

# Parthenogenesis in UK field populations of the tomato leaf miner, *Tuta absoluta*, exposed to the mating disruptor Isonet T

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## Abstract

**BACKGROUND:** The tomato leafminer, *Tuta absoluta* is a damaging pest of tomato crops worldwide. In the UK *T. absoluta* is controlled using an integrated pest management (IPM) strategy that includes pheromone-based mating disruption. However, some growers have reported a loss of efficacy of this technology, and there are concerns that *T. absoluta* may evolve resistance via changes in its capacity to reproduce asexually. In this study we investigated the reproductive capacity of virgin populations of *T. absoluta* collected from a UK glasshouse before (EVH2016) and after (EVH2019) the introduction of the mating disruptor Isonet T.

**RESULTS:** In line with earlier reports, we demonstrate that UK populations of *T. absoluta* can reproduce parthenogenetically, and observed a small but significant increase in the rate of parthenogenesis associated with the use of Isonet T. Marked differences in several other life history traits associated with reproduction were also observed between the two virgin populations, with the EVH2019 strain producing fewer eggs, a delayed onset of egg laying and increased lifespan.

**CONCLUSION:** The low rate of parthenogenetic reproduction seen in this study is unlikely to result in loss of efficacy of mating disruption. However, the observed changes in longevity and egg laying may allow *T. absoluta* to persist for longer within the crop, and, together with the increased rate of parthenogenesis, may reflect selection from the use of Isonet T. Thus, regular monitoring of the reproductive capacity of UK populations should be conducted, and mating disruption used only as part of IPM to avoid the emergence of resistance.

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**Keywords:** *Tuta absoluta*; resistance; Isonet T; mating disruption; parthenogenesis

## 1 INTRODUCTION

*Tuta absoluta* is a highly destructive pest of tomato crops globally. First identified in South America, *T. absoluta* reached the UK in 2009 and is now present in most of Europe, Africa, the Middle East and parts of Asia.<sup>1</sup> The economic impact of *T. absoluta* on the tomato growing industry can be profound, resulting in 100% yield loss if left untreated, with 60% of global tomato crops estimated to have been affected.<sup>1</sup> In the UK, growers have reported losses of up to £ 50 000 per hectare (Rob Jacobson pers. comm.). Control of *T. absoluta* has been achieved through the implementation of an integrated pest management (IPM) strategy, incorporating crop monitoring, biological control and application of pesticides. This strategy, once established, proved extremely effective, however, over time it became compromised due to the emergence of resistance to several of the insecticides used for control.<sup>2,3</sup> To restore full control through IPM, a mating disruptor was developed by Shin Etsu Chemical Co. Ltd and introduced to the market under the product name of Isonet T. This product works by inundating closed glasshouse environments with a synthetic version of the female sex pheromone of *T. absoluta* ((3E,8Z,11Z)-3,8,11-Tetradecatrienyl acetate).<sup>4</sup> When deployed, the high levels of synthetic pheromone in the environment prevent male moths

effectively detecting semiochemical concentration gradients emitted by females, inhibiting location of a mate and thus preventing reproduction. The incorporation of Isonet T into contemporary IPM proved a spectacular success and was found to eradicate outbreaks in as little as one generation.<sup>5</sup> This product not only eliminated yield losses, but also decreased reliance on chemical pesticides, thus providing additional environmental benefits. In spite of the remarkable success of Isonet T in many UK commercial tomato glasshouses, one grower in Evesham subsequently reported loss of effective control of *T. absoluta* by the product (Rob Jacobson pers comm.). *T. absoluta* at this site were also highly resistant to spinosad,<sup>3</sup> a pesticide used in previous

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IPM strategies, resulting in limited options for alternative chemical control.

The effectiveness of mating disruption in eliminating pest populations can be severely compromised if the target pest has the capacity to reproduce in the absence of sex. In this regard, research by Megido and Verheggen (2012) previously indicated that French populations of *T. absoluta* have the capacity to reproduce asexually through the process of deuterotokous parthenogenesis.<sup>6</sup> The authors found that 4 out of 20 virgin females laid viable eggs, and 57 larvae reached adulthood with a sex ratio of 1/1.5 (male/female). However, the fecundity of these adult moths was not reported. Following this, work by Abbes and Chermiti (2014) demonstrated that individual females from three virgin populations of Tunisian *T. absoluta* (30 individuals for each population) could lay eggs (30%, 13.33% and 50%), of which 11.36%, 16.67% and 17.02% hatched.<sup>7</sup> However, out of a collective 10 F1 female virgins only one egg was laid and this was not viable. Both of these studies demonstrate parthenogenetic reproduction in *T. absoluta*. It is therefore possible that the loss of efficacy of Isonet T in the Evesham glasshouse was caused, at least in part, by parthenogenetic reproduction circumventing the effectiveness of mating disruption.

Asexual reproduction in *T. absoluta* is likely tytoparthenogenetic, a process by which a small proportion of unfertilized eggs hatch spontaneously. Offspring survival of this reproductive mode is typically much lower than for sexual reproduction.<sup>8</sup> However, tytoparthenogenesis can result in a positive feedback loop in which males are lost through female-biased sex ratios and increasing mate limitation. As a result, the strength of selection for tytoparthenogenesis increases in concert with the proportion of tytoparthenogenetic offspring in the sexual population.<sup>9</sup>

In the current study we assessed the level of parthenogenetic reproduction in UK populations of *T. absoluta*, and looked for evidence of any potential shift in frequency or viability of parthenogenetic reproductive output as a result of selection through the use of Isonet T. The data provided by this study is of direct relevance to the control of UK populations of *T. absoluta* and the robustness of current IPM strategies for this pest.

## 2 MATERIALS AND METHODS

### 2.1 Insect cultures

Insects were collected from R & J Holts Sandylands Nurseries (Evesham) in 2016 and 2019, *i.e.* before and after the deployment of Isonet T. All insect stages were housed in bug dorm cages (MegaView science co.) in CE rooms at 24 °C 16L:8D and 65% humidity. Cultures were supplied with tomato plants (var. Money Maker) *ad libitum*. Insects were collected and sexed for experimentation at pupal stage.

### 2.2 Assessment of life history traits of virgin female *T. absoluta*

Sexing of pupae was confirmed with the use of a microscope according to differences in morphology described by Genc (2016).<sup>10</sup> One hundred female pupae were placed in individual chambers. A plastic beaker with a small hole in the bottom was placed in another plastic beaker containing 100 mL of water and fertilizer. The petiole of a tomato leaf was inserted through the hole with any left-over space blocked with cotton wool. A 1.5 mL plastic tube containing a sugar water solution, and bunged

with cotton wool, was placed in the chamber as a food source for the adult moth. The chambers were covered with a double layer of fine cloth mesh. Longevity of females was assessed, the number of eggs produced was counted daily and leaves were monitored for mining. Any offspring surviving to the pupal stage were sexed and placed in new chambers as described above and the same metrics recorded.

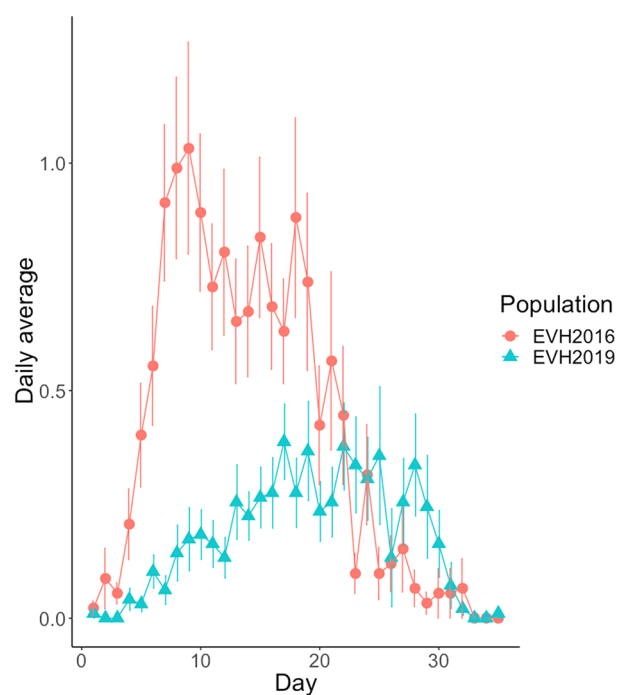
### 2.3 Statistical analysis

All statistical analyses were conducted in R. Data were tested for normality using Shapiro-Wilks normality test. As groups were not normally distributed, the Wilcoxon rank sum test was used to test significance. In all comparisons, two-tailed tests with  $P \leq 0.05$  were used to determine significance and reject the null hypothesis that no difference exists. Where outcomes were categorical,  $\chi^2$  tests were used to detect differences in the populations, and where assumptions of  $\chi^2$  were not met Fishers exact test was used.

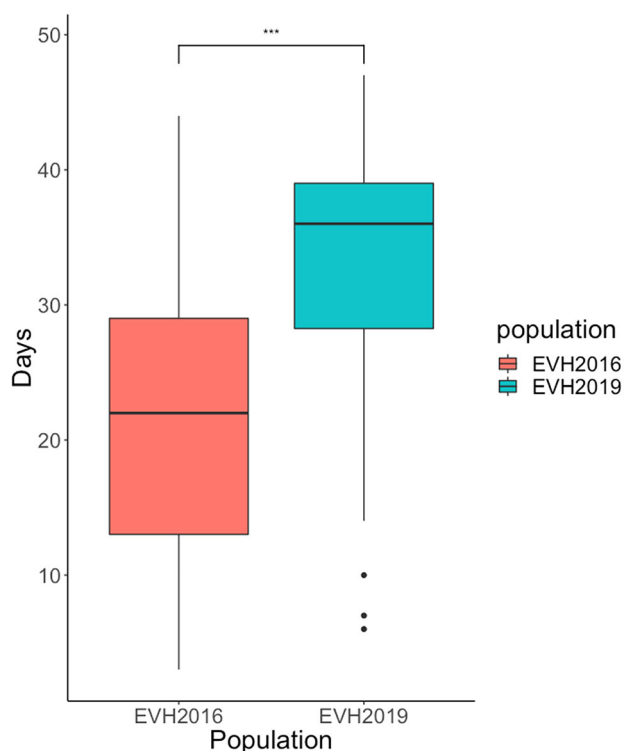
## 3 RESULTS

### 3.1 F0 virgin reproductive traits

There was no statistically significant difference between the numbers of females eclosing from the pupal stage between the populations collected before and after the deployment of Isonet T ( $\chi^2 = 2.6316$ ,  $df = 1$ ,  $P$ -value = 0.10). Ninety-two females eclosed successfully in the EVH2016 strain and 98 eclosed successfully in the EVH2019 strain. There was no significant difference between the numbers of individuals that laid eggs in each group ( $\chi^2 = 1.8601$ ,  $df = 1$ ,  $P$ -value = 0.17) with 84 females laying eggs in the EVH16 population compared to 82 females in EVH19.



**Figure 1.** Differences in average daily egg production by virgin females of the EVH2016 and EVH2019 strains over 35 days ( $W = 806$ ,  $P$ -value = 0.02). Error bars represent  $\pm$  SEM.

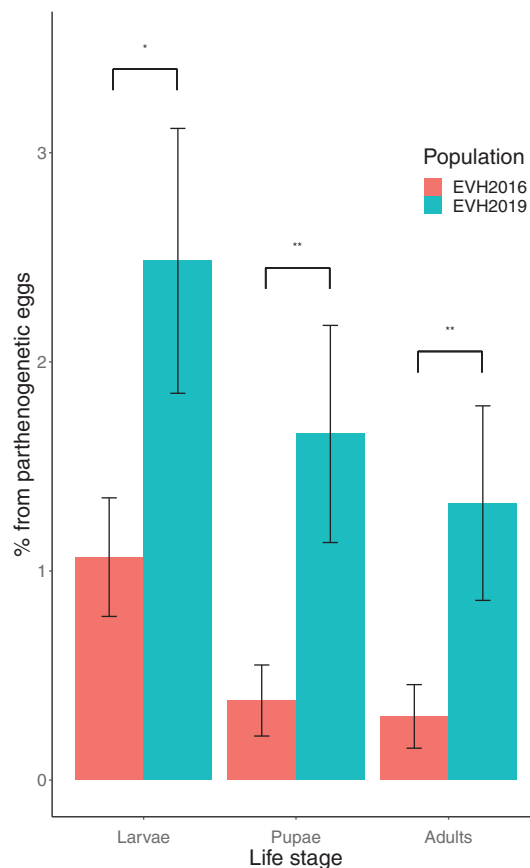


**Figure 2.** Box plots showing range of lifespan of virgin females of the EVH2016 and EVH2019 strains ( $W = 1682$ ,  $P$ -value  $< 0.001$ ).

There was a significant difference in the number of eggs laid between the two groups ( $W = 6149.5$ ,  $P < 0.005$ ) with EVH2016 laying 1313 eggs with an average of 14.27 eggs per individual and EVH2019 laying 604 eggs with an average of 6.16 eggs per individual. Figure 1 shows the differences between average daily increase in egg number across the two populations. Significant differences between the populations were also observed in lifespan ( $W = 1682$ ,  $P$ -value  $< 0.005$ ) with EVH2016 having an average lifespan of 21.28 days compared to 32.71 days in EVH2019 (Fig. 2). Significant differences were observed in the time it took females to start laying eggs ( $W = 3155.5$ ,  $P$ -value  $< 0.005$ ), on average EVH2016 started laying after 6.96 days compared to 10.32 days for EVH2019. No significant differences were observed between the populations in the total number of days the females laid eggs ( $W = 4929.5$ ,  $P$ -value = 0.27) with the average range being 8.42 and 7.39 for EVH2016 and EVH2019.

### 3.2 Parthenogenesis

Six females (7%) of the EVH2016 strain laid viable eggs, from which 14 larvae were detected (1% of eggs laid from the population). Five larvae survived to pupation with a sex ratio of 2:3 (males/females). Of these, three females and one male eclosed. These females laid 27, 57 and eight eggs and survived for 16, 27 and 31 days, respectively. No F1 virgins produced viable eggs. Eight virgin EVH2019 females laid viable eggs (8%) from which 15 larvae were detected (2.5% of all eggs), 10 of these developed into pupae (67%) with a sex ratio of 3:7 (males/females). From these six females and two males eclosed. These females laid 26, 0, 0, 7, 1 and 11 eggs and survived for 21, 11, 14, 13, 17 and 12 days, respectively. No F1 virgin females laid viable eggs.



**Figure 3.** Percentage differences in larvae ( $\chi^2 = 4.666$ ,  $df = 1$ ,  $P$ -value = 0.03), pupae ( $P$ -value  $< 0.01$ ) and adults ( $P$ -value = 0.01) from total number of eggs laid by virgin females of the EVH2016 and EVH2019 strains. Error bars display standard error for all proportions.

There was no significant differences in the number of active larvae, pupae or adults produced parthenogenetically by the two F0 female populations, however, there were significant differences between the populations when the likelihood of larvae, pupae and adults emerging from F1 eggs was compared. There was a 2.3-fold increase in the proportion of larvae ( $\chi^2 = 4.666$ ,  $df = 1$ ,  $P$ -value = 0.03), a 4.3-fold increase in proportion of pupae ( $P$ -value  $< 0.01$ ) and a 4.3-fold increase in proportion of adults ( $P$ -value = 0.01) in the EVH2019 strain compared to the EVH2016 strain (Fig. 3).

## 4 DISCUSSION

Our results reveal clear differences in life history traits associated with reproduction between two populations of virgin *T. absoluta* that were differentially exposed to mating disruption in the field. These included marked differences in the number of eggs laid, the start date of laying and lifespan. Both populations from Evesham had a low frequency of virgin females capable of laying viable eggs, and significant differences between these populations in the number of larvae, pupae and adults produced were not observed. However, a significant increase in their proportions as a function of eggs laid was observed.

What are the implications of these results for the resilience of control employing mating disruption against *T. absoluta* in the UK? Firstly, the very low levels of parthenogenesis observed in

both the Isonet T exposed and unexposed populations suggests changes in the rate of parthenogenesis, in isolation, are unlikely to explain the reduced levels of *T. absoluta* control reported at the Evesham glasshouse in 2019. A small but significant increase in the proportion of eggs developing parthenogenetically was observed in the EVH2019 population compared to EVH2016. However, the reduced total egg production of the former strain in comparison to the latter, resulted in no significant increase in total number of larvae, pupae and adults produced between the two strains. This mitigated any effect of the increased rate of parthenogenesis of the EVH2019 on population size. Furthermore, in both populations no first generation virgin females went on to produce viable eggs. This suggests that the ability of *T. absoluta* to persist over more than one generation by parthenogenesis is limited, however, given the small sample size of the second generation in this study, more extensive testing is required to confirm this.

Secondly, the marked differences in life history traits between virgin females of the EVH2016 and EVH2019 strains may also have relevance for IPM incorporating mating disruption. Evolutionary theory shows that life history traits are controlled by energetic trade-offs between intrinsically controlled factors such as reproductive output and longevity.<sup>11</sup> It is plausible therefore that in a mate-limited environment, selection would result in the diversion of energetic resources away from egg production and reallocation to longevity. The reduction in egg production would therefore be an antagonistic pleiotropic result of increased longevity - a strategy that would increase the likelihood of encountering a mate. Furthermore, the delay in average egg laying date observed in the EVH2019 strain would postpone energetic expenditure in reproductive output, facilitating longevity,<sup>12</sup> until a mating occurs, which in turn stimulates egg production.

Both parthenogenesis<sup>9</sup> and longevity<sup>13</sup> have been shown to result from populations that are male limited or at low density, which is consistent with our data. Furthermore, if parthenogenesis has a selective advantage in pheromone inundated environmental conditions, as suggested by the observed significant increase in its frequency, it could further proliferate as a result of a positive feedback loop. Specifically, males could be lost through a skewed parthenogenetic sex ratio, as observed in EVH2019. The overall reduction in egg production of this population could further drive parthenogenetic evolution, as a reduction in overall offspring produced from a low frequency of sexual encounters within the population would increase the relative fitness of any offspring produced through parthenogenesis. Thus, the increased rate of deuterotokous parthenogenesis in combination with a shift in longevity and egg laying date may allow the persistence of populations within the crop. This would allow populations to re-establish if there was any diminution of mating disruption. Furthermore, while sampling of additional field populations is required, our data supports the notion that there could be an inherited genetic component to asexual reproduction in *T. absoluta*, which can be selected for under conditions that limit sexual reproduction.

Finally, the changes in *T. absolutas'* life history traits may also have synergistic effects on the efficacy of biocontrol agents used as part of IPM in the UK. The predatory bug, *Macrolophus pygmaeus*, requires time to build effective population sizes in the crop. This means that pesticide applications are often required to knock back *T. absoluta* populations until the natural enemy is established. Later egg laying and longer life span of *T. absoluta*

would increase the time available for this process to occur. However, *M. pygmaeus* is polyphagous and can cause damage to the crop if densities are too high. Therefore a reduction in density of the applied biocontrol may be required to mirror any reduced reproductive output of *T. absoluta*.

A limitation of our study is the fact that, a) only two strains were tested and, b) these differed in the duration spent in culture under controlled environment conditions in the lab. In particular we cannot discount the fact that differences observed between the strains in certain life-history traits, at least in part, result from differences in acclimation to laboratory conditions. Thus further testing of field-strains is required to provide additional evidence that the findings reported in this study are associated with the use of pheromone-based mating disruption.

## 5 CONCLUSION

In summary, the observed increase in rate of parthenogenesis in UK glasshouse populations of *T. absoluta* following the deployment of Isonet T is unlikely to explain partial control loss of mating disruption. This news is especially welcome in light of *T. absoluta's* capacity for resistance to chemical control.<sup>3</sup> However, the marked differences in life history traits in UK populations that differ in exposure to mating disruption has implications for the current use of this control method, resulting in populations that may be more resilient at persisting within the crop at lower densities. Thus, further sampling of UK populations is warranted to examine the extent to which the modified life history traits identified in this study are observed in *T. absoluta* populations from glasshouses using Isonet T for control. Furthermore, the fact that deuterotokous parthenogenesis exists, the fact that populations are male restricted, and the fact that populations persist at low densities, does - according to evolutionary theory - provide the right circumstances for further changes in the rate of parthenogenesis to evolve.

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