Selection and Suitability of an Artificial Diet for 
*Tuta absoluta* (Lepidoptera: Gelechiidae) Based on Physical and Chemical Characteristics

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Abstract

*Tuta absoluta* (Meyrick, 1917) is a key tomato pest in South America and, recently, in Europe and Africa. To develop efficient control methods for this pest, adequate rearing protocols are desirable. As an alternative to tomato leaves (natural diet), we evaluated four artificial diets. Biological traits including larval and pupal viability and development time, pupal weight and deformations were assessed. Additionally, the optimum container size and larval density were evaluated. The diet based on casein, wheat germ and cellulose allowed the best development of *T. absoluta*, showing higher viability and no negative effects on larval instars and pupal weight. The best container was a glass tube measuring Ø 1 x h 6 cm, topped with waterproof cotton, with a density of three larvae. To evaluate the suitability of this diet, *T. absoluta* was reared during eight generations and life-table parameters were estimated for the F1, F3, F6, and F8 generations. The total viability (egg–adult) increased over the generations, reaching 75% in the eighth generation. Based on life-table estimations no differences among generations were found. The net reproductive rate (Ro) was higher than 40, the intrinsic rate of increase (rm) ranged between 0.08 and 0.11, the finite rate of increase (λ) was 1.1, the mean generation time (T) have a maximum of 44 d and doubling time ranged from 5.89–8.32 generations. These results indicated that a diet based on casein, wheat germ and cellulose was suitable for *T. absoluta* rearing in laboratory conditions.

Key words: Rearing technique; fitness; life table.

*Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) It is one of the most devastating tomato pests because it feeds on all aerial structures at all stages of plant growth, causing losses of 80–100% (Desneux et al. 2010; Escobar and Lee 2009; Estay 2009; Urbaneja et al. 2012, 2013) As well as on tomatoes, this pest has been reported from other economically important solanaceous plants including *Solanum tuberosum* (L.) (potato), *Solanum melongena* (eggplant) and weed plants like *Solanum nigrum* (European black nightshade), *Atropa belladonna* (belladonna), *Datura ferox* (L.) (fierce thorn–apple), *Nicotiana longiflora* Cav. (longflower tobacco), *Nicotiana glauca* Graham (tree tobacco), *Salpichroa origanifolia* (L.) Baill. (Lily of the valley vine), *Solanum americanum* Mill. (American black nightshade), *Solanum sisymbriifolium* Lam. (sticky nightshade), *Brugmansia arborea* (L.) (angel’s trumpet), *Solanum chenopodioides* Lam. (goosefoot nightshade), *Solanum saracoides* Sendtnr (hoe nightshade), *Solanum pygmaeum* and *Datura stramonium* (L.) (Jimson weed) (Bawin et al. 2015, Salas Gervassio et al. 2016) This lepidopteran was first reported in 1914 in Peru, and currently is a common pest found in South America (Jham et al. 2001) Since 2006, *T. absoluta* had invaded Europe and Africa, where it has caused significant economic losses (Desneux et al. 2011, Urbaneja et al. 2012, 2013).

The failure to control this pest may have a strong economic impact, and its recent history of introductions has increased the need for studies to develop strategies for its biological control, by the use of natural enemies, mainly the larval parasitoids such as *Apanteles gelechiidivoris* Marsh (Bajonero et al. 2008) or *Pseudapanteles dignus* (Muesebeck) (Luna et al. 2007), and microorganisms such as granulovirus PhopGV (Mascarín et al. 2010). For these studies it is necessary the production of large numbers of insects preferably with low costs and high performance (Zou et al. 2015). The rearing of *T. absoluta* in natural diet needs extensive manual labor and space to keep plants capable to support a significant pest population (Cely et al. 2010). The most used tomato varieties are “Santa Clara” and “IPA6” (Neves et al. 2003, Oliveira et al. 2009) in South America and “Marmande” (Chailleux et al. 2013, Han et al. 2015) and “Money maker” (Cuthbertson et al. 2013) in Europe.

Artificial diets have been essential to the development of knowledge in biology and ecology of many species of insect pests (Sorensen et al. 2012). Due to the possibility of producing a large...
number of insects, it has been possible to study nutritional processes, biochemical, behavioral and biological control methods of many insect pests (Parra 2012; Vanderzant 1974). Some artificial diets for *T. absoluta* have been tested. Giustolin et al. (1995) evaluated 10 diets for *T. absoluta* with different protein contents, and determined that a diet based on “Carioca” white beans, wheat germ, casein, yeast extract and soy protein was the most appropriate. Later, Mihsfeldt and Parra (1999) developed a diet based on white beans plus powdered tomato leaves that showed promise for rearing *T. absoluta* in laboratory conditions. However, the total viability obtained was <75%, which is proposed by Singh (1983) as the minimum for the use of a diet in the production of insects in the laboratory.

To understand the effect of external factors, in this case an artificial diet, in the development, survival and reproduction at the population level, life tables are a very important tool (Chi and Su 2006, Wittmeyer and Coudron 2001). They provided useful information to analyze the mortality factors in insect population and evaluate the relative importance of the numerous independent variables derived from field or laboratory experimentation (Kakde et al. 2014).

To improve rearing techniques for *T. absoluta*, the aim of this study was to select an artificial diet for *T. absoluta* based on physical and nutritional characteristics, assessing the insect’s biological development, the most suitable container and density in the selected diet, and evaluating insect development for eight successive generations.

### Material and Methods

**Rearing Stock of T. absoluta**

Individuals used in this experiment were obtained from the colony of *T. absoluta* maintained in the Laboratory of Insect Biology of the Department of Entomology and Acarology, Luiz de Queiroz College of Agriculture, University of São Paulo (ESALQ-USP), Piracicaba, Brazil. Rearing was initiated with a population collected in the state of São Paulo, Brazil, in 2012. *T. absoluta* was maintained in rearing cages 70- × 50- × 50-cm (height × width × depth) covered with voile containing tomato var. “Santa Clara” in a climate-controlled room (temperature 25 ± 2°C, relative humidity (RH) 70 ± 10% and a photoperiod of 14:10 (L:D) h. The plants were infested with adults, allowing oviposition for 24 h, after that, they were removed and the larvae allowed to develop until pupae formation. Obtained adults were transferred to new cages and were fed with honey droplets placed on the walls of the cage. Eggs were collected daily on individual tomato leaflets kept in a container with water and fastened at the top of the cage. Subsequently, the eggs were removed from the leaflets with a brush inside a glass recipient with distilled water, and then collected by filtration on polyester fabric. The eggs were placed in a Petri dish and kept in an incubator (temperature 25 ± 2°C, RH 70 ± 10% and a photoperiod of 14:10 (L:D) h until the larvae hatching.

#### Diets Evaluated

Four diets were compared (Table 1): D1: the diet of Mihsfeldt and Parra (1999) for rearing *T. absoluta*, based on the diet of Greene et al. (1976) "developed for *Anticarsia gemmatalis* Hübner; D2: the diet of Hensley and Hammond (1968) which is recommended for rearing *Diatraea saccharalis* (F.) and other lepidopterans; D3: the diet of Berger (1963) which was initially used for *Heliotis virescens* (F.) and contains cellulose, a component that does not provide nutrients but confers structural characteristics on the diet that may benefit the insect feeding; and D4: Berger’s (1963) diet, with the addition of lyophilized powdered tomato leaves. The tomato-leaf powder was obtained by lyophilizing 7-week-old leaves of the variety Santa Clara for 3 d in a lyophilizer (model Novalyph-NL150, Savant Instruments, Holbrook, NY), and then ground to a fine powder. Because of the small size of *T. absoluta*, the dry ingredients with larger particles were milled to homogenize each diet. The artificial diets were prepared as described by Parra (2013). Fresh tomato leaves were used as control (D5).

#### Development of T. absoluta on Artificial and Natural Diets

For each diet, 100 repetitions with three larvae each were used. The container used was an Ø1.0 × b 6.0 cm flat-bottom glass tube capped with water-repellent cotton. In each container about 4 ml of the diet tested was placed, taking care to form a thin film of diet on the walls of the container, increasing the area covered and available for the larvae to feed and simulating the natural conditions, where the larvae feeds on the leaf.

For the natural diet, the experimental unit was a plastic cup (Ø 8.5 cm × b 14), covered with thin veil to allow gas exchange. Each cup contained a tomato leaflet with the petiole submerged in water. In each experimental unit, three newly hatched larvae were placed, as for the artificial diet.

For the artificial diets, the mortality at 7 d after inoculation was evaluated, and for natural and artificial diets duration and viability of larval and pupal stages, pupal weight after 24 h, sex ratio (Butt and Cantu 1962, Sannino and Espinosa 2010) and percentage of deformations were estimated. With the adults obtained from each diet, 15 pairs were formed and placed in individual cages similar to those used to assess the natural diet. The egg viability was evaluated by collecting 20 eggs from each pair from the second day of oviposition, totaling 300 eggs per diet; these eggs were placed on filter paper in glass Petri cups containing tomato leaves (lyophilized).

### Table 1. Composition of artificial diets for rearing *T. absoluta*

| Component                  | D1  | D2  | D3  | D4  |
|----------------------------|-----|-----|-----|-----|
| White beans                | 37.5 g | –  | –  | –  |
| Wheat germ                 | 30.0 g | 27.0 g | 22.5 g | 22.5 g |
| Cellulose                  | –  | –  | 3.7 g | 3.7 g |
| Soy protein                | 15.0 g | –  | –  | –  |
| Saccharose                 | –  | 45.0 g | 26.2 g | 26.2 g |
| Choline chloride           | –  | 0.9 g | 0.7 g | 0.7 g |
| Wesson salts               | –  | 9.0 g | 7.5 g | 7.5 g |
| Casein                     | 15.0 g | 27.0 g | 26.2 g | 26.2 g |
| Brewer’s yeast             | 18.7 g | –  | –  | –  |
| Ascorbic acid              | 1.8 g | 3.6 g | 3.0 g | 3.0 g |
| Sorbic acid                | 0.9 g | –  | –  | –  |
| Methyl parahydroxybenzoate (Nipagin) | 1.5 g | 1.5 g | 1.5 g | 1.5 g |
| Tetracycline               | 56.5 mg | 0.2 mg | 0.5 mg | 0.5 mg |
| Formaldehyde 37%           | 1.8 ml | 0.4 g | 1.0 ml | 1.0 ml |
| Vitamin solution           | 4.5 ml | 9.0 ml | 7.5 ml | 7.5 ml |
| Agar                       | 11.5 g | 18.0 g | 9.0 g | 9.0 g |
| Water                      | 600.0 ml | 780.0 ml | 630.0 ml | 630.0 ml |
| Powdered tomato leaves     | 1.5 g | –  | –  | 4.0 g |

*Mihsfeldt and Parra (1999), based on Greene et al. (1976).*

*Hensley and Hammond (1968).*

*Berger (1963) modified.*

*Berger (1963) modified + powdered tomato leaves.*

*Niacinamide: 1.0 g; calcium pantothenate: 1.0 g; riboflavin: 0.50 g; thiamin: 0.5 g; pyridoxine: 0.25 g; folinic acid: 0.10 g; biotin: 0.02 mg; vitamin B12 (1,000 mg/ml): 2.00 ml.*
In order to assess whether the type of container affected the development of *T. absoluta*, the number of instars was determined. Eighty tubes of diet D3 were each inoculated with one larva. The head-capsule width was measured until pupation, using an ocular micrometer attached to a stereomicroscope (20×magnifications). The number of instars and the growth rate were determined by the mathematical model based on Dyar (1890), as recommended by Parra and Haddad (1989).

### Container Size for Rearing *T. absoluta*

In order to assess whether the type of container affected the development of *T. absoluta*, three sizes and types of container were compared. The first container “A” was a large glass tube of Ø 2.0 × 8.5 cm covered with waterproof cotton, which is commonly used for rearing Lepidoptera in Brazil. The second was similar to the first, but smaller, measuring Ø 1.0 × 6.0 cm, it was chosen due to the plastic material of the containers) to constant weight. The water content of the containers was calculated as a percentage (Colestino 2010):

\[
\text{Water content (\% base water)} = \frac{\text{Initial mass} - \text{final mass})\times 100}{\text{initial mass}}
\]

### Optimum Number of *T. absoluta* larvae per container

Densities of 1, 3, 6, and 12 larvae in each tube were tested. The experimental unit was a flat-bottom glass tube Ø 1.0 × 6.0 cm capped with water-repellent cotton containing the diet that gave the best results (D3). For each treatment, 30 experimental units were evaluated for the parameter of larval viability.

### Biological Development of *T. absoluta* on the Selected Diet for Eight Generations

*T. absoluta* development reared on the most appropriate diet, D3, was evaluated throughout the F1, F3, F6, and F8 generations. For each generation, 100 tubes were inoculated with three larvae each one. The viability in 7 d, larval and pupal viability and duration were evaluated. From the emerged adults, 25 pairs were formed and kept in identical cages to those described on “Development of *Tuta absoluta* on Artifical and Natural Diets” and fed with pure honey droplets (Bogorni and Carvalho 2006). The pre-oviposition period, longevity and number of eggs laid daily per female were recorded. For each pair, 20 eggs were separated from the second day and the egg viability was calculated. All tests were performed at a temperature of 25 ± 2°C, RH 70 ± 10% and a photoperiod of 14 h.

### Data Analysis

Data were analyzed by generalized linear models (GLMs) (Nelder and Wedderburn 1972) of the binomial type for viability and sex ratio data, and the Poisson type for duration, longevity, and number of eggs laid per female. The data were evaluated using a standard half-probability graph with simulated envelope (Demétrio and Hinde 1997, Hinde and Demétrio 1998). In case of significant differences, the Tukey multiple comparisons test was applied at 5% significance, using the glht function multicompar package with adjusted P.

With the collected data for the D3 diet, fertility life tables were constructed and the net reproductive rate (Ro), mean generation time (T), intrinsic rate of increase (rm), finite rate of increase (λ), and doubling time (DT) were calculated. These parameters were compared by a bootstrap analysis (Meyer et al. 1986) and all analyses were done in the statistical program R version 3.2.3.

### Results

**Development of *T. absoluta* on Artificial and Natural Diets**

Comparing the performance of the four artificial diets at 7 d after inoculation showed that diet D3 yielded approximately 77% of viability the highest value, followed by diet D2 (F = 11.972; df = 3,
Table 3. Sex ratio and weight of pupae of *T. absoluta* reared on five diets

| Diet       | Sex ratio<sup>a</sup> | Pupal weight (mg)<sup>b</sup> | Deformations<sup>%</sup> |
|------------|------------------------|-------------------------------|--------------------------|
|            | Females | Males       |                            |                          |
| D1<sup>c</sup> | 0.55    | 2.50 ± 0.18 b | 2.32 ± 0.107 b | 3.76                      |
| D2<sup>d</sup> | 0.44    | 2.51 ± 0.15 b | 2.20 ± 0.12 b  | 4.00                      |
| D3<sup>e</sup> | 0.40    | 3.33 ± 0.07 a | 3.34 ± 0.104 a | 3.33                      |
| D4<sup>f</sup> | 0.53    | 3.38 ± 0.09 a | 3.44 ± 0.069 a | 4.33                      |
| D5<sup>g</sup> | 0.50    | 3.64 ± 0.10 a | 3.48 ± 0.047 a | -                         |

<sup>a</sup>No difference in sex ratio (Chi² = 6.33, df = 4, *P* = 0.1753).
<sup>b</sup>Means followed by the same letter in the column do not differ significantly (GLM with quasi-Poisson distribution, followed by post hoc Tukey test; *P* < 0.05).
<sup>c</sup>Mihsfeldt and Parra (1999), based on Greene et al. (1976).
<sup>d</sup>Hensley and Hammond (1968).
<sup>e</sup>Berger (1963) modified.
<sup>f</sup>Berger (1963) modified + powdered tomato leaves.
<sup>g</sup>Tomato leaves.

Table 4. Larval viability (%) of *T. absoluta* reared in three types of containers and water loss of the diet per container

| Container type             | Larval viability (%)<sup>a</sup> | Water loss (%) |
|----------------------------|----------------------------------|----------------|
| Large glass tube (2.0 × 8.5 cm) | 43.33 ± 9.20 b                 | 15.75        |
| Small glass tube (1.0 × 6.0 cm)   | 66.66 ± 8.75 a                | 20.40        |
| Plastic cup (4.0 × 6.0 cm)        | 23.33 ± 7.85 c                | 13.23        |

<sup>a</sup>Means followed by the same letter in the column do not differ significantly (GLM with quasi-binomial distribution, followed by post hoc Tukey test; *P* < 0.05).

895; *P* < 0.001). Larvae fed on diets containing lyophilized tomato leaves (D1 and D4) did not reach a viability level of 60% 7 days after inoculation (Table 2).

The larval period lasted from 16 to 22 d on artificial diets, a longer period compared with the natural diet (*F* = 366.33; df = 4, 554; *P* < 0.001). Among the artificial diets, shorter larval and pupal periods were obtained in the diets containing lyophilized tomato leaves. The longest durations of larval (22 d) and pupal (9 d) stages were obtained with diet D3 (Pupa: *F* = 12.63; df = 4, 442; *P* < 0.001) (Table 2).

Larval viability was highest with the natural diet (91%); among the artificial diets, D3 allowed the highest viability (74%) (*F* = 35.155; df = 4, 895; *P* < 0.001). The pupal viability was >70% in all diets, being higher in the natural diet and D3 (*F* = 5.1308; df = 4, 560; *P* < 0.001). The egg viability exceeded 80% with all four diets; however, those registered with diets D3 and D4 reached 90%, and were equal to that obtained with the natural diet (*F* = 4.2583; df = 4, 1499; *P* < 0.001), (Table 2).

A 1:1 Sex ratio was registered in all treatments (Chi² = 6.33; df = 4; *P* = 0.1753) (Table 3). Pupal weight was about 3.5 mg when *T. absoluta* was fed with diets D3–D5, being heavier than those fed on diets D1 and D2. The deformation rates were <5% for all diets (Table 3).

**Determinant of Instar Number of *T. absoluta* on the Selected Diet (D3)**

Larvae of *T. absoluta* fed on diet D3 showed four instars which are the same observed when the insect develops on a natural diet. The

Table 5. Larval and pupal viability of *T. absoluta* reared at five densities per rearing container

| Number of larvae per container | Larval viability (%) | Pupal viability (%) |
|-------------------------------|---------------------|---------------------|
| 1                             | 68.75 ± 4.78 ab     | 90.9 ± 10.16 a      |
| 3                             | 87.5 ± 5.70 a       | 93.75 ± 5.84 a      |
| 6                             | 77.08 ± 7.76 ab     | 78.12 ± 10.30 a     |
| 9                             | 76.39 ± 4.60 ab     | 90.40 ± 4.83 a      |
| 12                            | 61.45 ± 1.35 b      | 98.43 ± 1.46 a      |

<sup>a</sup>Means followed by the same letter in the column do not differ significantly (GLM with quasi-binomial distribution, followed by post hoc Tukey test; *P* < 0.05).

widths of the head capsule were 0.13 ± 0.014, 0.19 ± 0.025, 0.34 ± 0.051, 0.49 ± 0.046, for the first to fourth instar, respectively.

**Container Size for Rearing *T. absoluta***

Larval viability of tomato pinworm measured in the small glass tube resulted in higher viability (66.7%) compared with the other two types of containers tested (*F* = 5.906; df = 2, 87; *P* = 0.027) (Table 4). Water loss was greater in the glass tube container (Table 4).

**Optimum Number of *T. absoluta* Larvae per container**

Larval viability was greater for a density of 3 T. absoluta larvae (87%), and decreased at lower and higher densities (*F* = 23.397; df = 4, 75; *P* = 0.0445). The pupal viability (80–90%) was not affected by the density (*F* = 0.2137; df = 4, 70; *P* = 0.091) (Table 5).

**Biological Development of *T. absoluta* on the Selected Diet for Eight Generations**

A viability of ca. 93% was reached during the first 7 d after feeding with the artificial diet in the eighth generation, being greater than in the previous generations (*F* = 18.492; df = 3, 1196; *P* < 0.001) (Table 6). The developmental period of eggs was longer in the first generation, decreasing in subsequent generations (*F* = 3.66; df = 3, 39; *P* < 0.001) (Table 6). The larval stage was longer in F1, then significantly shorter in F6 and F8 and the shortest in F4 (*F* = 262.63; df = 3, 918; *P* < 0.001). Generation F4 showed a longer pupal stage (a mean of 11.5 d) than the other generations assessed (*F* = 83.24; df = 3, 747; *P* < 0.001). The *T. absoluta* developmental time, considering from egg to adult were 34.7, 30.7, 33.0, and 31.8 in the first, fourth, sixth, and eighth generations, respectively.

The viability of the egg stage was highest in the first generation and decreased in subsequent generations ranging from 93 to 99% The larval and pupal stages showed no difference in the viability across the generations (larva: *F* = 3.491; df = 3.118; *P* = 0.0058; pupa: *F* = 0.4962; df = 3.952; *P* = 0.685). The total viability was >70% in the four generations studied.

*T. absoluta* female longevity ranged from 12 to 16 d throughout the generations studied when fed on the artificial diet. Fecundity was lowest in the first generation, and increased in subsequent generations up to 130 eggs per female (Table 7).

Life-table analyses for generations F1, F4, F6, and F8 (Table 8) showed no differences among generations. The net reproductive rate (Ro) was between 40 and 45, the intrinsic rate of increase (ri) ranged between 0.008 and 0.11, the finite rate of increase (λ) was...
In this study, we observed the development of *T. absoluta* from egg to adult on four artificial diets. The larval stage was the most critical in rearing *T. absoluta*, especially in the first days of development, as observed by Mihsfeldt and Parra (1999). High mortality was observed during the first 7 days after inoculation, this is frequently found on leaf-mining Lepidoptera, that usually present a mortality >32% in the first instar (Zalucki et al. 2002). Many factors influence this mortality when insects are reared on an artificial diet, such as the proportions of the different diets components and the physical conditions, as well as the water content, particle size of the mixture, and microbial agents, among others (Mihsfeldt and Parra 1999, Cohen 2004).

Diets containing lyophilized tomato leaves (D1 and D4) yielded low viability of young larvae (up to 7-d old). This ingredient was added as phagostimulant as stated previously by Mihsfeldt and Parra (1999). Although the tomato-leaf powder contains sugars and amino acids, which could stimulate the start of feeding (Awmack and Leather 2002), it is possible that secondary compounds that hinder the development of caterpillars were preserved by the lyophilization. For example some studies have not shown any benefits of the dry matter of herbaceous plants (Mcginnis and Kasting 1967). This ingredient does not provide nutrients to insects, but modifies the physical structure of the diet by making them consuming more dry matter in a way of a fibrous matrix. This was observed in *Spodoptera eridania* (Cramer) (Lepidoptera: Noctuidae), that increased its food intake on a diet rich in cellulose. The presence of cellulose in artificial diets for lepidopterans has been pointed out as producing a slower growth and lengthening the larval stage (Peterson et al. 1988, Timmins et al. 1988). This effect was observed for *T. absoluta* too, since when it developed in the D3 diet extended the larval period in 10 d compared with the natural diet. *T. absoluta* experimented an increased larval period (3–4 d) (Table 2) when fed on other artificial diets, which often occurs in insects reared on an artificial medium (Mendoza et al. 2016, Shen et al. 2006) and was previously observed by Mihsfeldt and Parra (1999) for *T. absoluta*, with a larval duration of 19 d in artificial diet. The pupal period of the insects developing on diet D3 was similar to that observed in the natural diet.

From this study, we could figure out that the best larval and pupal viability was obtained with a diet containing casein, wheat germ and cellulose. That fact suggests that this diet can supply the nutritional requirements of *T. absoluta*. The fertility was >85% for all the diets, and highest for D3 and D4, which were similar to the fertility rate obtained with the natural diet. High egg viabilities have previously been reported for this species (Bogorni and Carvalho 2006, Gonçalves-Gervásio et al. 1999) reared on both natural and artificial diets (Mihsfeldt and Parra 1999).

Interestingly, the diet that resulted better for *T. absoluta* larval and pupal viability, and fertility, yielded similar pupal weight to those from the D4 and natural diets, for both males and females, an indication that the nutritional conditions of the artificial diet produced similar patterns to the natural diet. Other positive aspect found in this study for the selected artificial diet D3 is that *T. absoluta* developed four larval instars on this diets, the same number as the insect has when it is reared on tomato leaves (Erdogan and Babaroglu 2014, Giustolin et al. 2002, Haji et al. 1988, Mihsfeldt and Parra 1999).

In the insect rearing process, a relevant aspect to be taken into account is the container’s features. The 1.0 × 6.0 cm flat glass tube, capped with water-repellent cotton, proved to be the most suitable for the development of *T. absoluta*. Small containers have been found better for rearing other Lepidoptera species as *Helicoverpa*

### Table 6. Viability with 7 d, duration and viability of *T. absoluta* on diet D3 (casein, wheat germ and cellulose) in the first, fourth, sixth, and eighth generations

| Parameter                  | Generation |
|----------------------------|------------|
| Viability with 7 d         | F1         |
| Duration (days)            | F4         |
| Viability (%)              | F6         |
|                            | F8         |
| Viability with 7 d         | 77.77 ± 2.52 b |
| Duration (days)            | 3.70 ± 0.221 a |
| Viability (%)              | 9.25 ± 1.15 b |
| Viability with 7 d         | 87.66 ± 1.62 ab |
| Duration (days)            | 3.00 ± 0.00 c |
| Viability (%)              | 11.55 ± 1.31 a |
| Viability with 7 d         | 83.00 ± 2.22 ab |
| Duration (days)            | 3.40 ± 0.16 b |
| Viability (%)              | 9.36 ± 1.10 b |
| Viability with 7 d         | 93.33 ± 1.44 a |
| Duration (days)            | 3.30 ± 0.15 b |
| Viability (%)              | 9.05 ± 1.67 b |
| Viability with 7 d         | 68.87 ± 0.42 |
| Duration (days)            | 69.97 ± 0.91 |
| Viability (%)              | 71.18 ± 0.70 |
| Viability with 7 d         | 74.66 ± 0.94 |
| Duration (days)            | 68.87 ± 0.42 |

### Table 7. Longevity and fecundity of *T. absoluta* reared for eight generations on a diet based on casein, wheat germ and cellulose

| Generation | Longevity (days) | Fecundity (eggs per female) |
|------------|------------------|-----------------------------|
| F1         | 12.80 ± 1.11 b   | 88.06 ± 13.89 b             |
| F4         | 11.77 ± 0.79 b   | 117.25 ± 10.73 a            |
| F6         | 15.97 ± 0.78 a   | 130.72 ± 12.32 a            |
| F8         | 13.96 ± 0.63 b   | 115.88 ± 8.33 a             |

### Discussion

*T. absoluta* fed on a diet that contains cellulose (as in D3) produced the highest viability in young larvae (7 d after egg hatching) (Table 2). Cellulose is a polysaccharide that comprises 20–40% of the dry matter of herbaceous plants (Mcginnis and Kasting 1967). This ingredient does not provide nutrients to insects, but modifies the physical structure of the diet by making them consuming more dry matter in a way of a fibrous matrix. This was observed in *Spodoptera eridania* (Cramer) (Lepidoptera: Noctuidae), that increased its food intake on a diet rich in cellulose. The presence of cellulose in artificial diets for lepidopterans has been pointed out as producing a slower growth and lengthening the larval stage (Peterson et al. 1988, Timmins et al. 1988). This effect was observed for *T. absoluta* too, since when it developed in the D3 diet extended the larval period in 10 d compared with the natural diet. *T. absoluta* experimented an increased larval period (3–4 d) (Table 2) when fed on other artificial diets, which often occurs in insects reared on an artificial medium (Mendoza et al. 2016, Shen et al. 2006) and was previously observed by Mihsfeldt and Parra (1999) for *T. absoluta*, with a larval duration of 19 d in artificial diet. The pupal period of the insects developing on diet D3 was similar to that observed in the natural diet.

From this study, we could figure out that the best larval and pupal viability was obtained with a diet containing casein, wheat germ and cellulose. That fact suggests that this diet can supply the nutritional requirements of *T. absoluta*. The fertility was >85% for all the diets, and highest for D3 and D4, which were similar to the fertility rate obtained with the natural diet. High egg viabilities have previously been reported for this species (Bogorni and Carvalho 2006, Gonçalves-Gervásio et al. 1999) reared on both natural and artificial diets (Mihsfeldt and Parra 1999).

Interestingly, the diet that resulted better for *T. absoluta* larval and pupal viability, and fertility, yielded similar pupal weight to those from the D4 and natural diets, for both males and females, an indication that the nutritional conditions of the artificial diet produced similar patterns to the natural diet. Other positive aspect found in this study for the selected artificial diet D3 is that *T. absoluta* developed four larval instars on this diets, the same number as the insect has when it is reared on tomato leaves (Erdogan and Babaroglu 2014, Giustolin et al. 2002, Haji et al. 1988, Mihsfeldt and Parra 1999).

In the insect rearing process, a relevant aspect to be taken into account is the container’s features. The 1.0 × 6.0 cm flat glass tube, capped with water-repellent cotton, proved to be the most suitable for the development of *T. absoluta*. Small containers have been found better for rearing other Lepidoptera species as *Helicoverpa*
Table 8. Life-table parameters for *T. absoluta* reared for eight generations on diet D3, based on casein, wheat germ, and cellulose (L. C. 95%)

| Generation | Ro\(^{a}\) | Rm\(^{b}\) | \(\lambda\) | T\(^{d}\) | DT\(^{e}\) |
|------------|------------|------------|-------------|-----------|-----------|
| F1         | 45.019 (21.06–82.97) | 0.117 (0.08–0.14) | 1.124 (1.09–1.15) | 32.405 (30.26–34.92) | 5.89 (4.80–7.89) |
| F4         | 45.164 (25.24–6.37) | 0.099 (0.07–0.12) | 1.104 (1.08–1.12) | 38.395 (35.88–41.59) | 6.98 (5.78–8.79) |
| F6         | 45.170 (21.41–93.11) | 0.109 (0.08–0.15) | 1.116 (1.08–1.16) | 34.689 (32.08–37.92) | 6.310 (4.56–8.59) |
| F8         | 40.930 (17.40–83.02) | 0.0833 (0.06–0.10) | 1.086 1.06–1.10 | 44.558 (42.43–46.96) | 8.320 (6.75–12.01) |

\(^{a}\)Net reproductive rate.
\(^{b}\)Intrinsic rate of increase.
\(^{c}\)Mean duration of one generation.
\(^{d}\)Duration time. Parameters compared by bootstrap analysis (10,000 repetitions).

The smaller the container the higher surface-to-volume ratio (García et al. 2006), and consequently, a greater water loss is expected. Water content is a determining factor in the quality of an artificial diet. In the case of *T. absoluta*, the dry surface favored its development, probably due to its feeding habits and a reduction of free water which limited the presence of pathogens. The physical characteristics of the diet are clearly important for *T. absoluta*, as reported by Mihsfeldt and Parra (1999).

The larval density per rearing container influenced the survival of *T. absoluta*. Thus, three larvae per tube yielded, larval and pupal viabilities >80 and 90%, respectively, meanwhile lower (one larva per tube) or greater (>3 larvae per tube) densities reduced, larval viability although without affecting the pupal viability (Table 5). The higher mortality at the higher density may be related to the increased humidity provided by larval excrement.

When assessing the biological parameters of *T. absoluta* in the selected diet, in generations F1, F4, F6, and F8, the length of the egg-adult period decreased with the generations (Table 6), from 34.67 d for the F1 to 31.79 d for the F8 which shows an adaptation to the artificial diet with the passing of the generations. Although the life cycle of this species is reported to be 22–30 d with the natural diet (Barrientos et al. 1998), the results obtained with this artificial diet are not far from these values. The major difference occurred in the larval period which lasted from 20 to 22 d, while in the natural diet lasts 14 d (Desneux et al. 2011, França et al. 2000, Urbaneja et al. 2013).

The viability 7 d after inoculation increased with the generations; while the larval and pupal viabilities were equal over the generations, larval viability without affecting the pupal viability (Table 5). The higher mortality at the higher density may be related to the increased humidity provided by larval excrement.

In conclusion, an artificial diet based on wheat germ, casein and cellulose provides optimal nutritional conditions for rearing *T. absoluta* with a total viability of 75%, without affecting the development and reproduction of the insects for eight successive generations, based on life table parameters. A smaller container (a 

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