Role of plant age in the resistance of *Lycopersicon hirsutum* f. *glabratum* to the tomato leafminer *Tuta absoluta* (Lepidoptera: Gelechiidae)

G.L.D. Leite¹, M. Picanço, R.N.C. Guedes *, J.C. Zanuncio

*Departamento de Biologia Animal, Universidade Federal de Viçosa, Viçosa, MG 36571-000, Brazil*

Accepted 7 July 2000

**Abstract**

The objective of this work was to study the role of plant age in the resistance of tomato, *Lycopersicon hirsutum*, to the tomato leafminer *Tuta absoluta* (Meyrick). Determination of the levels of tridecan-2-one and undecan-2-one in *L. hirsutum* were made at three different ages (2–4 months after germination), as well as the leaf area, and the density and types of trichomes present on *L. hirsutum* and *L. esculentum*. This information gathered was correlated with the following biological characteristics of *T. absoluta*: rates of oviposition and egg eclosion; length of egg, larval and pupal stages; mortality in the larval and pupal stages; pupal weight and proportion of females. *T. absoluta* showed higher oviposition and egg eclosion, as well as lower mortalities and shorter larval and pupal periods in *L. esculentum* than in *L. hirsutum*. The proportion of females obtained was also higher on *L. esculentum*. The rate of egg eclosion and proportion of females as well as the length of the larval stage and the mortality of larvae appeared to be affected by plant age. A higher proportion of females, mortality of larvae and length of the larval period were obtained with older plants of *L. hirsutum*, while the rate of egg eclosion was higher for *L. hirsutum* plants that were 3 months old. For *L. esculentum*, plant ageing increased larval mortality. It seems that the glandular trichome density in *L. hirsutum* increased with plant age leading to an increase in the levels of tridecan-2-one, which slows larval development. © 2001 Elsevier Science B.V. All rights reserved.

**Keywords:** Tomato plant; Tomato leaf miner; Tridecan-2-one; Undecan-2-one
1. Introduction

The tomato leafminer, *Tuta (=Scrobipalpuloides) absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is considered as one of the key pests of tomato *Lycopersicon esculentum* in South America. Its control is based on intensive insecticide applications (Guedes et al., 1994; Picanço et al., 1996a,b). An alternative strategy to reduce *T. absoluta* populations and their losses would be the development of resistant commercial varieties. The wild tomato *Lycopersicon hirsutum f. glabratum* (PI 134417) has been reported as resistant to this leafminer (Giustolin and Vendramim, 1994; Leite et al., 1995). The resistance of *L. hirsutum* to *T. absoluta* is usually attributed to the allomones tridecan-2-one and undecan-2-one present in the leaf glandular trichomes of this tomato species, which are absent in *L. esculentum* (Giustolin and Vendramim, 1994), but there is very little work on the subject.

One of the limitations of using *L. hirsutum f. glabratum* (PI 134417) as a source of resistance is that the resistance is affected by the plant growth and leaf senescence. Schalk and Stoner (1976) and Kennedy and Sorenson (1985) found that reduced resistance of *L. hirsutum* to Colorado potato beetle is due to the reduction of the concentration of tridecan-2-one as leaves become older. The objectives of this study were to identify the types and densities of trichomes, quantify tridecan-2-one and undecan-2-one, and determine the effect of age (2–4 months after emergence) on the leaf area of *L. hirsutum f. glabratum* (PI 134417) and how it affects the biology of *T. absoluta*.

2. Material and methods

Tomato plants were grown in 5 l polyethylene pots in greenhouse conditions. The average minimum and maximum temperature were 11.7 ± 0.8 and 35.0 ± 0.4°C, respectively, and the photoperiod was 11:13 (L:D) during the investigation. No supplemental light or heat was used. The 2 × 3 factorial experiment was established in a randomized blocks design with 12 replicates for the assessment of plant-related traits and 28 replicates for the assessment of insect biological traits. Each replicate was constituted by one tomato plant in a 5 l polyethylene pot. The influence of the following factors on the resistance of *T. absoluta* were studied: tomato species (*L. esculentum* cv. Santa Clara and *L. hirsutum f. glabratum* (PI 134417)) and plant age (2–4 months after emergence). The daily recorded insect biology data (rates of oviposition and egg eclosion; length of egg, larval and pupal stages; mortality in the larval and pupal stages; pupal weight and proportion of females) as well as the levels of tridecan-2-one, undecan-2-one, trichome density and leaf area were determined in leaves from each third of the plant height and the average of these determinations was
calculated for each plant and used for the statistical analyses. Two months old plants presented flowers and small fruits sometimes, and 3 months old plants presented flowers and non-mature fruits, while 4 months old plants presented flowers and mature fruits.

Soil chemical analysis was made prior to the beginning of the experiment. After this analysis, 200 mg of N and 100 mg of K/kg of soil were used for soil fertilization. K was added as potassium chloride, which was mixed to the soil 1 month before starting the experiment. The N source was urea, added to the pots as an aqueous solution 1 week before sowing. After the plants reached 2 months of age, the fertilization with urea through aqueous solution was maintained, being added to each pot a total of 0.30 g of urea. The N was applied in a single dose for the 2 months old plants, but only 90 and 80% of the recommended N dose was applied before sowing for the 2 and 4 months old plants, respectively. The remaining N dose was applied as aqueous solutions in the 3 and 4 months old plants after 30 and 60 days of sowing, respectively. The plants were daily irrigated and enough water was provided to form a film at the soil surface of each pot.

Groups of five 1 day old eggs of *T. absoluta* were placed on the third leaflet fully expanded from each third of the plants with a total of 15 eggs/plant and 420 eggs/treatment. The plant branches containing the leaves with the insect eggs (in their surface) were enclosed in cloth bags (0.2 m × 0.28 m) and the length of the egg incubation period, rate of eclosion, larval mortality, number of small and large mines (mines smaller or larger than 0.5 cm in length were classified as small or large mines, respectively) (Picanço et al., 1995), length of the larval stage (days), pupal weight and deformation, and sex ratio were daily evaluated. The male and female pupae were identified, separated and placed in white 500 ml plastic pots covered with cloth and incubated at 25 ± 0.5°C and a 12 h photoperiod until adult emergence (Giustolin and Vendramim, 1994). The pupae were daily inspected until adult emergence for determination of the pupal mortality and the length of the pupal period.

Another set of plants maintained in similar conditions to those used for the insect studies were used for plant-related determinations. For extraction of tridecan-2-one and undecan-2-one, 5 g of leaves were collected from each plant in the morning and used for extraction with 50 ml of hexane (distilled) for 24 h. The solution was decanted, dried over sodium sulfate and evaporated to dryness at 30°C in a rotatory evaporator. The oil was redissolved in 1 ml of hexane and analyzed by gas chromatography using OV 17 (1%) packed in a 2 m glass column. The chromatograph (Instrumentos GC, São Paulo, Brazil) was programmed from 150 to 220°C at 6°C/min. The injector and detector were maintained at 260 and 280°C, respectively. The flow rates of H₂/N₂/air were 30, 30 and 300 ml/min, respectively. The tridecan-2-one and undecan-2-one concentrations in the leaf hexane extracts were determined using standard
calibration curves for both compounds (99% pure) obtained from Aldrich (Milwaukee, WI). Three independent evaluations were made for each of the 12 replicates.

Trichome density was evaluated on three leaflets from each of the 12 replicates for a total of 36 leaflets/treatment. Each leaflet was individually collected and stained for 3 min in fast green dye (Johansen, 1940). These leaflets were collected at the same period from the plants at the ages studied. The trichome density (trichomes/mm²) was calculated using a light microscope and by counting the number of trichomes present per leaflet area in both surfaces (Channarayappa et al., 1992). Twenty-four fields of 0.6 mm² were analyzed in the mid-part of each leaflet area between the main vein and the leaflet edge. Leaflet area of three leaves collected in the morning was determined using a leaf area meter LI-COR model LI-3000 (Lincoln, NE). Three independent evaluations were made for each of the 12 replicates.

The trichomes were classified as glandular or non-glandular in both genotypes for the purpose of this work, despite the existence of seven distinct trichome types within the genus Lycopersicon (Channarayappa et al., 1992). This was done because the accessions of L. hirsutum show almost only glandular trichomes (types I, IV, VIc, and VII), mainly of the type VI (producer of tridecan-2-one and undecan-2-one), unlike L. esculentum which shows mainly non-glandular trichomes (types III, Va, and Vb) (Channarayappa et al., 1992). L. hirsutum does contain other allelochemicals important in insect plant interactions such as α-tomatine, α-humulene, chlorogenic acid and rutin, but their concentrations in the leaves are rather small (Elliger et al., 1981). The main leaf allelochemicals of L. hirsutum are tridecan-2-one and undecan-2-one which are present mainly on type VI glandular trichomes and have been correlated to insect pest resistance (Dimock et al., 1982; Dimock and Kennedy, 1983; Kennedy and Sorenson, 1985; Lin et al., 1987; Giustolin, 1991). The results on T. absoluta biology and attack, and tomato leaf size, trichome density, and concentrations of tridecan-2-one and undecan-2-one were subjected to two-way analysis of variance and the Scott–Knott multiple range test ($P < 0.05$) whenever appropriate (Scott and Knott, 1974). Regression analysis were carried out separately for L. hirsutum and L. esculentum correlating trichome density and concentrations of tridecan-2-one, undecan-2-one with plant age and biological characteristics of T. absoluta.

3. Results

There was no significant difference ($P < 0.05$) of undecan-2-one concentration (0.0015% based on fresh weight) in L. hirsutum as a function of plant age. However, trichome density (mainly of type VI glandular trichomes) and,
consequently, production of tridecan-2-one increased with plant age in *L. hirsutum* (Fig. 1). Trichome density (mainly of non-glandular trichomes) also increased with plant age in *L. esculentum* (Fig. 1), but there was no effect of plant age on leaf area in any of the tomato species studied.

The highest rates of egg eclosion (56.5 and 61.3%), lowest larval mortality (72.8 and 77.6%) and shortest larval (22.9 and 25.8 days) and pupal (7.3 and 8.0 days) development were observed in plants at 2 and 3 months of age, respectively.

Fig. 1. Effect of plant age on trichome density for *L. esculentum* and *L. hirsutum* (A), and relationship between level of tridecan-2-one (% weight fresh) and trichome density for *L. hirsutum* (B). The symbols represent the average of 12 replicates and the vertical bars indicate standard errors of the mean.
Table 1
Egg incubation period (days), egg eclosion (%) and proportion of females (No. females/(No. females + males)) of T. absoluta in leaves of L. esculentum and L. hirsutum of different ages (N indicates the number of insects and n indicates the number of experimental units used for each plant species in each analysis)

<table>
<thead>
<tr>
<th>Plant age</th>
<th>Egg incubation period</th>
<th>Egg eclosion</th>
<th>Proportion of females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L. esculentum (N = 488; n = 84)</td>
<td>L. hirsutum (N = 548; n = 84)</td>
<td>L. esculentum (N = 1260; n = 84)</td>
</tr>
<tr>
<td>2 Months</td>
<td>6.4 aA</td>
<td>6.5 aA</td>
<td>61.8 aA</td>
</tr>
<tr>
<td>3 Months</td>
<td>6.5 aA</td>
<td>6.4 aA</td>
<td>63.0 aA</td>
</tr>
<tr>
<td>4 Months</td>
<td>6.4 aA</td>
<td>6.5 aA</td>
<td>59.0 aA</td>
</tr>
<tr>
<td>Mean</td>
<td>6.4 a</td>
<td>6.4 a</td>
<td>61.3 a</td>
</tr>
</tbody>
</table>

*Means followed by the same low case letter in a row or the same capital letter in a column do not differ significantly by the Scott–Knott multiple range test (P < 0.05).*
days) periods, number of small mines (2.8 and 25.8) and large mines (8.3 and 11.3) per leaf, and higher proportions of females were observed in *L. esculentum* in comparison with *L. hirsutum* (Tables 1 and 2). However, no negative effects of *L. hirsutum* were observed at different ages on the length of the egg incubation period (6.4 days), pupal mortality (25.3%) and weight (3.1 mg), as well as pupal (6.5%) and adult malformation (0.0%) of *T. absoluta* (Tables 1 and 2).

The ageing of *L. hirsutum* plants led to higher mortality of *T. absoluta* larvae, longer larval periods, higher number of small mines and lower number of large mines, besides higher proportion of females (Tables 1 and 2). In *L. esculentum*, ageing apparently led also to a higher larval mortality and smaller number of large mines (Table 2). The length of the pupal period was similar for females and males of *T. absoluta* in both tomato species (7.2 and 7.3 days, respectively, when on *L. esculentum*, and 7.4 and 8.4 days, respectively, when on *L. hirsutum*).

**Table 2**

Length of larval and pupal periods (days), mortality of larvae and pupae (%), and number of small (<0.5 cm) and large (>0.5 cm) mines of *T. absoluta* reared on leaves of *L. esculentum* and *L. hirsutum* from plants of different ages (*N* indicates the number of insects and *n* indicates the number of experimental units used for each plant species in each analysis)

<table>
<thead>
<tr>
<th>Plant age</th>
<th>Length of larval period (days)</th>
<th>Length of pupal period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>L. esculentum</em> (N = 133; <em>n</em> = 44)</td>
<td><em>L. hirsutum</em> (N = 123; <em>n</em> = 40)</td>
</tr>
<tr>
<td></td>
<td><em>L. esculentum</em> (N = 103; <em>n</em> = 33)</td>
<td><em>L. hirsutum</em> (N = 92; <em>n</em> = 30)</td>
</tr>
<tr>
<td>2 Months</td>
<td>22.5 bA(^a) 23.1 aC 7.1 bA 8.3 aA</td>
<td>22.9 b 25.8 a Pupal mortality (%)</td>
</tr>
<tr>
<td>3 Months</td>
<td>23.3 bA 26.3 aB 6.9 bA 8.5 aA</td>
<td>35.2 aA 18.6 aA</td>
</tr>
<tr>
<td>4 Months</td>
<td>23.5 bA 32.9 aA 7.8 bA 8.5 aA</td>
<td>96.4 aA 11.5 aA</td>
</tr>
<tr>
<td>Mean</td>
<td>22.9 b 25.8 a 7.3 b 8.0 a</td>
<td>72.8 b 77.6 a 22.8 a 25.3 a</td>
</tr>
</tbody>
</table>

No. of small mines/leaf  

<table>
<thead>
<tr>
<th></th>
<th><em>L. esculentum</em> (N = 133; <em>n</em> = 33)</th>
<th><em>L. hirsutum</em> (N = 133; <em>n</em> = 44)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>L. esculentum</em> (N = 103; <em>n</em> = 33)</td>
<td><em>L. hirsutum</em> (N = 92; <em>n</em> = 30)</td>
</tr>
<tr>
<td>2 Months</td>
<td>63.5 bB 69.8 aB 35.2 aA 18.6 aA</td>
<td>21.3 aA 36.8 aA</td>
</tr>
<tr>
<td>3 Months</td>
<td>68.5 aB 66.5 aB 21.3 aA 36.8 aA</td>
<td>7.4 aA 11.5 aA</td>
</tr>
<tr>
<td>4 Months</td>
<td>86.3 bA 96.4 aA 7.4 aA 11.5 aA</td>
<td>22.8 a 25.3 a</td>
</tr>
<tr>
<td>Mean</td>
<td>72.8 b 77.6 a 22.8 a 25.3 a</td>
<td>8.3 b 11.3 a</td>
</tr>
</tbody>
</table>

\(^a\) Means followed by the same low case letter in a row or the same capital letter in a column do not differ significantly by the Scott–Knott multiple range test (*P* < 0.05).
4. Discussion

Dimock et al. (1982) reported a higher level of undecan-2-one (0.066 % on fresh weight) in *L. hirsutum f. glabratum* (PI 134417) than that observed in this study, probably because of the winter effect on our experiment (Nichoul, 1994). The shorter photoperiod of winter days reduces the number of type VI trichomes which are involved in undecan-2-one production (Nichoul, 1994). In addition, type VI trichome density and exudate production are higher during the spring than during the fall due to differences in photoperiod between these seasons (Nichoul, 1994). On the other hand, the level of tridecan-2-one observed here (0.30 % on fresh weight) was similar to the level reported by Dimock et al. (1982) (0.37 % on fresh weight).

A low number of small mines probably reflects the host suitability to the caterpillars. Thus, when adequate food is available, the caterpillars lodge in the leaf mesophyll and consume it increasing the size of the mines and transforming the small into large galleries. *L. hirsutum* does not seem to be a suitable host to the leafminer probably due to the antixenotic and antibiotic effect of tridecan-2-one, as observed on the Colorado potato beetle by Kennedy and Sorenson (1985), in addition to the possible existence of deterrent substances in the leaf mesophyll of this tomato species (Lin and Trumble, 1986). The larvae of this leafminer insect pest usually leave their mines to move over the leaf surface during sunny hours (Moore, 1983). Such behavior favors the exposure of the insects to the noxious substances of the leaf glandular trichomes of *L. hirsutum*. In a free choice test performed by Picanço et al. (1995), no significant difference in the number of leaf galleries formed by *T. absoluta* was observed between *L. esculentum* and *L. hirsutum*. However, Leite et al. (1995) showed that despite the lower oviposition of *T. absoluta* in *L. hirsutum*, the larvae made higher number of galleries in this genotype than in *L. esculentum*.

There was no effect of undecan-2-one on *T. absoluta* unlike what was observed by Giustolin (1991). Giustolin (1991) observed that with the addition of 0.03% of undecan-2-one in the insect diet, there was a decrease in larval mortality and length of the larval period, as well as an increase in pupal weight and adult longevity. When 0.06% of undecan-2-one was added to the diet, only 8.6% of *T. absoluta* caterpillars reached the next developmental stage, but no other effect on the insect biology was observed. In our study the amount of undecan-2-one observed was lower (ca. 40×) than the amount necessary to cause harmful effects on the leafminer, therefore it is not surprising the absence of undecan-2-one effects in our experiment. In addition, it seems that this compound is less toxic than tridecan-2-one and active in fewer insect species (Farrar and Kennedy, 1991).

The increase in trichome density with plant age is related to an increase in the leaf levels of tridecan-2-one in *L. hirsutum* which is expected since this
compound is present in leaf glandular trichomes (Fig. 1). The higher levels of tridecan-2-one associated with older plants of *L. hirsutum*, are related to a slower larval development of *T. absoluta* (Fig. 2) when compared with insects reared on *L. esculentum*. These results suggest that commercial varieties of tomato having *L. hirsutum* as source of resistance may be more resistant to the leafminer especially during the plant reproductive stage, the critical period of attack by this pest in tomato plants (Ullé and Nakano, 1994).

The higher male (than female) mortality in the larval stage (evaluation performed after pupation) in *L. hirsutum* than in *L. esculentum* suggests that male...
larvae are more susceptible to tridecan-2-one than female larvae. This may be due to an increased concentration of tridecan-2-one/mg weight in male larvae as a consequence of their smaller weight and the higher relative penetration rate of the allelochemical through the male cuticle since the males have lower body volume and consequently higher specific area than the females.

The shorter pupal development period of females of *T. absoluta* and the higher proportion of females in the population suggests an adaptative advantage of the insect species decreasing the period of the female exposure to adverse conditions (Coelho and França, 1987). Giustolin and Vendramim (1994) reported lower pupal weights (of both males and females) for *T. absoluta* reared on *L. hirsutum* than on *L. esculentum*. This could be due to antibiosis of tridecan-2-one on *T. absoluta* caterpillars when feeding in *L. hirsutum* since the type VI glandular trichomes which produce this compound are not present in *L. esculentum*.

5. Conclusion

*T. absoluta* showed better development in *L. esculentum* than in *L. hirsutum*. The increase in trichome density with plant age led to an increase in the leaf levels of tridecan-2-one in *L. hirsutum* and these higher levels of tridecan-2-one may have led to a slower larval development of *T. absoluta* when compared with insects reared on *L. esculentum*. These results suggest that commercial varieties of tomato having *L. hirsutum* as source of resistance may be more resistant to the leafminer especially in their reproductive stage, the critical period of attack by this pest in tomato plants.

Acknowledgements

Appreciation is expressed to Dr. Patrick De Clerk, from the University of Gent, Belgium, and two anonymous referees for reviewing this manuscript and providing helpful comments and the Brazilian Government (CNPq) for financial support.

References


