Studies on the development of a mating disruption system to control the tomato leaf miner, *Tuta absoluta* Povolny (Lepidoptera: Gelechiidae)

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Running title: Mating disruption of Tomato Leaf Miner

Abstract

BACKGROUND: The tomato leaf miner (*Tuta absoluta* Povolny) has rapidly colonized the whole Mediterranean and South-Atlantic coasts of Spain, and it has become a key problem in both outdoor and greenhouse crops. New control methods compatible with biological control are required and mating disruption appears to be a perfect method in current agriculture as it is an environmentally-friendly and residue-free technique. IPM packages tested have included the use of pheromones to detect populations, but there has not been much previous research on mating disruption of *T. absoluta*. In this work, pheromone doses varying from 10 to 40 g ha^{-1}, emitted at a constant rate over four months, were tested in greenhouses with different levels of containment, in order to evaluate the efficacy of mating disruption on *T. absoluta*.

RESULTS: Trials on containment level revealed that the flight of *T. absoluta* was satisfactorily disrupted with an initial pheromone dose of 30 g ha^{-1}, and levels of damage did not significantly differ from those in reference plots with insecticide treatments. Later efficacy trials confirmed our previous experiences, and release studies showed that control of damage and flight disruption were taking place when releasing, at least, 85 mg pheromone per ha per day.

CONCLUSION: Effective control using pheromone application against *T. absoluta* can be achieved, in greenhouses with high containment levels, for 4 months, with initial doses of 30 g ha^{-1}. Further research must be conducted in order to evaluate the prospect of outdoor application of mating disruption systems.

Keywords: *Tuta absoluta*, mating disruption, pheromone, mesoporous dispensers

1 INTRODUCTION

Since the first detection in Spain of *Tuta absoluta* Povolny (Lepidoptera: Gelechiidae) in 2006 (Castellón, Eastern Spain), the tomato leaf miner (TLM) has invaded the whole Mediterranean and South-Atlantic coasts of the Iberian Peninsula and many other interior regions of Spain.[1] Due to its rapid colonization and the high levels of damage it wreaks, intervening in the control of *T. absoluta* has become a key issue for tomato production, both in outdoor and greenhouse crops. Being a moth of the Gelechiidae family, *T. absoluta* is a leaf miner that attacks tomato plants in all stages of development, damaging the stems, apices, flowers and fruits, in addition to mining the leaves.[2] In high densities, TLM is able to cause significant production losses in tomato crops.[3]

The control of this pest requires expensive treatments with translaminar active chemicals, or the repeated application of chemicals in order to affect larvae outside the galleries. However, some investigators have proven the development of resistance to some insecticides in this moth [4-6] and the repeated use of the few authorized active ingredients could hasten the appearance of such resistance. It must be taken into account that, in many cases, these insecticides could also affect natural enemies, making the consolidation of biological control systems impossible. In fact, a broad variety of parasitoids and predators have been reported attacking egg, larval or pupal stages of *T. absoluta*.[1,2,10-13] Spanish tomato producers have been encouraged to control virus vector insects, such as thrips and whiteflies, by augmentative releases and conservation of beneficial insects in greenhouses, as these vector insects have become resistant to many insecticides.[7-9] Thus, alternate means of suppressing TLM populations are needed in order to prevent the deleterious side effects of repeated applications of insecticides. IPM packages could include other cultural, biotechnological and biological methods, such as application of entomopathogenic fungi and nematodes,[14,15] and treatments with *Bacillus thuringiensis*, whose efficacy has already been demonstrated.[16-19]

Pest management tactics could also include the introduction of techniques based on pheromones. Since virgin TLM females release a sex pheromone that strongly attracts males,[20] efforts were directed towards identifying it. *T. absoluta* pheromone was characterized as (3*E*,8*Z*,11*Z*)-tetradecatrienyl acetate (TDTA hereafter).[21,22] This component represents about 90% of the volatile material found in the sex gland of calling males, but a minor component (~10%) was identified as (3*E*,8*Z*)-tetradecadienyl acetate (TDDA).[23,24] These findings permitted the development of pheromone dispensers in order to test attract and kill[25] or mating disruption[26] control methods against TLM. Up until now, mating disruption has been tested in South America with doses ranging from 10 to 80 g ha^{-1}, in outdoor plots of under 200 m^2, without success in controlling TLM and without studies of pheromone release. Several companies have developed pheromone dispensers to detect and monitor *T. absoluta* populations but there is only one reported experiment of mating disruption in Spain, testing doses from 0.15 to 2 g ha^{-1}, with unsuccessful results.[27]

Our present work shows the results of mating disruption field trials testing pheromone doses from 10 to 40 g ha^{-1}, emitted at a constant rate over four months. Mating disruption was tested in minimum-containment mesh greenhouses, as well as in high-containment glass greenhouses. Efficacy and requirements in order for mating disruption to be successful are discussed.

2 MATERIALS AND METHODS

2.1 Mesoporous pheromone dispensers

New mesoporous pheromone dispensers were developed for the field trials carried out in the present work. All of them were formulated based on a mesoporous material,[29,30] supplied by Ecologia y Protección Agrícola SL (Valencia, Spain). The dispensers were cylindrical tablets 9 mm in diameter, of various lengths and initial loads: 20, 60 and 80 mg (T20, T60 and T80, respectively). The formulations contained the main compound of the *T. absoluta* sex-pheromone 3,8,11-tetradecatrienyl acetate (TDTA), supplied by Ecologia y Protección Agrícola SL (Valencia, Spain).

The dispensers were hung inside polypropylene (PP) baskets, also supplied by Ecologia y Protección Agrícola SL (Valencia, Spain). The PP baskets were 50 mm wide and 90 mm long, and the pheromone was released through a 6×5 mm mesh. The pheromone basket had a hanger at the top to attach it to the trellis strings.

2.2 Containment level trials

2.2.1 Low-containment trial. An initial study was conducted to evaluate the prospects of mating disruption applied to *T. absoluta* in three commercial mesh greenhouses growing tomatoes (*L. sculentum*, var. Valenciano), located in El Perelló (Valencia, Spain). The trial covered the whole summer cycle of the crop, from 4th June to 26th August 2008. Plots were arranged in two 500m^2 and one 300m^2 minimum-containment mesh greenhouses (9×6 threads cm^{-2}) as follows: each 500 m^2 greenhouse was divided, with the same mesh, into two 250 m^2 plots in order to apply two different pheromone doses in four separate plots: three 250 m^2 plots with 10 g ha^{-1} and the fourth to test a higher dose of 40 g ha^{-1}. To obtain the aforementioned doses per ha, two different mesoporous mating disruption dispensers were developed, with loads of 20 mg and 80 mg TDTA (T20 and T80, respectively). Mating disruption treatment was installed following transplantation on 4th June, together with monitoring pheromone traps. In all plots, pheromone dispensers were installed at a density of 500 dispensers ha^{-1}, distributed inside the greenhouse attached to trellis strings, at a height of at least 1.8 m. The 300 m^2 mesh greenhouse was the reference plot with conventional insecticide treatments.

Insecticide treatments were applied in accordance with weekly assessments, when the percentage of live stages of *T. absoluta* exceeded 10%. The reference plot had the conventional treatments used by the grower: Indoxacarb (Steward® Indoxacarb 30% (Du Pont Ibérica SL, Barcelona, Spain) at 100 g ha^{-1}, applied on 25th June, 12th July and 1st August) alternated with Spinosad (Spintor* 480 SC (Dow AgroSciences Ibérica, Madrid, Spain) at 300 g ha^{-1} applied on 7th July). In view of the results, the mating disruption plots were also treated with Indoxacarb on 15th July and 1st August, as successful control of the pest had not been achieved.

2.2.2 High-containment trial. In a second experiment in 2009, a dose of 30 g ha^{-1} (with T60 mesoporous dispensers, loaded with 60 mg of TDTA) was tested in a 1000 m^2 plastic greenhouse, property of Fundación Ruralcaja, located in Paiporta (Valencia, Spain). This was a high-containment greenhouse, which included a mesh cover (10×14 threads cm^{-2}) on ventilation windows and double doors. The crop was tomato, var. Valenciano, in hydroponic substrate, begun in January 2009. Mating disruption dispensers were applied on 4th March 2009, at the same density and position described in the low-containment trial above. The trial was conducted on the crop until 20th July (20 weeks). Monitoring pheromone traps were placed on 19th January to obtain population data prior to pheromone application.

A second 1000 m^2 greenhouse, with the same crop and containment features as the first, was used as reference plot, using the conventional chemical control applied by the grower. Unlike the first trial, treatments with *Bacillus thuringiensis* Ber., var. *Kurstaki*;

(Bt hereafter) at 0.13% (Costar, Syngenta Agro SA, Madrid, Spain), were applied to combat any presence of *T. absoluta* live stages, in accordance with weekly assessments. It was applied on 16th and 30th March, 20th April and 8th June 2009 in the Reference plot. In addition, Indoxacarb (100 g ha^{-1}) was applied when the threshold of 15-20% of plants with live stages was exceeded (8th May and 20th May in the Reference plot).

2.3 Efficacy trials

After performing preliminary trials and having checked the influence of greenhouse containment level, two new efficacy trials were carried out in order to precisely evaluate the mating disruption technique with the T80 mesoporous dispensers.

2.3.1 First trial: 2009. The first efficacy trial was conducted in a high-containment greenhouse property of the company Tomspring, located in Alicante (Spain). Four plots of tomato (var. Valenciano) were arranged inside a 2600 m^2 mesh greenhouse (16×10 threads cm^{-2}) with a plastic cover. Following plantation, plastic sheets were used to divide up the plots, as shown in Figure 1.

Pheromone treatment began on 8th October 2009, and the trial lasted the entire crop cycle, which was 20 weeks (until February 2010). Pheromone dispensers were applied at 500 dispensers ha^{-1} (dose of 40 g ha^{-1}), with the same method and positioning as described for the previous experiments. Monitoring pheromone traps were also installed on 8th October.

Chemical treatments in the Reference plot were applied in accordance with the weekly assessment of live stages of *T. absoluta*: one treatment with Indoxacarb (100 g ha^{-1}) on 13th October, one with Etofenprox (Trebon 30 LE (Certis, Alicante, Spain) at 0.1% applied on 24th November) and seven applications of *B. thuringiensis* (Bt at 0.05% applied on 13th and 27th October, 3rd, 10th, 17th, 24th November, and the 22nd December).

2.3.2 Second trial: 2010. The mating disruption efficacy trial was performed in one 1000 m^2 and three 250 m^2 high-containment glasshouses. The crop was tomato, var. Valenciano in hydroponic substrate. The trial consisted of three MD plots and two reference plots, arranged as follows: the 1000 m^2 glasshouse was divided by a thermal blanket into two plots of 500 m^2 each, in order to set up a mating disruption plot and a reference plot for conventional treatments. Two of the other three 250 m^2 glasshouses were set up for mating disruption treatment, and the remaining glasshouse also acted as a reference plot. Monitoring pheromone traps were placed on 25th January 2010, while MD pheromone dispensers were applied on 8th February, at 500 dispensers ha^{-1} (40 g ha^{-1}) density according to the usual method and placing. This trial covered the tomato cycle up to the harvest, which was after 23 weeks.

The 500 m^2 reference plot had four *B. thuringiensis* treatments (Bt at 0.13%, applied on 3rd May, 24th May, 26th June and 2nd July), in accordance with the weekly assessment of live stages. Meanwhile, the remaining plots only received the final treatment, applied on 2nd July.

2.4 Evaluation of treatment efficacy

In all cases, in order to evaluate the efficacy of mating disruption, three commercial Delta traps, with sticky bases, supplied by Biagro SL (Valencia, Spain), were placed in each untreated or pheromone treated plot. Each trap was baited with commercial pheromone monitoring lures from Pherobank™ (Wageningen, The Netherlands). The evaluation was made by comparing the moth trap catches from the mating disruption plots with those obtained from the reference plots. Captures on sticky bases were recorded and replaced weekly, whereas the monitoring lures were replaced every 40 days. The absence of trap catches during mating disruption treatment is a good indication of the effectiveness of the technique, but crop damage assessment provides the final proof.[28] In order to assess the percentage reduction in males captured in pheromone traps between the MD and reference plots, the mating disruption index (MDI) was calculated according to the following formula:

$$MDI = (1 - (x/y)) \times 100$$

where x is the number of males captured in the MD plots and y is the number of males captured in the control plots.

To assess crop damage, 40 plants from the central area of each plot were randomly evaluated weekly, and the number of galleries and live stages of *T. absoluta* (eggs, pupae and larvae) were recorded. Treatment efficacy results were given as a percentage of plants with live stages. For efficacy trials, the percentage of damaged fruits was also recorded by revising every fruit from the 40 selected plants in the first week of harvest.

2.5 Pheromone release profiles

In parallel with the greenhouse trials, additional dispensers were simultaneously aged in nearby areas inside greenhouses of the same type, for 90 days in 2008, 124 days in 2009 and 164 in 2010 trial. Residual TDTA content was extracted at different ageing times. Three dispensers for ageing time were extracted by solvent-extraction, at 40ºC during 2 h, with magnetic agitation and dichloromethane as solvent.

TDTA content was measured by gas chromatography with flame ionization detector (GC/FID) using a Clarus®500 gas chromatograph (PerkinElmer Inc., Wellesley, USA). Extracts were analyzed and quantification was made using dodecane as internal standard. All injections were made onto a ZB-5 (30 × 0.25mm × 0.25 mm) column (Phenomenex Inc., Torrance, CA), held at 120ºC for 2 min and then, raised at 20ºC/min^{-1} up to 260ºC, maintained for 3 min. The carrier gas was helium at 1.5 ml min^{-1}. The amounts of pheromone and the responses were connected by fitting a linear regression model, y = a+bx, where y is the amount of pheromone and x is the ratio between pheromone and dodecane responses.

The residual pheromone load, called P (µg), for each dispenser was fitted by polynomial regression. The independent variable was the number of days that dispensers had been installed in the plot, which was called t (days).

2.6 Statistical analysis

Moth catches in pheromone baited traps, per trap and week, were analyzed using a one-way ANOVA model, followed by an LSD test ($P<0.05$), to assess the significance of differences observed in captures between treatments.

Contingency tables and the Pearson's chi-square (χ^2) test were used to test the correlations between treatments in regard to the number of damaged plants with live stages of *T. absoluta*. A significant chi-square statistic ($P<0.05$) is evidence for the existence of differences. Analysis was performed with SPSS 16.0.1 software (SPSS Inc., Chicago, IL).

3 RESULTS

3.1 Low-containment level trial: El Perelló 2008

Figure 2a shows population dynamics of *T. absoluta*, as number of moths captured per trap and day (MTD), for the different plots set up in the 2008 trial. For statistical analysis, data from weeks 1 to 5 were grouped into a single period in order to homogenize the data, together with that from weeks 9 and 10. One-way ANOVA performed with sqrt-transformed catch data showed that there were no statistical differences among catches obtained in the Reference plot and those obtained in any of the mating disruption plots ($F=0.52$; $df=2,53$; $P=0.52$). Therefore, none of the pheromone treatments (T20 and T80) had achieved a reduction in moth population in comparison with the Reference plot with chemical control, and average MDI values were around 40% during some periods.

The most important issue is the evolution of the percentage of plants with live stages throughout the different assessments, which is depicted in Figure 2b. From March to June, no live stages of *T. absoluta* were found in any plot. Up to the beginning of July, chemical treatments were only applied to the Reference plot (indicated by black arrows in Fig. 2b), in the 3rd July assessment it was found that around 20% and 10% of the plants in the mating disruption plots (T20 and T80, respectively) had been attacked by *T. absoluta*. Thus, the presence of live stages had increased in the pheromone treated plots but not in the chemical Reference plot. According to the level of damage, it was decided to perform Indoxacarb treatments in all the plots, which happened on 12th July and 1st August. The 12th July treatment managed to restrain the increase of population as observed in the 17th July assessment (Fig. 2b), with no significant differences between treatments (χ^2, P=0.822). However, at the end of the cycle (31st July assessment in Fig. 2b), the damage level in the Reference plot was significantly lower than that in the pheromone treatment plots (P=0.018), which means that pheromone dispensers had not achieved the disruption of *T. absoluta* population, with any of the pheromone doses tested.

3.2 High-containment level trial: Paiporta 2009

Before commencing MD treatment in the 6th week, 5 and 29 moths were captured in MD and Reference plots, respectively, over 5 weeks. However, data up to 14th week were not employed for statistical analysis, as population levels were not high enough to provide reliable information (Fig. 3a). Therefore, statistical analysis was performed with data from the 15th to 25th weeks, when TLM populations had increased sufficiently in order to detect the differences between plots, which were statistically significant in the mentioned period ($F=98.89$; $df=1,58$; $P<0.001$). Average catches in the Reference plot were significantly higher, which means that the pheromone dispensers installed had on this occasion a presumptive disruptive effect on the moth's flight, obtaining an average MDI of 94.4%.

Regarding damage assessment, the presence of live stages of TLM in both pheromone-treated and Reference plots is shown in Figure 3b. During March no recordings of live stages were obtained in any of the plots. Damage significantly increased in the Reference plot in April, but the applications of Indoxacarb, on 8th and 20th March, together with the application of Indoxacarb+Bt on 8th June, reduced the presence of TLM. Regarding the mating disruption plot (MD), the percentage of damaged plants only exceeded 5% at certain times and the same level of attacked plants was recorded in the Reference plot during June. Damage levels ranged from 2–5% in the MD plots during the last month without any additional chemical treatment, being significantly lower in comparison with the Reference plot ($P=0,006$).

3.3 Efficacy trial: Alicante 2009

TLM population levels were different both during and following November (9th week in Fig. 4a), so they were compared separately. Mean captures from the three MD plots differed significantly from those obtained in the Reference plot ($F=34.37$; $df=1,75$; $P<0.001$) up to the 9th week (Fig. 4a), obtaining an average MDI of 84.6%.

During November, the Reference plot received one treatment with Etofenprox and four with Bt. These treatments affected larval instars and achieved a population reduction in the Reference plot. As a consequence, differences between the MD and Reference plots were not so evident at the end of the cycle (10th to 21st weeks; $F=4.03$; $df=1,108$;

P=0,047). However, captures in MD Plots were higher than expected during the last week, which could signify the dispenser having reached the end of its lifespan.

Damage assessments showed that no live stages of *T. absoluta* were detected in the Reference plot throughout the period under study (Fig. 4b), and any presence of *T. absoluta* in the MD plots was only detected after several assessments. However, live stages never exceeded the threshold of 5% presence on plants and the differences between the MD and Reference plots were not significant in any case according to χ^2 test (P>0.05). No damaged fruit was observed throughout the trial in either the pheromone-treated or Reference plots.

3.4 Efficacy trial: Paiporta 2010

TLM populations were virtually zero, both in the 500 m^2 and 250 m^2 plots, up to the 14th week (Fig. 5a). Twenty-one and 49 moths were captured in 14 weeks in the MD and Reference plots, respectively. From this date, statistical analysis showed that average captures obtained in MD plots differed significantly from those of Reference plots (F=31.96; df=1,148; P<0.001) up to the date of harvest. Thus, a flight disruption effect was taking place in the pheromone treated plots, and the average MDI was 83.2%. However, a changing trend was observed in all the MD plots, as captures were higher than expected during the two last weeks of the trial. This could signify the dispenser having reached the end of its lifespan.

The average percentage of plants with live stages of *T. absoluta* in the MD plots was lower than that in the Reference plots up until June. Specifically, the 26th April assessment (Fig. 5b) showed no significant differences in the percentage of plants with live stages between the pheromone treated and reference plots (P=0.174). From April up until the end of May, the percentage of damaged plants did not exceed 8% in the plots with mating disruption treatments, and differed significantly from the plot with conventional control in the May recordings (P=0.008). By contrast, from mid-June to the end of the cycle, plant damage in the MD plots increased, and damage did not differ from that in the Reference plots. In the same way, at the end of the trial, an average of 1.94% of tomatoes were damaged in the Reference plots, whereas 2.89% was recorded in the MD plots. The results of the July assessment would also indicate the dispenser having reached the end of its lifespan, with a consequent loss of efficacy.

3.5 Pheromone release profiles

Release profiles of TDTA were studied for the different dispensers tested each year. The residual pheromone load was fitted by linear regression in all cases (Fig. 6a). For T80 dispensers in 2008, a linear equation was obtained (eq. 1). Data at t=0 were not fitted properly, which is explained by the higher emission rate during the first few days, until the dispenser reaches an equilibrium with the environment. So these values were disregarded, along with three outliers, resulting in a coefficient of determination of R^2=0.974. Data considered outliers were disregarded according to normal probability plots and residuals of regression analysis.

$$P_{T80} = 59{,}715 - 0{,}4913x \qquad (1)$$

Residual pheromone load of T20 dispensers was also fitted to a linear model (eq. 2 in Fig. 6b), disregarding data at t=0, resulting R^2=0.901.

$$P_{T20} = 15{,}275 - 0{,}1322x \qquad (2)$$

In view of the results from containment-level trials, it was decided to employ the high load dispensers with some changes in the formulation. In the case of the dispensers employed in 2009, the linear model (eq. 3 in Fig. 6c) resulted in R^2=0.859, with three discarded outliers. However, these dispensers still contained 65.8% of their initial TDTA load after 124 days of trial.

$$P_{T60} = 61{,}953 - 0{,}1716x \qquad (3)$$

Finally, for the T80 employed in 2010, it was observed that these dispensers stopped emitting after 121 days of ageing, so average pheromone contents at 143 and 164 days were the same as at 121 days (Figure 7D). Thus, these final data were discarded and release profile was fitted to a linear model (eq. 4 in Fig. 6d), resulting in a coefficient of determination of R^2=0.915.

$$P_{2010}= 81{,}738 - 0{,}2427x \qquad (4)$$

Thus, equations 1 to 4 fitted to linear models, which means that pheromone is released at a constant rate during the period under study and mean emission rates are assumed to be the value of their slopes: 491.3 and 132.2 µg day^{-1} for T80 and T20, respectively, in the 2008 trial, while 171.6 and 242.7 µg day^{-1} were the release values for T60 in 2009 and T80 in the 2009-2010 efficacy trials, respectively.

The percentage of residual pheromone load at the end of the study period must be taken into account. Dispensers T80 and T20 had residual percentages of 21.5 and 18.7% respectively. By contrast, formulations employed in 2009 and 2010 still contained more than 60% of their initial pheromone loads after 124 days, which would be a poor feature for an efficient dispenser.

4 DISCUSSION

The interruption in pheromone communication of *T. absoluta* in pheromone treated plots has been demonstrated by Michereff and coworkers,[25] but they highlighted the need for more detailed pheromone studies to achieve a more effective mating disruption in TLM. In this previous experiment, doses up to 80 g a.i. ha^{-1} were tested, with dispensers being replaced every 4 weeks and a density of application greater than the 500 dispensers ha^{-1} employed in this study. Our present work contributes new efficacy trials for mating disruption in greenhouses applied to TLM and a new mesoporous pheromone dispenser for this purpose.

The main conclusion drawn from our containment-level trials was that mating disruption could not be achieved, with the tested pheromone doses, when a tomato crop is grown outdoors or in low-containment greenhouses. Low isolation meshes or the existence of holes in greenhouse covers allow the immigration of moths from outside the pheromone-treated areas, maintaining high population densities and increasing the likelihood of casual mating. Immigration of mated females is the reason of mating disruption failures in open field environments and the need of treatments in huge areas to avoid edge effect. Few studies have focused on the use of mating disruption in indoor facilities (such as greenhouses and storages), even though indoor applications provide natural boundaries that limit outdoor constraints.[31,32] Nowadays, the application of outdoor mating disruption for *T. absoluta* is not affordable due to the need of high pheromone amounts to be applied in wide areas and its excessive cost. Currently, the cost of pheromone synthesis is crucial for this technique to be applied. Commercial price of the pheromone is 900 € g^{-1} but this cost can be reduced to 30 € g^{-1} when it is synthesized at industrial scale (Ecología y Protección Agrícola SL pers. comm.). Tomato crop conducted in greenhouses allows mating disruption to be applied using lower pheromone doses in small plots.

In the first years of our field trials, there was not enough quantity of pheromone available to have true replicates. This was the case of Paiporta 2009 trial, where three different locations inside the same greenhouse were considered for statistical analysis. Once this preliminary test showed efficacy against TLM, and due to the big impact caused by this pest in Spain, true replicates were conducted in 2009-2010 in two different locations, Alicante and Paiporta.

According to our results, in low-containment greenhouses, damage was not reduced in plots treated with two different pheromone doses (10 and 40 g ha^{-1}), in comparison with a reference plot with four chemical treatments. However, when containment level increased, as in the case of Paiporta 2009 trial, damage did not significantly differ from that in the reference plot with four Bt and three Indoxacarb treatments and male flight was satisfactorily disrupted with about 30 g ha^{-1} of TDTA. Therefore, MD treatment was as effective as chemical control, with the mesoporous dispensers, for at least 4 months, when applied in greenhouses protected with a double door and a more closely-woven mesh. This would confirm the importance of the degree of containment on the success of pheromone treatment on the TLM, as it prevents the migration of pests. The following efficacy trials conducted in Alicante and Paiporta, in 2009 and 2010 respectively, achieved good control of damage at the end of the cycle, what finally supported results of flight disruptions. An average MDI of 84% was obtained in Alicante when moth population peaked at the beginning of November, before Bt and Etofenprox treatments were applied in the Reference plot. In Paiporta, average MDI was 83% from the 14th week until the end of the experiment.

As stated by other authors, it is difficult to link a reduction in moth catches with an equal reduction in damage.[33,34] For many moth species, it is especially difficult to obtain a relationship between male catches and plant damage.[35] Regarding plant damage in our present work, control of *T. absoluta* with MD was as effective as nine conventional treatments applied to the reference plot in Alicante, and damage was controlled in the 2010 Paiporta MD plots up to June, as observed in the 1st July assessment (Figure 5b). Due to the high containment level of these glasshouses in Paiporta, moth migrations did not occur, so the depletion of the pheromone dispensers, reaching the end of their lifespan, could be the reason for this reduction in efficacy.

Focusing our attention on the dispensers' release profiles, in 2008, formulations T80 and T20 did not prove effective under the particular conditions of the field trial, but release profiles were fitted to linear models and residual pheromone amounts were of about 20%. T60 in Alicante disrupted moth's flight and controlled damage for at least 120 days, with a linear release profile, emitting an average of 171.6 µg day^{-1}, despite having a high residual level of pheromone (65.8% after 124 days). In 2010, T80 also had a lifespan of 4 months (121 days) with a residual pheromone load of 61.6% and an average release value of about 240 µg day^{-1}. However, this 4 month period was not sufficient to control TLM in Paiporta 2010 up to the harvest, which is evidenced by the increase in damage from June onwards (Figure 6b). Therefore, some key changes should be made to dispenser formulation to ensure the disruption of pheromone communication. The percentage of residual pheromone load must be reduced, as it is known that the pheromone synthesis is the main cost for mating disruption implementation and pheromone must not be wasted. As it has been proven that TLM flight and damage could be controlled by releasing at least 170 µg day^{-1}, the lifespan should be extended in order to achieve this level of emission for almost 6 months, to cover longer crop cycles and maintain pheromone doses of at least 30 g ha^{-1}. This value is consistent with Michereff et al,[25] who found interruption in male orientation in plots treated with 35 to 50 g ha^{-1} of sex pheromone for *T. absoluta*. There are some experiences on mating suppression to manage other Gelechiidae species, including *Pectinophora gossypiella* (Saunders), *Tecia solanivora* Povolny, *Sitotroga cerealella* (Olivier) and the tomato pinworm *Keiferia lycopersicella* (Walsingham).[33,34,36,37] Specifically, *P. gossypiella* has been widely controlled by mating disruption in cotton fields with 78 g ha^{-1} of gossyplure, and tests with *T. solanivora* have been successful by applying 86 g ha^{-1}.

Regarding treatment application procedure, pheromone dispensers were hung a few days after plantation in all cases, when the tomato plants were 10-15 cm high. With the proper formulation in the dispensers, this early application was sufficient to protect the crop throughout the season. Several authors have demonstrated that early pheromone applications prevent Lepidoptera populations from increasing at mid-season,[34,38,39] which can result in high-yield losses. Therefore, mating disruption of the first emerging moths is crucial in order to affect development of the subsequent generations throughout the season.

In conclusion, this study revealed that effective pheromone application against *T. absoluta* can be achieved, in high containment greenhouses for 4 months, with doses of 30 g TDTA per ha employing new mesoporous dispensers. These dispensers were not replaced throughout the season and provided suitable emission rates, but formulations must be improved to avoid high residual loads and the subsequent waste of active ingredients. On the other hand, under the current conditions of pheromone synthesis and the affordable pheromone doses, the application of mating disruption needs high-containment degree greenhouses in order to succeed and be competitive with insecticide control. Therefore, research must be directed at reducing the price of pheromone synthesis and to evaluate the prospects of the outdoor application of mating disruption systems to control *T. absoluta* damage in outdoor tomato.

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Figure footnotes

Figure 1. Arrangement of the different plots inside the greenhouse for the 2009 efficacy trial (Alicante, Spain). The arrow indicates the entrance of the greenhouse. Mating disruption plots are referred to as 'MD1', 'MD2', 'MD3', and 'Reference' is the control plot with conventional chemical treatments.

Figure 2. Results from low-containment level trial conducted in El Perelló (Valencia, Spain) in 2008. **(a)** Captures of *Tuta absoluta*, as moths per trap and day (MTD), in commercial monitoring traps for pheromone treated plots (T80 and T20) and the Reference plot. The arrow indicates the date when pheromone dispensers were applied. **(b)** Damage level obtained in the mentioned plots, as percentage of plants with *T. absoluta* live stages (eggs, pupae or larvae). Black arrows indicate the dates when Indoxacarb and Spinosad were applied to the Reference plot and grey arrows indicate Indoxacarb applications on the pheromone treated plots.

Figure 3. Results from high- containment level trial conducted in Paiporta (Valencia, Spain) in 2009. **(a)** Captures of *T. absoluta*, as moths per trap and day (MTD), in commercial monitoring traps for the pheromone treated plot (MD) and the Reference plot with conventional chemical treatments. **(b)** Damage level obtained in the mentioned plots, as percentage of plants with TLM live stages (eggs, pupae or larvae). Black arrows indicate the dates when Indoxacarb treatments were applied and discontinuous arrows indicate Bacillus *thuringiensis* (Bt) applications, all of which in the Reference plot.

Figure 4. Results from the efficacy trial conducted in Alicante (Spain) in 2009. **(a)** Average captures of *T. absoluta*, as moths per trap and day (MTD), in commercial monitoring traps for the pheromone treated plots (MD) and the Reference plot with conventional chemical treatments. **(b)** Damage level obtained in the mentioned plots, as percentage of plants with TLM live stages (eggs, pupae or larvae). The black arrow indicates Etofenprox application in the Reference plot and discontinuous arrows indicate the dates when Bt was applied, all of which in the Reference plot.

Figure 5. Results from the efficacy trial conducted in Paiporta (Valencia, Spain) in 2010. **(a)** Average captures of *T. absoluta*, as moths per trap and day (MTD), in commercial monitoring traps for the pheromone treated plots (MD) and the Reference plots with conventional chemical treatments. **(b)** Damage level obtained in the mentioned plots, as percentage of plants with TLM live stages (eggs, pupae or larvae). Black discontinuous arrows indicate the dates when Bt was applied in the Reference plot. The grey arrow indicates Bt application on the MD plots at the end of the trial.

Figure 6. Release profiles of (3E,8Z,11Z)-tetradecatrienyl acetate (TDTA), the major *T. absoluta* pheromone component, from the four dispensers employed for the different trials: **(a)** T80 dispenser from low-containment trial 2008, **(b)** T20 dispenser from low-containment trial 2008, **(c)** T60 dispenser from high-containment trial 2009, **(d)** T80 dispenser from efficacy trials 2009-2010. Fitted lines describe the TDTA content of the dispenser (mg) versus time (number of days of ageing).