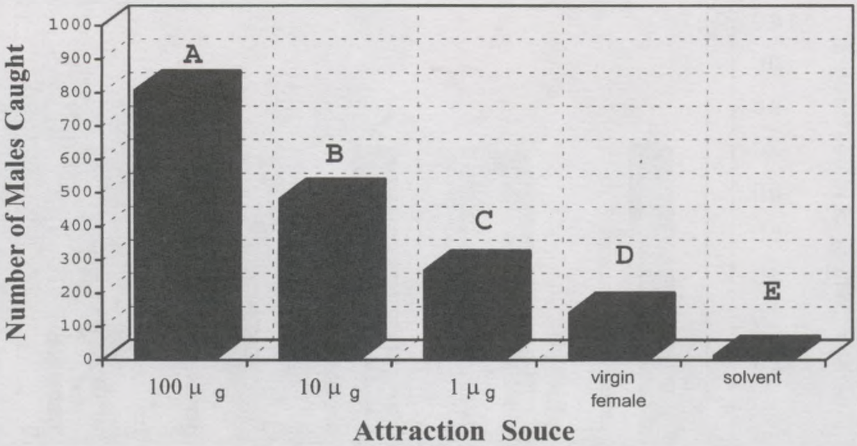

FIG. 4. Average number of males of *T. absoluta* caught per morning per trap in a staked tomato crop in Araguari, MG, Brazil, during 1994, with different concentrations of the synthetic TDTA. Histograms followed by the same letter are not significantly different ($\alpha = 0.05$, Scott-Knott test, $N = 5$).

(Table 1). Insect infestation was lower during the evaluation period in the industrial tomato crops compared to the staked tomato crops. Insect capture with synthetic pheromone baits was affected by temperature. Earlier capture generally took place when the temperature was lower ($r = -0.79$, $P < 0.01$). However, no effect of relative humidity was noted ($r = 0.79$, $P = 0.14$).

Height of Traps. In the industrial tomato crops, when the soil was prepared for tilling, traps placed at a height of 20 cm above ground presented the best results, capturing the highest number of *T. absoluta* males (Figure 5). When the tomato plants were about 20 cm high, traps placed at heights of 20, 40, and 60 cm captured

TABLE 1. AVERAGE NUMBER ($N = 4$) OF MALES OF *Tuta absoluta* CAUGHT PER MORNING PER TRAP, IN DELTA TRAPS BAITED WITH 1 μG OF SYNTHETIC TDTA IN A FIELD WITH STAKED TOMATO CROP (ARAGUARI, MG, BRAZIL, 1994)

Evaluation date	5-6 AM	6-7 AM	7-8 AM	8-9 AM	General average
April 8	13.98cAB	511.91aA	190.00Ba	3.48dA	111.02A
April 9	22.90cA	432.48aB	48.53bB	0.00dA	69.03B
April 10	2.30aC	6.69aC	5.68aC	2.00aA	3.93C
April 11	5.96aBC	9.98aC	0.46bC	0.00bA	2.97C
General average	9.83c	151.79a	36.27b	1.06c	

Averages followed by the same small letter in a row or capital letters in a column did not differ significantly (Duncan test, $P < 0.05$).

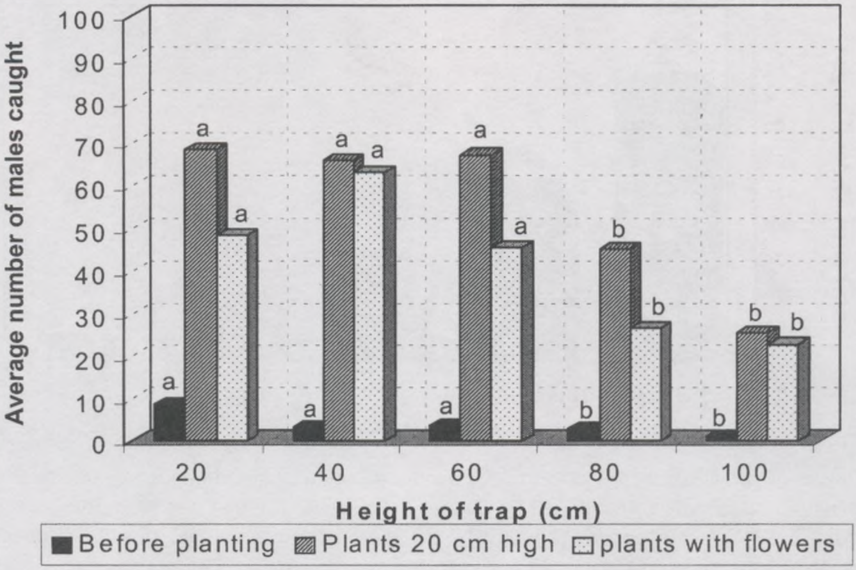

FIG. 5. Average number of males of *T. absoluta* caught per morning per trap in three different stages of an industrial tomato crop in Petrolina, PE, Brazil, during 1994. Each trap was baited with 1 μg of synthetic TDTA. In each stage, histograms followed by the same letter are not significantly different ($\alpha = 0.05$, Scott-Knott test, $N = 5$ for each stage).

a higher number of insects. However, the results obtained from different heights were not significantly different.

DISCUSSION

Wind-Tunnel Bioassays. Typical responses were recorded in wind-tunnel bioassays. Wing fanning represents a state of high sexual excitement in males (Cardé, 1984) and most of the time resulted in landing on the pheromone source. Males flew towards the source impregnated with 0.1, 1.0, and 10 μg of TDTA, as well towards virgin females, whereas there was no significant difference between 0.01 μg and solvent (Figure 1). Similar results have been reported by Hickel et al. (1991), working with virgin females.

Response to Trap Design in the Field. The best results were obtained with the CICA-R trap, which was probably due to its completely open shape. These results were in agreement with those of Wyman (1979), working on monitoring of *Keiferia lycopersicella* using synthetic pheromone. He concluded that a high trap efficiency was related to ease access of insects to traps. The cost of each unit of the CICA-R trap, Delta trap and its sticky cardboard, CICA-Q trap, and PVC trap was of US$2.95, 5.00, 7.00, and 1.92 (not including the sticky cardboard), respectively.

Concentrations of TDTA in the Field. Each higher concentration of TDTA trapped more insects, possibly due to greater amounts of pheromone released. Tumlinson et al. (1994) have reported a higher release rate of the synthetic pheromone of *Manduca sexta* (L.) in the field from the rubber septa impregnated with higher amounts.

Periodicity of Male Captures. In the staked tomato crops, the results on periodicity of male captures were in agreement with those of Uchôa-Fernandes et al. (1995b), who used virgins female as baits. The difference in the number of insects captured in the two areas was most likely due to the lower insect infestation during the evaluation period in the industrial tomato crops compared to the staked tomato crops.

The influence of temperature on pheromone catch is in agreement with that reported by Cardé and Roelofs (1973), for *Holomelina immaculata* (Rearkirt), for which the temperature influenced both the initiation and periodicity of captures. Cardé et al. (1974) confirmed that temperature can modify the calling period or the male response. McNeil (1991) reported that several factors affect the emission and reception of pheromones, mainly temperature, which affected the calling period of the females and, consequently, the male response.

In summary, these results suggest that a partial application of low cost CICA-R traps loaded with 100 μg of the synthetic pheromone (TDTA) for monitoring *T. absoluta* populations. In soil prepared for sowing, the height of the trap should be 20 cm, and for plants that are around 20 cm high, the traps should be placed at a height of 20–60 cm and should remain in place until harvest. With growing plants, the height of the traps should be about 60 cm, since a higher degree of attack takes place in the medium and top parts of the canopy (Coelho and França, 1987; Pratissoli, 1995; Picanç et al., 1995).

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