

First evidence of deuterotokous parthenogenesis in the tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)

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Abstract The tomato leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae), originates from South America and is now considered to be one of the most damaging invasive pests of tomatoes in the Mediterranean Basin countries of Europe and North Africa. The preventing pest mating control methods include: (1) the use of synthetic pheromones for male attraction and annihilation inside insecticide-containing traps; (2) mating disruption by saturating the atmosphere with sex pheromones which alter the ability of males to locate females; and (3) massive applications of sterile males to alter the overall reproductive success of the pest population. However, all these methods achieve only a poor success rate in controlling *T. absoluta* populations under greenhouse conditions. Sex pheromone management and sterile insect techniques are both based on an important biological trait: the insect must breed through sexual reproduction. Here, we report for the first time laboratory evidence of deuterotokous parthenogenesis, an asexual reproduction where both males and females are produced from unfertilized eggs. We discuss the consequences for *T. absoluta* control strategies.

Keywords Asexual reproduction · Insect pest management · Sex pheromone management · South American tomato moth

Introduction

The tomato leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae), is now considered to be one of the most damaging pests of tomato production, both in open fields and under greenhouse conditions in South America, where it is originated, and in the West Palearctic region, where it was introduced in 2006 (Desneux et al. 2010). This invasive moth can cause yield losses as high as 100 % (Desneux et al. 2011). It is estimated that a total of 84.9 % (3.7 M ha) and 87.4 % (133.7 M tons) of world tomato-cropped surface and world tomato production, respectively, are now directly threatened by *T. absoluta* and could be infested in the near future (Desneux et al. 2011).

In addition to the traditional use of insecticides, environmentally sound control strategies have been developed, including the use of natural enemies (parasitoids, predators, entomopathogens, and nematodes) or resistant varieties of tomato (Desneux et al. 2010; de Oliveira et al. 2012). Other alternative control methods are aimed at preventing pest mating, either by use of synthetic pheromones for male annihilation technique or mating disruption, or by use of the sterile insect technique. The male annihilation technique involves the use of a high density of male lures combined with an insecticide to reduce the male population, and thereby mating, while the mating disruption method consists in saturating the atmosphere with a synthetic pheromone, altering the ability of males to locate females and mate, thereby causing a long-term reduction in population density (Cunningham 1989; Carde and Minks 1995; Carde 2007; Cocco et al. 2012). The sterile insect technique consists of sustained mass-releases of sterile males of the pest species to introduce sterility into wild populations, and thereby control their reproduction (Klassen and Curtis 2005).

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The sex pheromone management and sterile insect techniques are based on an important biological trait: the insect must breed through sexual reproduction. Even if large numbers of male individuals can be caught by coupling pheromone releasers with use of insect trapping devices, the success of pheromone-based control strategies is usually low (Hassan and Al-Zaidi 2010). The same poor results are also observed in pheromone-based controls of *T. absoluta* (Michereff Filho et al. 2000; Vacas et al. 2011); one of the hypotheses for this failure could be that the pest used asexual, or parthenogenetic, reproduction.

Parthenogenesis can be defined as the production of an embryo from a female gamete without any genetic contribution from a male gamete (Mittwoch 1978), and is relatively rare in the order of Lepidoptera (Suomalainen 1962; Lynch 1984). It is currently found in several psychids and is called thelytokous parthenogenesis because only females are produced (Grassé 1949; Lynch 1984). Parthenogenesis in psychids is also called geographic parthenogenesis, with reference to the fact that parthenogens tend to occupy ranges different from those of their close, sexually reproducing, relatives (Grassé 1949; Lynch 1984). Parthenogenetic forms are the exception among other families of Lepidoptera and are termed accidental, or tycho-, parthenogenesis (Grassé 1949; Ball 2001). Around 20 species belonging to several families of Lepidoptera (Bombycidae, Gelechiidae, Gracillariidae, Lasiocampidae, Liparidae, Nepticulidae, Saturniidae, Sesiidae, Sphingidae) are known to be able to use parthenogenetic reproduction (Trehan and Bagal 1944; Portier 1949; Suomalainen 1962; Mitter and Futuyma 1977; Lynch 1984; Menken and Wiebosch-Steeman 1988; Mozuraitis et al. 2002). In these families of Lepidoptera, when parthenogenesis occurs, it produces an equal proportion of both sex, and is called deuterotokous parthenogenesis (Portier 1949).

The aim of this study was to evaluate the capacity of *T. absoluta* to reproduce parthenogenetically in laboratory conditions.

Experimental section

Plant and insect cultures

Tomato (*Lycopersicon esculentum* cv. MoneyMaker) plants were cultivated in a greenhouse (25 ± 5 °C) in plastic pots (20 cm diameter \times 20 cm height) filled with loam (Peltracom—VP113BIO, Belgium) and grown under a 16L: 8D photoperiod.

Two hundred and third instar larvae of tomato leafminers, *T. absoluta*, were collected in a commercial tomato plantation located in Saint Andiol (France) in July 2011, and were kept under laboratory conditions at 24 ± 1 °C, 60–70 % RH and

16L: 8D photoperiod on tomato plants (cv. MoneyMaker), placed in various net cages (46.5 cm \times 46.5 cm \times 46.5 cm). Fresh plants were provided three times a week to the caterpillar until pupation. Five generations were established on the host plant before experiments began.

Experimental setup

Under laboratory conditions, twenty female pupae from insect cultures (F0) were isolated in petri dishes filled with moistened filter paper and a single tomato (cv. MoneyMaker) leaf. Individuals were sexed following the method of Coelho and França (1987). After adult emergence (F0), females were left in petri dishes for their whole life. The number of eggs laid on the tomato leaf and the proportion of hatched eggs were evaluated daily until adult death.

Twenty pairs of male and female pupae from the same insect cultures (F0) were isolated in another set of 20 petri dishes, also filled with moistened filter paper and a single tomato (cv. MoneyMaker) leaf. After adult emergence (F0), males and females were left together in petri dishes for their whole life. After visual observation of the first mating, the number of eggs laid on a tomato leaf and the proportion of hatched eggs were evaluated daily until adult death. If a second mating was observed, additional eggs were not taken into account. Water was supplied to each petri dish every 2 days to optimize adult survival.

Each neonate larva (F1) was isolated and placed individually in a petri dish filled with moistened filter paper and one single tomato leaf. Their food was changed every day and water was added to humidify the filter paper until pupation. F1 pupae were sexed and kept in petri dishes filled with moistened filter paper and a single tomato (cv. MoneyMaker) leaf. After adult emergence (F1), females were kept individually in petri dishes. The number of eggs laid on each tomato leaf and the proportion of hatched eggs were evaluated daily until adult death.

Experiments were carried out under laboratory conditions (24 ± 1 °C, 65 ± 5 % RH, 16L: 8D photoperiod) monitored by an automatic datalogger (HOBO RH/TEMP 8K, Onset, Bourne, MA, United States).

Statistical analyses

One-way ANOVAs were applied to compare the mean number of eggs laid by mated and unmated females, and by the F0 and F1 parthenogenetic females. Natural logarithm transformations were applied to the data before statistical analysis. Chi-square tests of association were applied to compare the percentage of mortality between fertilized and unfertilized eggs. Chi-square tests of association were also applied to compare the sex ratio between adults emerging from fertilized and unfertilized eggs. All tests were

performed using Minitab® (v.16) software (<http://www.minitab.com/fr-FR/default.aspx>).

Results

Of the 20 isolated F0 virgin females, 19 laid unfertilized eggs with an abundance ranging from 1 to 95 eggs and an average of 10.7 eggs per female: the overall total was 203 eggs. Four females laid viable eggs, totaling 81 eggs (corresponding to a survival rate of 39.9 %). Normally developed caterpillars emerged from unfertilized eggs and 57 of these larvae (corresponding to a survival rate of 70.4 %) reached the adult stage with a sex ratio of 1/1.5 (male/female).

Among the 20 F0 isolated couples, 13 females laid fertilized eggs with an abundance ranging from 21 to 85 eggs and an average of 50.6 eggs per female, for a total of 658 eggs, of which 493 hatched as larvae (corresponding to a survival rate of 74.9 %). The sex ratio corresponded to 1/2 (male/female) for adults from fertilized eggs.

The average number of eggs laid by mated females (50.6 ± 5.01) was statistically higher than that observed for unfertilized females (10.7 ± 4.84) ($F_{1,21} = 14.788$; $P = 0.001$). Moreover, the mortality of unfertilized eggs (60.1 %) was statistically higher than the mortality of fertilized eggs (25.1 %) ($\chi^2 = 59.67$; $P < 0.001$). We observed no difference for sex ratio between adults emerging from unfertilized eggs and from fertilized eggs ($\chi^2 = 0.53$; $P = 0.465$).

The number of eggs laid by 20 parthenogenetic F1 females was subsequently analyzed: 10 females (75 %) laid unfertilized eggs with an abundance ranging from 4 to 71 eggs and an average of 21.2 eggs per female, from a total of 212 eggs.

The mean number of eggs laid by the parthenogenetic F1 generation (21.2 ± 7.16) was found to be statistically similar to the mean number of eggs laid by the parthenogenetic F0 generation (10.7 ± 4.84) ($F_{1,18} = 0.54$; $P = 0.470$).

Discussion

The development of both male and female adults from unfertilized eggs demonstrated deuterotokous parthenogenetic reproduction of *T. absoluta* under laboratory conditions. This parthenogenesis can be described as tytoparthenogenesis or accidental parthenogenesis. It is found in species for which the primary reproduction mode is sexual but, in the absence of males, females are able to produce eggs without mating (Grassé 1949; Menken and Wiebosch-Steeman 1988). Here, we report tytoparthenogenetic reproduction under laboratory conditions but its occurrence in natural populations of *T. absoluta* remains

to be clarified. Moreover, if parthenogenetic individuals are actually found in natural populations, the capacity of *T. absoluta* to generate and maintain sufficient genetic variation for its persistence should be evaluated, as suggested by Chevasco et al. (2012) for *Dahlica fennicella* (Lepidoptera: Psychidae). Some questions also remain about the origin of this tytoparthenogenesis in *T. absoluta*. It could be considered as a classical automictic tytoparthenogenesis, similar to known tytoparthenogens, or it could be attributed to microbial manipulation by a bacterial endosymbiont such as *Wolbachia*, recently identified in *T. absoluta*, which has the capacity to modify the reproduction of its host into parthenogenetic reproduction (Ball 2001; Beukeboom 2012; Škaljac et al. 2012).

Directive 2009/128/EC promotes the use of integrated pest management and alternative approaches or techniques, such as non-chemical alternatives to pesticides (Cocco et al. 2012). To respond to environmental requirements, several programs based on mating management (such as mass trapping, mating disruption, and male sterility programs) are under development (Hassan and Al-Zaidi 2010; Morrison et al. 2011; Cocco et al. 2012) but asexual reproduction in natural populations of *T. absoluta* could have strong implications for the efficiency of these management strategies. However, although *T. absoluta* is able to reproduce parthenogenetically, sexual reproduction seems to lead to a better overall fitness, as mated females lay more eggs than unmated ones, as suggested by our laboratory observations. Unfertilized egg mortality was also found to be higher than that of fertilized eggs. Further studies on the fitness of *T. absoluta* parthenogenetic lineages are clearly needed to confirm the adaptive character of this type of reproduction. Traditionally, low genetic diversity and reduced evolutionary potential have been linked to parthenogenetic reproduction but, unfortunately for effective control of *T. absoluta*, no parthenogenetic moth species show signs of deterioration in their populations (Chevasco et al. 2012).

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