Tomato fruit size, maturity and α-tomatine content influence the performance of larvae of potato tuber moth *Phthorimaea operculella* (Lepidoptera: Gelechiidae)

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Abstract

Various physical and chemical properties of host plants influence insect larval performance and subsequent adult fitness. Tomato plants are relatively new hosts to the potato tuber moth, *Phthorimaea operculella* (Zeller), with the fruit being its preferred feeding site. However, it is unclear how the biochemical and physical properties of tomato fruits relate to potato tuber moth performance. Significant amounts of α-tomatine were detected in maturing green and ripening fruits of cherry (cv. Ceres) and processing (cv. Serio) types of tomatoes whereas none was detected in a fresh market variety (cv. Marglobe), at comparable stages. α-Tomatine is negatively and significantly correlated with development rate (head capsule size) of larvae reared in the fruits of the cherry and processing type tomatoes. Generally, survival, growth and development were significantly superior for larvae reared in the ripening fruits of the fresh market cultivar. At this stage, the fruits of this cultivar are also the largest. Based on these results it is concluded that fruit α-tomatine content, as well as fruit size and maturity, all affect performance of *P. operculella* larvae in the fruits of cultivated tomatoes.

Keywords: herbivory, insect–plant interactions, *Phthorimaea operculella*, potato tuber moth, secondary plant compounds, tomatine, tomato

Introduction

Host plant properties influence growth, development and survival of larvae with direct implication for adult fitness (Tikkanen *et al.*, 2000; Coll & Yuval, 2004). Plant allelochemicals play an important role in the ecology and physiology of phytophagous insects (Bloem *et al.*, 1989; Smith *et al.*, 1994; Panda & Khush, 1995; Duffey & Stout, 1996; Rodriguez-Saona & Trumble, 1999). That the potato tuber moth

*Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), is widely adapted to its traditional potato and tobacco host plants (Rothschild, 1986; Kroschel & Koch, 1996; van Vuuren *et al.*, 1998) is attributable, at least in part, to its ability to cope with α-solanine and α-chaconine, the major glycoalkaloids in potato (Morgan *et al.*, 1983) and nicotine and nornicotine alkaloids in tobacco (Panda & Khush, 1995). These constitutive allelochemicals were shown to be toxic to numerous other herbivorous insects (reviews by Valkonen *et al.*, 1996; Yamamoto *et al.*, 1998).

The steroidal glycoalkaloid α-tomatine is the major constitutive allelochemical in tomatoes (Juvic *et al.*, 1982a,b). When incorporated in artificial diet it was found to be toxic to tomato fruitworms (Bloem *et al.*, 1989; Stamp &
Phthorimaea operculella is not widely adapted to tomatoes; both avoidance of tomatoes (Fenemore, 1980; van Vuuren et al., 1998) and, conversely, acceptance, have been reported (Gilboa, 1994). However, such differences may have been due to inherent variations in host susceptibility to P. operculella, and we questioned whether differences in α-tomatine contents in tomato genotypes might have influenced this behaviour. Fruit infestation has been reported in all tomato fruit stages: immature green, ripening and ripened fruits; the latter were found to be more significantly infested (Mulatu, 2003). Fruit size also affected the field infestation level of tomato cultivars by P. operculella (Mulatu, 2003), which may imply that fruit size and maturity stage affect its performance on tomatoes. The size of mature fruits varies greatly among tomato genotypes. The most commonly recognized categories of mature fruit size are cherry (small rounded), processing (medium and often oval) and fresh market (large and often rounded) types. Phthorimaea operculella commonly feeds in the fruit central core. The amount of tissue it can ingest therefore depends very much on the size of the fruit. Based on wet weight, 10 g of central core fruit tissue was obtained from 40, 10 and 2 ripening fruits of cherry, processing and fresh market tomato cultivars, respectively (B. Mulatu, unpublished data). Such variation might affect the biological performance of developing P. operculella larvae.

While feeding in a tomato fruit, P. operculella normally avoids all fruit parts outside the central core tissue. Phthorimaea operculella larvae die soon after accidentally coming in contact with the inner fluid within the loculi bearing the developing seeds (B. Mulatu, personal observation) and this is one factor that might be restricting larvae to feed only on fruit core placental tissue. Additionally, feeding on small-sized fruits might force P. operculella larvae to shift to nearby fruits to complete development, thus exposing the developing larvae to diverse mortality factors prevailing in their immediate environment, such as desiccating temperature, predators and parasitoids.

Tomato is a newly acquired host of P. operculella (Mulatu et al., 2004). The present study was performed in order to evaluate the possible effects of tomato fruit α-tomatine content, size and stages of maturity, on several descriptors of P. operculella fitness.

Materials and methods

Feeding assays

Survival, growth and development of P. operculella larvae were determined on intact tomato fruits in the field. Fruits were examined in plots of cherry, processing and fresh market tomatoes (Ceres, Serio and Marglobe cultivars, respectively) laid out in a randomized complete block design with four replicates. Cultivars of the three tomato types were selected to represent a wide range of fruit traits, such as size, chemistry, etc. Ten bunches of fruits (one per plant) were tagged at random in each plot and caged after making sure that there were no larval tunnels under the calyx (P. operculella entry point to the fruits). Lightweight cages, constructed of polystyrene foam and plastic mesh, were used to enclose the fruit. Neonates of P. operculella (<24 h old), at twice the number of fruits in a bunch, were introduced into each cage and left undisturbed in the field for two weeks. All the fruits were then collected, dissected and the numbers of infested fruits and surviving larvae, their body weight and head capsule sizes, were recorded. The head capsule width was measured using a stage micrometer at 14× magnification, after freeze-killing the larvae. The larval head capsule, being heavily sclerotized, is not influenced by handling and physical conditions and its morphometrics are therefore indicative of larval development. The tests were performed on immature green, maturing green and ripening fruits of the three cultivars.

Quantification of α-tomatine

Tomato fruit samples

Tomato fruit samples were prepared from core tissue excised from groups of three replicates of 50 fruits each, randomly chosen from immature green, maturing green and ripening stages of each cultivar. A 20 g fresh weight sample from each group was taken, homogenized in 50 ml absolute ethanol and stored at −20°C until chemically analysed.

Alpha-tomatine extraction

Alpha-tomatine extraction from tomato fruit core tissue was performed as described by Friedman et al. (1994). Briefly, the core tissue homogenates were filtered and the ethanol extracts evaporated to dryness under vacuum. One hundred milligrams of the dried extract was dispersed in 2 ml of 1% aqueous acetic acid and kept at room temperature for 4 h. The suspensions were then centrifuged for 20 min at 5000 rpm and the supernatants filtered through Whatman GF/C. The pellets were resuspended, dispersed as above in 2 ml of 1% aqueous acetic acid, centrifuged and filtered and filtrates combined with the first extracts. C18 Sep-Pak cartridges (Waters) were conditioned with 2 ml absolute methanol and washed with 2 ml ddH2O. Aqueous tomato extracts (c. 4 ml) were applied and allowed to percolate through the cartridge by gravity. The loaded cartridges were then washed with 2 ml water followed by 1 ml of 30:70 acetonitrile:1% aqueous NH4OH and then 1 ml ddH2O to eliminate excess ammonium ions. Alkaloids were then eluted with 2 ml of 70:30 acetonitrile-pH3 citric acid/dissodium phosphate buffers. The organic solvent was evaporated and the aqueous residues were extracted twice into water-saturated butanol. Samples were dried under a gentle flow of N2. The residue was brought to 1 ml with 25% acetonitrile, 15% methanol, 100 mM sodium phosphate (monobasic) pH 3 in HPLC-quality water (HPLC running buffer) and filtered through a 0.45 μm HV membrane. This filtrate was subjected to isocratic HPLC (Merck-Hitachi LaChrom system) on a LiChrosphere-100 RP-18 column in the above solvent and the effluent monitored at 200 nm. Retention time of fruit-derived α-tomatine was compared to the mobility of commercial α-tomatine (Sigma).

The identity of the fruit-derived material was verified by acid hydrolysis of α-tomatine to yield the aglycone tomatidine (Friestman et al., 1998): 100 mg of the dried fruit-derived ethanol extracts containing α-tomatine were suspended in 2 ml of 1 N HCl in glass vials and heated for 70 min at 95–100°C in a water bath. After cooling, the mixture was neutralized with 1 N NaOH and partitioned five times with 2 ml of benzene. The combined benzene solutions were washed five times with 2 ml of water. The benzene was then evaporated to dryness and the residue re-dissolved in 1 ml of the eluent used for HPLC of α-tomatine, filtered
through 0.45 μm HV membranes and chromatographed as above. The peak retention time (t_R) of the commercial α-tomatine was 15.2 min and that extracted from tomato fruits was 15.9 min. The t_R of plant-derived tomatidine and tomatidine prepared from a commercial source of α-tomatine were 12.8 min.

A standard curve of commercial α-tomatine quantity, relative to HPLC peak area, was prepared. Alpha-tomatine was quantified from this standard curve in the fruits of the three tomato cultivars.

**Statistical analysis**

Two-way ANOVA were run on the effect of cultivar and fruit stage on survival, growth, and development of *P. operculella* larvae, and on the α-tomatine contents in the fruit core tissue at the three fruit stages of the three cultivars (SAS, 2001). Correlation analyses tested for a relation between fruit α-tomatine contents and parameters of larval performance developing on these fruits (SAS, 2001). Means were compared using Tukey-Kramer honest significance difference test (5%). Means ± SE are reported.

**Results**

**Survival of *P. operculella* larvae in tomato fruits**

Whereas the mean percent of *P. operculella*-infested fruits did not differ significantly among the three cultivars on the immature green and maturing green fruits, significantly more ripening fruits were infested of the fresh market fruits than those of the other cultivars (F2,115 = 12.56, P < 0.001) (fig. 1a). The interactive effect of cultivar and fruit stage on the level of *P. operculella* infestation was not statistically significant.

In contrast, fruit stage and tomato cultivar interacted significantly in their effects on larval survival (F2,313 = 3.02, P < 0.018). On the immature green fruits, more larvae survived on the cherry and fresh market cultivars than the processing cultivar (F2,117 = 8.48, P < 0.001). Significantly more larvae survived on the fresh market on maturing green and ripening fruits (F2,115 = 5.6, P < 0.004 and F2,313 = 26.4, P < 0.001, respectively) than on the cherry and the processing cultivars (fig. 1b). Larval survival on cherry and processing cultivars did not differ significantly among fruit stages (cherry: F2,116 = 0.69, P > 0.5; processing: F2,116 = 2.54, P < 0.08). In contrast, a significantly higher percentage of larvae survived in the ripening fruits (F2,117 = 12.84, P < 0.001) than the immature green and maturing green fruits of the fresh market cultivar.

**Growth and development**

The effect of fruit stage on larval growth differed significantly on the three tomato cultivars (interactive effect: F2,313 = 7.8, P < 0.001). Larval weight was consistently and significantly higher on immature green (F2,107 = 24.9, P < 0.001), maturing green (F2,108 = 14.2, P < 0.001) and ripening (F2,107 = 60.4, P < 0.0001) fruits of the fresh market than the other cultivars (fig. 2a). Within-cultivar comparison of larval body weight on fruits from the three fruit categories were significantly higher on green mature than immature green and ripening fruits of the cherry (F2,109 = 11.45, P < 0.0001) and processing cultivars (F2,100 = 4.43, P < 0.01). Larval weight on the fresh market cultivar was significantly higher on ripening than green fruits (F2,111 = 11.5, P < 0.001).

Fruit stage and tomato cultivar interacted significantly in their effects on larval head capsule width (F2,313 = 3.26, P < 0.012). Larval head size was significantly and consistently larger for those reared on the fresh market cultivar at the three fruit stages than on the respective fruit stages of cherry and processing cultivars (immature green F2,108 = 26, P < 0.001; maturing green F2,108 = 9.84, P < 0.001; and ripening F2,105 = 30.3, P < 0.001 fruits, fig. 2b). Within cultivars, head capsule widths were significantly larger for larvae reared on ripening fruits than those on developing fruits (cherry F2,108 = 9.21, P < 0.001; processing F2,109 = 9.34, P < 0.001; and fresh market F2,111 = 9.75, P < 0.001 cultivars).

![Fig. 1. (a) Percent-infested fruits (mean±SE) and (b) percent larval survival of *Phthorimaea operculella* on fruits of cherry (■), processing (□) and fresh market (■) tomatoes (mean±SE). Within fruit category, columns with same letters do not differ significantly (Tukey-Kramer at P < 5%).](image-url)
The α-tomatine level in fruit core tissue was significantly dependent on the interactive effect between fruit stage and tomato cultivar ($F_{6,18} = 9.85, P < 0.0001$). In immature green fruits, the α-tomatine content was significantly higher in the fruit core tissue of the processing cultivar than in the cherry and the fresh market cultivars (Table 1). However, the α-tomatine content at this fruit stage was not correlated significantly with larval survival, body weight and head capsule width. The α-tomatine content increased in maturing green fruits of the cherry cultivar and decreased in the processing and fresh market cultivars, with no traceable amounts found in the fruit core tissue of the latter cultivar (Table 1). At this fruit stage, the α-tomatine content was significantly and negatively correlated only with larval head capsule width. Alpha-tomatine levels in ripening fruits core tissue remained higher in the cherry cultivar and no α-tomatine was found in the processing tomato cultivar (data not shown).

**Discussion**

That similar infestation levels were recorded in pre-ripening stages of the fruits in all three tested tomato cultivars suggest that at these stages, all the cultivars were similarly accepted by larvae of *Phthorimaea operculella*. Infestation level of ripening fresh market fruits, however, was significantly higher compared to the other cultivars. It appears that factors found in cherry and processing cultivars render the fruits unsuitable for *P. operculella* larvae. These factors in the ripened fruits may have been retained from the pre-ripening stages, different new factors, or both. The high survival of *P. operculella* larvae in the ripening fruits of the fresh market cultivar and the significantly higher growth rate of larvae on these fruits are consistent with this notion. Head capsule size, which relates to the development stage of lepidopteran larvae, was also found to be significantly higher in this same cultivar. The above results indicate that, in general, the performance of larvae of *P. operculella* show fruit maturity stage-dependence, which was particularly pronounced in the fresh market type with the highest survival, growth and development rates of larvae on ripening fruits.

Of the three tested genotypes, the fresh market cultivar possesses the largest fruit, which apparently provides more core tissue for the developing *P. operculella* larva. This affords the developing larvae the opportunity to remain and complete their development within a single fruit, thus avoiding the possible risks of desiccation, predation and parasitism that might occur when larvae exit from one fruit to another. This may explain the higher number of larvae surviving in the ripening fruits of the fresh market cultivar of the three cultivars examined. Higher levels of fruit infestation were observed in both green and ripening fruits of the fresh market tomato (Mulatu, 2003). Apparently, fruit size is an important determinant of the performance of *P. operculella* larvae in tomato fruits. The suggested importance of fruit size and maturity level on *P. operculella* larvae should be

**Table 1.** Alpha-tomatine level (mean ± SE) in the fruit core tissue of cherry, processing and fresh market tomato cultivars and its correlation with measures of larval performance of *Phthorimaea operculella*.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Immature green</th>
<th>Maturing green</th>
<th>Ripening</th>
<th>P&lt;sub&gt;F2,18&lt;/sub&gt;</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceres (cherry)</td>
<td>9.30 ± 2.80</td>
<td>27.4 ± 20.7</td>
<td>132.8 ± 23.9</td>
<td>0.001</td>
<td>0.33</td>
</tr>
<tr>
<td>Serico (processing)</td>
<td>69.9 ± 11.1</td>
<td>11.5 ± 1.90</td>
<td>88.8 ± 22</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Marglobe (fresh market)</td>
<td>2.50 ± 1.60</td>
<td>0</td>
<td>0</td>
<td>0.007</td>
<td></td>
</tr>
</tbody>
</table>

Correlation coefficients (P-values) with larval performance:

- Survival (%): $-0.99 (0.09)$, $-0.98 (0.13)$, $-0.97 (0.16)$
- Larval body wt: $-0.56 (0.62)$, $-0.88 (0.31)$, $-0.99 (0.10)$
- Head capsule size: $-0.45 (0.71)$, $-0.99 (0.02)$, $-0.99 (0.02)$

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**Fig. 2.** (a) Body weight and (b) head capsule width of *Phthorimaea operculella* larvae recovered from fruits of the cherry (C), processing (■) and fresh market (■) tomatoes (means ± SE). Within fruit category, columns with same letters do not differ significantly (Tukey-Kramer at $P < 0.05$).
qualified because these traits were confounded with tomato fruit type in the present study. Several cultivars should be tested for each fruit type before a more general trend might be found in the effects of fruit size and maturity level on P. operculella larvae.

The absence of traceable amounts of α-tomatine in the maturing green and ripening fruits of the fresh market type and the better performance of P. operculella larvae in its fruits is highly suggestive of the importance this glycoalkaloid might have in affecting the performance of P. operculella larvae. The development of larvae was significantly slower in the cherry and the processing cultivars in which α-tomatine was found in significant amounts even in green maturing and ripening fruits. Leonardi et al. (2000) also found significantly lower amounts of tomato glycoalkaloids (α-tomatine and dehydrotomatine) in salad type tomatoes (fresh market cultivars) even when they were harvested at the green-orange stage. This was also the case in the present study, wherein the fresh market cultivar had no traceable amount of the glycoalkaloid in its maturing green and ripening fruits. In the cherry type in contrast, a significant amount of α-tomatine remained even in the ripened fruits. Perhaps as a result, the correlation of α-tomatine content with head capsule width was found to be significantly negative in maturing green and ripening fruits of the cherry type in contrast, a significant amount of the glycoalkaloid in its maturing green and ripening fruits. In the cherry type in contrast, a significant amount of α-tomatine remained even in the ripened fruits. Perhaps as a result, the correlation of α-tomatine content with head capsule width was found to be significantly negative in maturing green and ripening fruits of the cherry and processing cultivars. Leonardi et al. (2000) reported that cultivar, ripening stage and growing condition play a pivotal role in determining the amount of glycoalkaloid in tomato fruits. In the present study, the three cultivars were grown under the same environmental conditions. The observed significant differences in α-tomatine content were therefore dependent on both plant genotype and fruit ripening stage. In general, it can be concluded that fruit α-tomatine content, size and maturity stage, are all important factors that affect the performance of P. operculella larvae in tomato fruits.

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References


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