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Inherited sterility in *Tuta absoluta* (Lepidoptera: Gelechiidae): Pest population suppression and potential for combined use with a generalist predator

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Abstract

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) is one of the most devastating pests of tomato. We studied whether partially sterile *T. absoluta* males and fully sterile females were capable of suppressing wild populations of this moth in semi-controlled conditions. After irradiating *T. absoluta* pupae with 200 Gy, emerged males and females were released at 10:1 (treated:untreated) and 15:1 over-flooding ratios inside field cages containing tomato plants. The number of eggs and larvae produced was recorded once per wk during 3 mo. An over-flooding ratio of 10:1 caused a decline in larvae production compared with the untreated control cages, but these differences were not statistically significant. Using an over-flooding ratio of 15:1, the moth population in the cages with irradiated insects decreased significantly compared with those in the untreated control cages. In addition, the possibility of combining inherited sterility and a natural enemy as a strategy to manage this pest was investigated. We studied in no choice and choice tests the predation behavior of females of the egg predator *Tupiocoris cucurbitaceus* (Hemiptera: Miridae) on eggs derived from the following parental *T. absoluta* crosses: $♀_U × ♂_U$ (control), $♀_U × ♂_I$ and $♀_I × ♂_U$ (where U = untreated, I = irradiated). In the no choice test, females of the *T. cucurbitaceus* readily consumed *T. absoluta* eggs regardless of their origin. In the choice test, *T. cucurbitaceus* females consumed similar numbers of eggs of untreated parents and eggs oviposited from crosses in which the male had been irradiated. However, the mirid females preyed on significantly more eggs—roughly 20% more—from irradiated females than on eggs from untreated females. We conclude that it is possible to achieve a certain level of suppression of a *T. absoluta* wild population through the release of irradiated insects, and that it is technically feasible to combine the use of the predator *T. cucurbitaceus* with inherited sterility to control this moth pest.

Key Words: tomato leafminer; F_1 sterility; X radiation; *Tupiocoris cucurbitaceus*; biological control

Resumen

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) es una de las plagas más devastadoras de los cultivos de tomate. Se estudió si machos parcialmente estériles y hembras totalmente estériles de *T. absoluta* son capaces de suprimir poblaciones salvajes de esta polilla en condiciones semicontroladas. Después de la irradiación de pupas de *T. absoluta* a 200 Gy, machos y hembras emergidos fueron liberados con una relación de 10:1 (tratados:no tratados) en el interior de jaulas que contenían plantas de tomate. El número de huevos y larvas producido fue registrado una vez a la semana durante tres meses. El experimento se repitió con una relación de 15:1. El uso de una relación de 10:1 causó una disminución de la población de larvas en comparación con las jaulas control, pero estas diferencias no fueron estadísticamente significativas. Cuando la relación fue 15:1, la población de la polilla en las jaulas con los insectos irradiados disminuyó significativamente en comparación con las jaulas control. Además, se analizó la posible compatibilidad de la esterilidad heredada y el control biológico como estrategia para el control de esta plaga. Se estudió en pruebas sin elección y con elección si los huevos provenientes de los siguientes cruzamientos parentales de *T. absoluta*: $♀_U × ♂_U$ (control), $♀_U × ♂_I$ y $♀_I × ♂_U$ (donde U = no irradiado, I = irradiado) son igualmente aceptados para el consumo por hembras de *Tupiocoris cucurbitaceus* (Hemiptera: Miridae). En la prueba sin elección, las hembras de *T. cucurbitaceus* consumieron un elevado número de huevos de *T. absoluta*, independientemente de su origen. En la prueba de elección, las hembras de *T. cucurbitaceus* consumieron tanto huevos provenientes de parentales no tratados como huevos de parentales con el macho irradiado. Sin embargo, las hembras del mírido predaron significativamente más huevos de hembras irradiadas que huevos de hembras no tratadas. Se concluyó que es posible alcanzar un cierto nivel de supresión de una población salvaje de *T. absoluta* mediante la liberación de insectos irradiados y que es factible combinar el uso del predador *T. cucurbitaceus* con IS para controlar esta polilla plaga.

Palabras Clave: polilla del tomate; esterilidad F_1; radiación X; *Tupiocoris cucurbitaceus*; control biológico

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) is one of the most devastating pests of tomato crops. It is native to South America, but recently it has spread throughout the world (Desneux et al. 2010, 2011). In 2006, this pest was first reported in Spain (Urbaneja et al. 2007) and is now considered a key agricultural threat to European and North African tomato production (Desneux et al. 2010, 2011). Control of *T. absoluta* infestations is a challenge as the larvae are protected inside the leaf mesophyll. In South America, the intensive use of insecticides to control *T. absoluta* has led to the development of pesticide resistance to several products (Lietti et al. 2005; Desneux et al. 2010;

Guedes & Picanço 2011; Reyes et al. 2011). Moreover, the inappropriate or extensive use of insecticides negatively affects the performance of beneficial insects (Croft 1990; Desneux et al. 2007). As such, there is growing pressure from consumers, farmers and the community to develop new or to improve current control tactics that are harmless for the environment and socially accepted (Bloem & Carpenter 2001; Soopaya et al. 2011).

The sterile insect technique (SIT) is an environmentally friendly control tactic that is based on the release of sterile insects to control individuals of the same species (LaChance 1985). Lepidopteran species are more resistant to the sterilizing effects of radiation than insects of any other order (LaChance 1985; Bakri et al. 2005). As a consequence, the greater amount of radiation required to achieve full sterility in males may reduce their competitiveness and performance in the field (Suckling et al. 2011). One approach to reduce these negative effects is the use of inherited sterility (IS) or F_1 sterility (Proverbs & Newton 1962; Knipling 1970, 1979; LaChance 1985; Carpenter et al. 2005).

Since female moths in general are more sensitive to radiation than males, the radiation dose is adjusted in programs that incorporate an IS component so that treated females are completely sterile and males are partially sterile, but radiation-induced deleterious chromosomal rearrangements are inherited by the F_1 generation. Matings of these released irradiated males with wild fertile females results in reduced egg hatch and offspring that are predominately males and sterile. The major benefit of an IS approach is that the lower radiation dose used for the released parental insects impairs the quality and competitiveness of the released males in the field to a lesser extent than a fully sterilizing dose (Carpenter et al. 2005).

Successful eradication also requires sufficient depression of fertility from matings between irradiated lineage and wild partners, and that sufficient numbers of irradiated males are released to overcome the natural rate of increase of the wild population. The potential of the IS as a control tactic to suppress wild populations of pest moths has been investigated in many laboratory and field-cage studies (Bloem & Carpenter 2001; Bloem et al. 2005). The objective of most of these field cage studies was to determine an effective over-flooding ratio (number of irradiated moths to untreated moths) to be released in order to suppress a wild population of a pest.

The overall effectiveness of the IS technique at reducing lepidopteran pest populations has been demonstrated in operational programs against the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) in the United States and northern Mexico, the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) in Canada (Bloem *et al.* 2005), the false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) in South Africa (Nepgen 2014) and the Australian painted apple moth, *Teia anartoides* Walker (Lepidoptera: Lymantriidae) in New Zealand (Suckling et al. 2007).

The development of the SIT to target key lepidopteran pests requires improvements that increase the quality control of mass-rearing, irradiation, shipping, release and field assessment activities. Moreover, mass-reared insects used in SIT programs need to be of high quality to ensure the efficacy and efficiency of the sterile insects when released in the field (Bloem et al. 1998, 1999; Calkins & Parker 2005; Vreysen et al. 2006, 2007b).

The use of IS has many advantages including species specificity and compatibility with the use of other area-wide control tactics, such as biological control using parasitoids or predators, mating disruption, cultural control methods, and the use of bio-rational pesticides (Carpenter 2000; Dyck et al. 2005). Many studies have shown that the IS technique can be successfully combined with biological control by parasitoids such as *Trichogramma* spp. (Mannion et al. 1994, 1995; Zhang

& Cossentine 1995; Carpenter et al. 1996). Various *Trichogramma* spp. have been reported and studied as natural enemies of *T. absoluta* in South America and Europe (Parra & Zucchi 2004; Pratissoli et al. 2005; Desneux et al. 2010; Zappalà et al. 2012). Also, in Europe, mirid predators are now commonly used as biocontrol agents of *T. absoluta* (Urbaneja et al. 2009; Calvo et al. 2012; Bompard et al. 2013). In Argentina, the mirid bug, *Tupiocoris cucurbitaceus* (Spinola) (Hemiptera: Miridae) has been collected on tomato crops (del Pino et al. 2009), and although it has been frequently associated with whiteflies in the field, it is also a predator of *T. absoluta* eggs (López et al. 2012).

Recently, we analyzed the effects of ionizing radiation on *T. absoluta* that provided the basis for a feasibility study on the IS technique as a potential control tactic for this species (Cagnotti et al. 2012). We determined that 200 Gy was the optimum dose for *T. absoluta* for use in an area-wide integrated pest management (AW-IPM) program that has an IS component.

In this work, we examined whether partially sterile *T. absoluta* males and fully sterile females had the suitable quality to mate wild moths and suppress populations of *T. absoluta* in semi-controlled conditions at 2 over-flooding ratios. We also investigated if eggs oviposited by irradiated *T. absoluta* parents were equally suitable for consumption by *T. cucurbitaceus* females to assess the potential compatibility of both control strategies.

Materials and Methods

LABORATORY COLONIES

The research was carried out at the Insectario de Investigaciones para Lucha Biológica, (IILB), Instituto de Microbiología y Zoología Agrícola (IMYZA), Instituto Nacional de Tecnología Agropecuaria (INTA), Castelar, Buenos Aires Province, Argentina. *Tuta absoluta* used in these studies were obtained from colonies maintained in this laboratory. The larvae of *T. absoluta* had been reared on tomato plants for about 45 generations and the colony was periodically refreshed by adding wild insects that were collected from local greenhouses. The colony was maintained in a controlled environment room at 24–28 °C and 60–85% RH.

Adults and nymphs of *T. cucurbitaceus* used in the experiments were obtained from the colony reared at the IILB. They were originally collected in 2009 from tomato plants in greenhouses located in La Plata, Buenos Aires. The species was identified by Diego Carpintero from the Museo Argentino de Ciencias Naturales Bernardino Rivadavia, Buenos Aires, Argentina, and voucher specimens were preserved in the collection of the IILB. Using previous experience of mirid rearing (Agustí & Gabarra 2009) the bugs were reared on tobacco plants (*Nicotiana tabacum* L.) and fed on *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae) eggs.

RADIATION TREATMENT PROCEDURES

Pupae of *T. absoluta* were separated by sex following the criteria used by de Coelho & França (1987). They were placed in Petri dishes (1.5 cm high and 5 cm diam) and irradiated 48–72 h before adult emergence with a dose of 200 Gy. The radiation treatment was given at IGEAF (Institute of Genetics-INTA) in a Constant Potential X-Ray System Mg 160 Philips at a dose rate of 39.11 Gy / min. The characteristics of the irradiator were: anode voltage 120 kVp, current 15 mA, beam quality Cx120 approx. 0.07 mm aluminum (at 160 kV). For dosimetry, the PTW model UNIDOS® E was used as a dosimeter, and calibration was done with secondary standards dosimetry (CRRD) by the regional reference center located at Centro Atómico Ezeiza, Buenos Aires, Argentina. It was calibrated by the method of substitution in terms of air

kerma by the supplier with the expanded 1.2 percentage uncertainty in the calibration factor. Traceability to last calibration was 14 Jun 2011. The ion chamber was a Farmer-Type Ionization chamber with a volume of 0.6 cm^3 (TN30010-2339 PTW-Freiburg) that had the following characteristics, i.e., measuring quantity: air kerma (Kair), calibration factor: Nk = 4.938 × 107 Gy/C.

POPULATION SUPPRESSION BY IRRADIATED *TUTA ABSOLUTA* MALES AND FEMALES AND THEIR PROGENY

Experiment 1. Effects of an Over-flooding Ratio of 10:1 Treated:Untreated *T. absoluta* Moths

The experiment was carried out in field cages (0.80 m high, 0.55 m wide, 0.60 m long) made of fine-mesh cotton organdy that were deployed in a greenhouse (4.5 × 6 m), Hurlingham Buenos Aires Province, Argentina. Four potted tomato plants each with 6–8 leaves were placed in each field cage. Plants were irrigated regularly and received no other treatment during the experiment. After irradiation, *T. absoluta* adults (48–72 h old) were released into the cages and this resulted in an over-flooding ratio of 10:1 treated:untreated moths (30 ♀$_I$:30 ♂$_I$:3 ♀$_U$:3 ♂$_U$) (I = irradiated; U = untreated). Likewise in control cages, untreated males and females were released in the following ratio: 0 ♀$_I$:0 ♂$_I$:3 ♀$_U$:3 ♂$_U$. Control and treatment cages were replicated 5 times and the experiment was initiated on Mar 2012. Population growth for each treatment was analyzed by recording the number of eggs and larvae. To this end, one leaf per plant stratum (upper, middle, lower) was randomly chosen and observed once per week during 3 months. If necessary because of damage to the plants caused by larval feeding, tomato plants were added weekly to the cages.

Experiment 2. Effects of an Over-flooding Ratio of 15:1 Treated:Untreated *T. absoluta* Moths

The procedure described above was repeated with an over-flooding ratio of 15:1 treated:untreated moths. Irradiated and untreated insects were released as follows: 45 ♀$_I$:45 ♂$_I$:3 ♀$_U$:3 ♂$_U$. Untreated males and females were released in the same ratio as in the previous experiment for the control group (0 ♀$_I$:0 ♂$_I$:3 ♀$_U$:3 ♂$_U$). The control group was replicated 10 times, whereas the treatment cages were replicated 5 times. The experiment was initiated in Oct 2012. The same variables as mentioned in experiment 1 were recorded during 3 months.

STATISTICAL ANALYSIS IN EXPERIMENTS 1 AND 2

The effects of treatment and time (explanatory variables) on the number of eggs, small and large larvae per cage were evaluated in each period of peak production with Generalized Linear Models using the R software version 2.13.1 (http://www.r-project.org/). We used the function Generalized Least Squares of the Linear and Nonlinear Mixed Effects Models (Gaussian family and Identity link function). Data were normalized using the Box-Cox transformation. An error structure was chosen where a correlation between the dates was assumed with the shape of a compound symmetric matrix. The structure of the variances-covariances matrix was evaluated with Akaike's criterion (AIC) and Likelihood Ratio Tests were built to assess the factor effects.

TUPIOCORIS CUCURBITACEUS PREDATION ON EGGS FROM UNTREATED AND IRRADIATED PARENTS

Late 5th instar mirid *T. cucurbitaceus* nymphs were randomly collected from the colony and isolated individually in a glass tube where they were provided with water on a moistened cotton wad for a period of 24 h until adult emergence.

NO CHOICE EXPERIMENT

Consumption of eggs by *T. cucurbitaceus* females was evaluated on 3 types of *T. absoluta* eggs, i.e., eggs from untreated parents or from parents with one irradiated member (female or male). After irradiation, the emerged *T. absoluta* adults were separated into couples by the following host crosses: ♀$_U$ × ♂$_U$ (untreated control), ♀$_U$ × ♂$_I$ and ♀$_I$ × ♂$_U$ (where U = untreated, I = irradiated). All the couples were individually placed in a cylindrical acetate cage (24.5 cm high by 7.5 cm diam) containing a tomato plant where they were allowed to mate and lay eggs. These eggs (24–48 h old) were collected with a fine brush and were used in the predation studies. Two hundred eggs obtained from *T. absoluta* couples of each cross were transferred to the surface of a tomato leaflet (approx. 3 × 2 cm) using a stereo microscope. This leaflet was placed on the bottom of a Petri dish (9 cm diam and 1.5 cm deep) that had been lined with a filter paper. A newly emerged *T. cucurbitaceus* female was introduced into this arena for 24 h. After this exposure, the number of partially or fully consumed eggs was recorded using a stereo microscope. Preyed eggs can be easily distinguished because predation leaves an empty egg cuticle. We performed 10–11 replicates for each experimental host cross. The experiment was carried out in a controlled chamber at 25 ± 2 °C, 44–80% RH and a photoperiod of 14:10 h L:D. These environmental conditions were measured with a TFA digital thermo-hygrometer model 30.5003 (Dostmann GmbH & Co.KG, Germany). The variable number of preyed eggs was analyzed using a one-factor analysis of variance (ANOVA) with type of cross as source of variation with Statistica for Windows (Statsoft 2000).

CHOICE EXPERIMENT

We studied the preference of *T. cucurbitaceus* females for *T. absoluta* eggs from untreated parents and from parents with one member irradiated. The *T. absoluta* eggs and predator experimental specimens were obtained as described above. In this experiment each newly emerged *T. cucurbitaceus* female was exposed to 400 eggs of *T. absoluta*, 200 from the untreated control cross (♀$_U$ × ♂$_U$) and the remaining 200 from one of the crosses with one of the parents irradiated (♀$_U$ × ♂$_I$ or ♀$_I$ × ♂$_U$). The eggs (24–48 h old) were placed with a brush on a tomato leaflet that had been divided in 4 quadrants, each holding 100 eggs. Ten replicates were carried out with *T. absoluta* eggs oviposited from each of the crosses, ♀$_U$ × ♂$_U$ and ♀$_U$ × ♂$_I$, and 8 replicates with eggs from each of the crosses, ♀$_U$ × ♂$_U$ and ♀$_I$ × ♂$_U$. Mirid females were removed after 24 h of exposure. Then, the number of partially or fully consumed eggs was recorded using a stereo microscope. The experiment was conducted in a controlled chamber at 25 ± 2 °C, 40–65% RH and a photoperiod of 14:10 h L:D. Data were analyzed using a paired *t* test with Statistica for Windows (Statsoft 2000).

Results

POPULATION SUPPRESSION BY IRRADIATED MALES AND FEMALES AND THEIR PROGENY UNDER SEMI-CONTROLLED CONDITIONS

Experiment 1. Effects of an Over-flooding Ratio of 10:1 Treated:Untreated *T. absoluta* Moths

Average (± SE) minimum/maximum temperature and relative humidity during this experiment were 11.4 ± 0.8/ 32.4 ± 1.2 ºC and 37.6 ± 2.5/ 83.3 ± 1.3% respectively. During the 3 months of the experiment the population completed 2 generations. The 3 variables recorded (number of eggs, small and large larvae) showed 2 periods of peak production over the 12 week experimental period (Fig. 1).
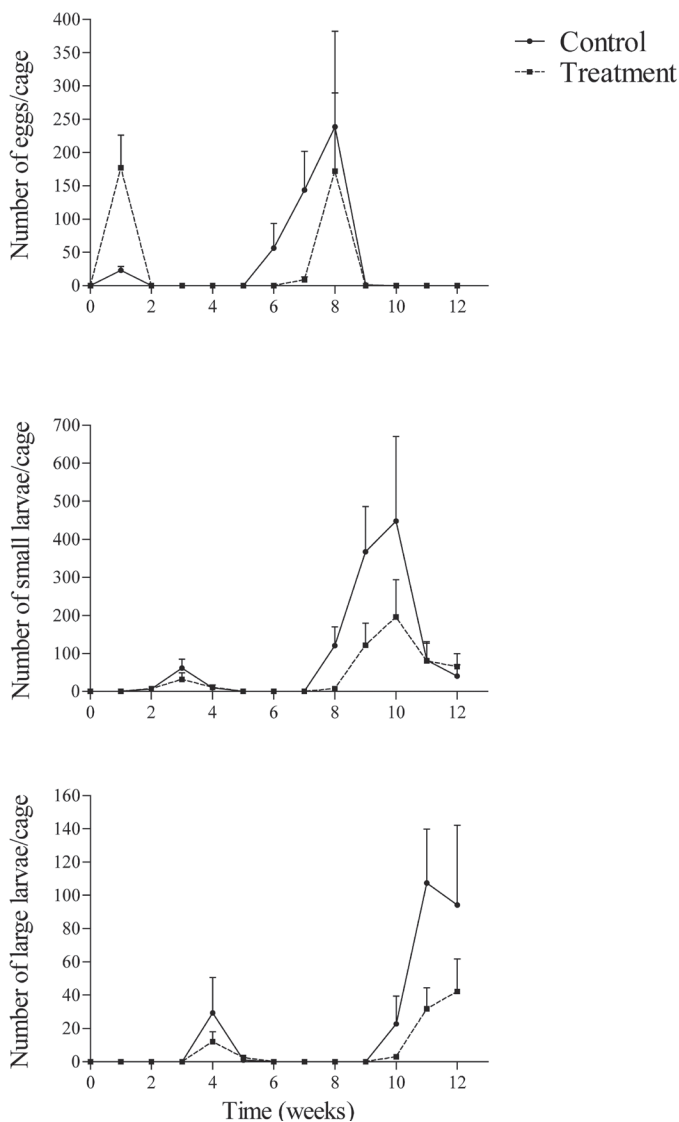
The number of large larvae obtained was not significantly different between the untreated control group and the treatment group for both peak production periods (first production period: F = 0.023; df = 1,16; P = 0.88; second production period: F = 2.66; df = 1,26; P = 0.11). With respect to production over time, there were significant differences in the number of large larvae among weeks for both production periods (first production period: F = 11.07; df = 1,16; P = 0.0043; second production period: F = 9.40; df = 1,26; P = 0.0050) (Fig. 1c).

Experiment 2. Effects of an Over-flooding Ratio of 15:1 Treated:Untreated *T. absoluta* Moths

Average (± SE) minimum/maximum temperature and relative humidity during this experiment were 14.9 ± 0.05/ 37.7 ± 1.0 ºC and 26.6 ± 1.4/ 79.7 ± 2.2%, respectively. During the 3 months of the experiment the population completed 3 generations. The number of eggs and larvae showed 3 production periods during the experimental period (Fig. 2).

The untreated control group produced less eggs in the first production period and more eggs in the second and third periods in comparison
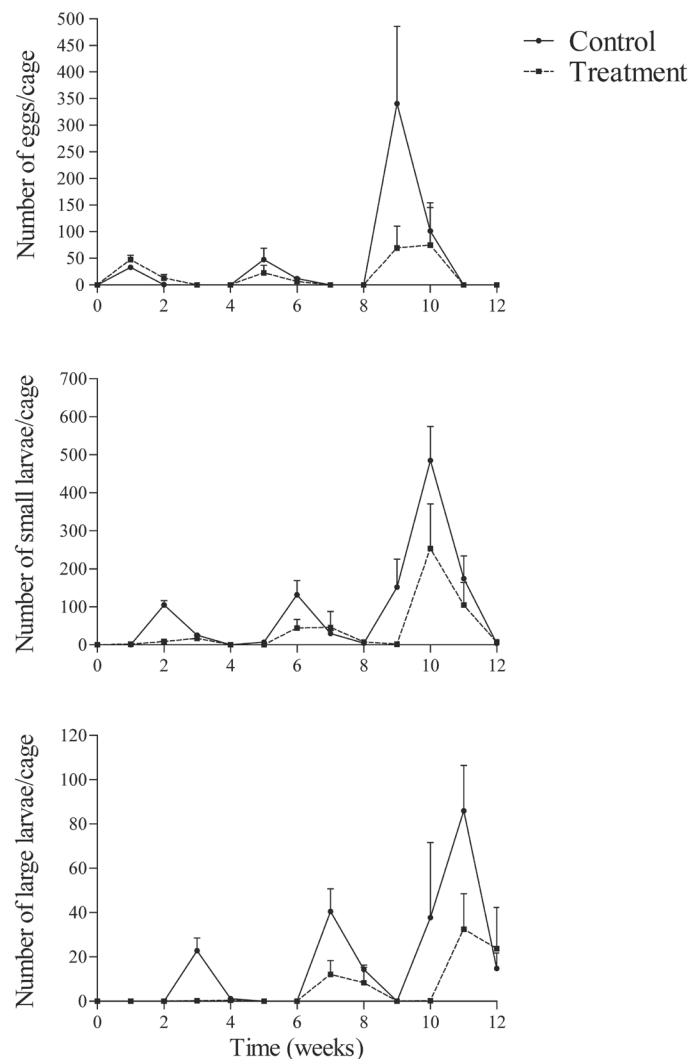
Fig. 1. Population suppression by irradiated individuals and their progeny over 12 weeks at a 10:1 irradiated:untreated ratio. a) Number of eggs/ cage, b) number of small larvae /cage and c) number of large larvae/cage (mean ± SE). Only 1 release of irradiated moths was made, i.e., at the beginning of the experiment. For details see text on Experiment 1.

Significant differences in the number of eggs in both peak production periods were observed between the treatment and the untreated control group (first production period: F = 22.93; df = 1,8; P = 0.0014; second production period: F = 4.39; df = 1,36; P = 0.043). In the first period, more eggs were produced in the treatment cages as compared with the control cages. Conversely, during the second peak production period, more eggs were produced in the untreated control cages. During the second peak production period, there were no significant differences in the number of eggs among weeks (F = 0.0058; df = 1,36; P = 0.93) (Fig. 1a).

More small larvae were observed in the untreated control as compared with the treatment group during both peak production periods; however, these differences were not significant (first production period: F = 0.05; df = 1,26; P = 0.83; second production period F = 3.14; df = 1,46; P = 0.083). With respect to production over time, there were no significant differences in the number of small larvae among weeks of both peak production periods (first production period: F = 1.56; df = 1,26; P = 0.22; second production period: F = 0.18; df = 1,46; P = 0.67) (Fig. 1b).

Fig. 2. Population suppression by irradiated individuals and their progeny over 12 weeks at a 15:1 irradiated:untreated ratio. a) Number of eggs/cage, b) number of small larvae /cage and c) number of large larvae/cage (mean ± SE). Only 1 release of irradiated moths was made, i.e., at the beginning of the experiment. For details see text on Experiment 2.

with the treatment group, but the differences were only significant for the first peak production period (first production period: F = 10.11; df = 1,26; P = 0.0038; second production period: F = 3.81; df = 1,26; P = 0.062; third production period: F = 2.64; df = 1, 26; P = 0.12). With respect to production over time, significant differences were observed among weeks only in the first production period (first production period: F = 74.78; df = 1,26; P < 0.0001; second production period F = 0.50; df = 1,26; P = 0.48; third production period: F = 2.15; df = 1,26; P = 0.15) (Fig. 2a).

Significantly more small larvae were produced in the untreated control cages than in treatment cages for all 3 production periods (first production period: F = 7.45; df = 1,41; P = 0.0093; second production period: F = 5.29; df = 1,41; P = 0.027; third production period: F = 4.11; df = 1,56; P = 0.047). With respect to production over time, significant differences were observed among weeks in all production periods (first production period: F = 23.46; df = 1,41; P < 0.0001; second production period F = 10.62; df = 1,41; P = 0.0022; third production period: F = 4.84; df = 1,56; P = 0.032) (Fig. 2b).

The number of large larvae was significantly different between the untreated control group and the treatment group for the first 2 production periods (first production period: F = 72.93; df = 1,13; P < 0.0001; second production period: F = 9.01; df = 1,26; P = 0.0059). With respect to production over time, there were no significant differences among weeks during the second production period (F = 1.72; df = 1,26; P = 0.20). During the third production period, the number of large larvae was significantly different among weeks and marginally different between treatments (F = 7.56; df = 1,41; P = 0.0088; and F = 3.31; df = 1, 41; P = 0.076, respectively) (Fig. 2c).

TUPIOCORIS CUCURBITACEUS PREDATION ON EGGS FROM UNTREATED AND IRRADIATED PARENTS

No Choice Experiment

The number of *T. absoluta* eggs consumed by *T. cucurbitaceus* was not affected by the origin of the *T. absoluta* eggs (type of parental cross) (F = 1.37; df = 2,28; P = 0.27) (Fig. 3). *Tupiocoris cucurbitaceus* preyed upon an average of 147.4 ± 9.5 (mean ± SE) *T. absoluta* eggs oviposited by untreated *T. absoluta* moths, and upon an average of 135.8 ± 8.8 and 127.2 ± 7.7 eggs produced from the $♀_u × ♂_i$ and $♀_i × ♂_u$ crosses, respectively.

Choice Experiment

In the choice test mirid females showed no preference for eggs oviposited by untreated *T. absoluta* moths or for eggs produced from the cross were the male had been irradiated (t = 0.48; df = 1,9; P = 0.64) (Fig. 4). In this case, on average 97.3 ± 6.7 (mean ± SE) eggs from untreated *T. absoluta* moths were preyed upon by *T. cucurbitaceus* and an average of 94.3 ± 10.2 eggs from the cross, $♂_i × ♀_u$. However, the predation was different when eggs were offered from the cross $♀_i × ♂_u$ (t = -3.07; df = 1,7; P = 0.02) (Fig. 5). Eggs produced by irradiated females were preferred compared with those from untreated females. An average of 77.2 ± 5.0 (mean ± SE) *T. absoluta* eggs were preyed upon that originated from untreated moths compared with 99.1 ± 6.3 eggs that were produced from the $♀_i × ♂_u$ cross.

Discussion

One of the main challenges in an AW-IPM project that incorporates the IS technique is to determine the number of sterile moths that need to be released to obtain the desired effective over-flooding ratio, i.e., the ratio of irradiated to wild moths that will enable suppression of the wild pest population. Since moth quality (ability to fly and mating competitiveness) could be affected by the ionizing radiation during the sterilization procedure, the use of lower doses might require the release of a greater number of partially sterile males in the field to control the wild pest population. In our field cage experiments, more eggs were produced in the treatment cages with irradiated insects in the first production period than in the untreated control group due to the larger number of females introduced in to the cages. Moreover, during the second production period, more eggs were produced in the control cages than in the treatment cages irrespective of the sterile to untreated ratio, reflecting the effects of the inherited sterility. Although an over-flooding ratio of 10:1 caused a decline in larval produc-
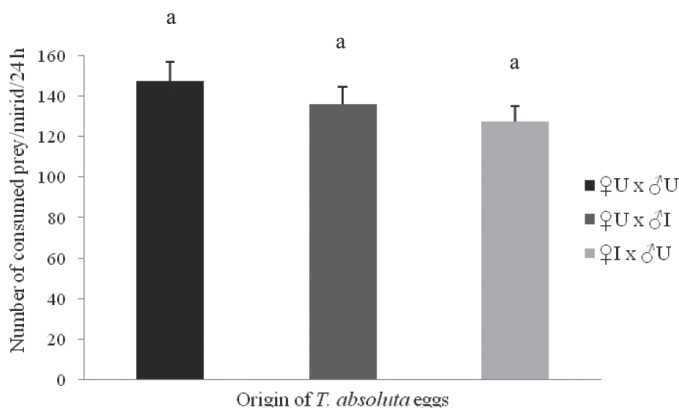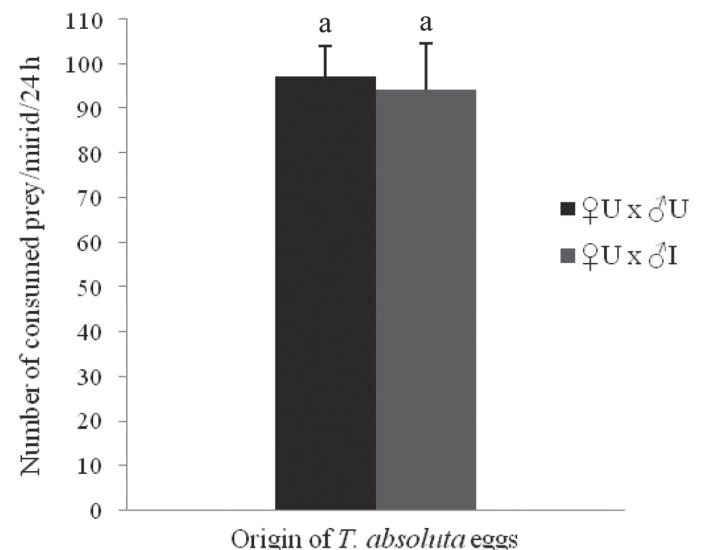

Fig. 3. Prey consumption in the no-choice test. Number of *T. absoluta* eggs consumed per *T. cucurbitaceus* (mean ± SE) individual in 24 h from following *T. absoluta* eggs crosses: $♀_u × ♂_u$, $♀_u × ♂_i$ or $♀_i × ♂_u$ The same letter above the 3 error bars indicates that there were no significant differences in the numbers of eggs consumed based on the crosses from which they had originated (ANOVA, P > 0.05). U = untreated, I = irradiated.

Fig. 4. Prey consumption in the choice test involving progeny of irradiated males. Number of *T. absoluta* eggs from following *T. absoluta* eggs crosses, $♀_u × ♂_u$ and $♀_u × ♂_i$, consumed per *T. cucurbitaceus* (mean ± SE) individual in 24 h. The same letter above the 2 error bars indicates that there was no significant difference in the numbers of eggs consumed based on the crosses from which they had originated, even though the male parent had been irradiated in 1 of the crosses (paired t tests, P > 0.05). U = untreated, I = irradiated.
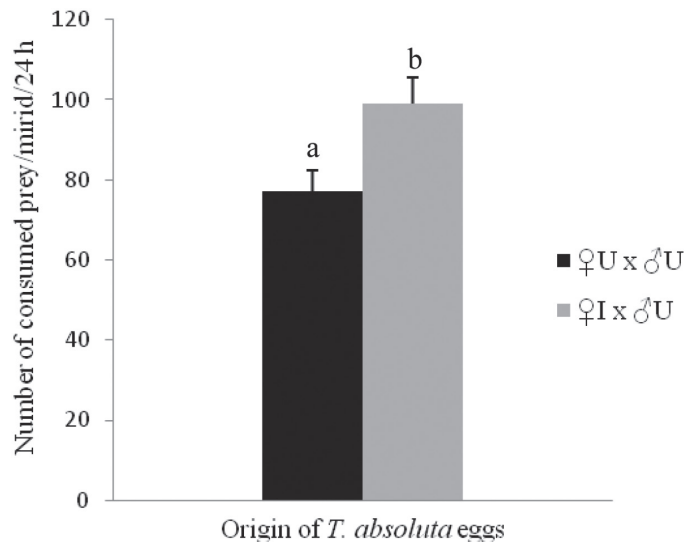
Fig. 5. Prey consumption in the choice test involving progeny of irradiated females. Number of *T. absoluta* eggs consumed per *T. cucurbitaceus* (mean ± SE) individual in 24 h from the following *T. absoluta* eggs crosses, $\female_U \times \male_U$ and $\female_I \times \male_U$. The different letters above the 2 error bars indicates that there was a significant difference in the numbers of eggs consumed based on the crosses from which they had originated, even though the female parent had been irradiated in 1 of the crosses (paired t tests, $P < 0.05$). U = untreated, I = irradiated.

tion as compared with the untreated control group, these differences were statistically not significant. This was probably due to the variability in the data sets. However, increasing the treated:untreated moth ratio to 15:1 resulted in significantly fewer small and large larvae as compared with the untreated control cages across the 3 generations studied. These results indicate that sub-sterile males were as competitive as untreated males in mating with untreated females under the experimental conditions, and were also successful in producing sterile F₁ progeny that reduced the wild population growth over time.

Optimal over-flooding ratios seem to be different depending on the lepidopteran pests, as evidenced from other field cage studies. A treated:untreated over-flooding ratio of 5:1 in field cages was sufficient to effectively suppress wild populations of the cactus moth, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae), the potato tuber moth, *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae) and the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) (Nguyen Thi & Nguyen Thanh 2001; Saour 2004; Hight et al. 2005). Experiments with the litchi stem-end borer, *Conopomorpha sinensis* Bradley (Lepidoptera: Gracillariidae) in field cages showed that the release of irradiated male adults at a 10:1 treated:untreated ratio could effectively control a natural population of this pest (Hu et al. 2012). However, for *P. gossypiella* a treated:untreated over-flooding ratio of 20:1 was necessary to cause a significant reduction of the wild population of this pest (Ahmad et al. 2002). Differences in the over-flooding ratios of moth species to achieve the suppression of their populations can be explained by differences in their intrinsic characteristics (reproduction rate, longevity, radiation damage, etc.). The potential reduced quality of the sterile moths resulting from stress related to rearing methods, irradiation dose, handling, transport and release processes will also affect the optimal over-flooding ratio for each species.

In most open field studies that evaluated the potential of IS to control lepidopteran pests, the over-flooding ratios of irradiated to untreated individuals necessary to achieve population suppression were much greater than those observed in field cage studies (more than 60:1 for *P. gossypiella*, 40:1 for *C. pomonella*, 100:1 for *T. anartoides*)

(Bloem et al. 2005). This might be related to higher mortality in an open field situation due to inclement weather, predation and the need for a better dispersal to find a female to mate as compared with field cage conditions. Based on our results, we can therefore expect that the numbers of sterile male *T. absoluta* needed for the release will need to be higher than those required to obtain an over-flooding ratio of 15:1 before we can expect a reduction in the wild population of *T. absoluta* in tomato crops. Further studies under greenhouse or open field conditions are required to test this hypothesis.

The IS technique is a species-specific tactic that is especially effective when it is combined with other methods of control such as mating disruption, mass-trapping, habitat removal, host-plant resistance and biological control (Bloem et al. 2005). Carpenter et al. (2005) described the synergism between IS and natural enemies. Hence, the level of population suppression of *T. absoluta* achieved by the IS technique alone could be improved by combining it with the use of natural enemies like the mirid predator *T. cucurbitaceus*. Our predation assays indicate that *T. cucurbitaceus* females were indeed able to consume large numbers of *T. absoluta* eggs, regardless whether they originated from untreated crosses or from a cross where one of the parents had been irradiated. In the choice experiment, the predator female preyed as many eggs from untreated parents as from non-irradiated females mated to irradiated males. However, the mirid female preyed significantly more eggs from irradiated females than eggs from untreated females. There might have been some external cues, which possibly may be indicative of the internal quality of the eggs that influenced the predator in its selection of the eggs. It is known that nutritional quality of the prey is an important characteristic that can influence the predator's choice as the greater the nutritional value of the prey, the better the predator's fitness (better survival, greater fecundity, etc.) (Eubanks & Denno 2000). Hagler et al. (1992) found that mirid predators consumed more young eggs than older eggs, and they concluded that this is due to the fact that young eggs have more vitellus content than older eggs. The vitellus is the major protein source in the egg, and is gradually consumed by the developing embryo. Since eggs oviposited by irradiated *T. absoluta* females are infertile (Cagnotti et al. 2012), it is likely that they contain more vitellus than fertile ones, and therefore the eggs from irradiated females may be preferred by the *T. cucurbitaceus* predator. Further research is necessary to analyze the levels of this protein and other nutritive elements in the *T. absoluta* eggs from different origins to clarify this issue.

The integration of the SIT or IS with natural enemies into an effective integrated pest management approach can only be successful either if the natural enemy does not negatively impact the irradiated insects and their progeny more severely than those of the wild pest population, or if the release of irradiated insects does not negatively impact the efficacy of the natural enemy (Carpenter 1993). The use of such a combined strategy for the control of *T. absoluta* seems to have potential in view of the preference shown by the predator *T. cucurbitaceus* for sterile *T. absoluta* eggs, which could be used to maintain the predator's population in the field at basic levels. In addition, we could expect a successful integration of both techniques of control because the predator consumed a great and equal quantity of eggs laid by untreated moths or females mated with irradiated males. Moreover, it is worth mentioning that more eggs were consumed by *T. cucurbitaceus* than by the European predators, *M. pygmaeus* and *N. tenuis* (Arnó et al 2009; Urbaneja et al. 2009; Chailleux et al. 2013).

Based on results presented herein, we can conclude that the released irradiated insects had the appropriate quality to achieve a certain level of suppression of wild populations of *T. absoluta* and to produce suitable eggs to be consumed by the predator *T. cucurbitaceus*. Thus, it is technically feasible to combine the use of this natural enemy with IS to control this moth pest.

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