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Some Ecological and Behavioral Aspects of the Tomato Leaf Miner Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae)

***Hussein Samir Abd El-Rahman Salama*[1*], *Ismail Abdel-Khalek Ismail*[2], *Mohammed Fouda*[3], *Ibrahim Ebadah*[2], *Ibrahim Shehata*[2]**

1 – National Research Center, Agricultural and Biological Research Division, Pests and Plant Protection Dept., EGYPT
2 - National Research Center, Plant Protection Dept., Dokki, Giza, EGYPT
3 - Al. Azhar University, Faculty of Science, Zoology Dept., Nasr city, Cairo, EGYPT
*Corresponding author: hsarsalama@hotmail.com

Abstract. Preliminary investigations were carried out to determine the insect behavior of *Tuta absoluta* towards different factors. Observations showed that the moth is nocturnal in habits. It is most active at dusk and dawn and rest among leaves of the host plant during the day, showing greater morning-crepuscular activity. It prefers to infest leaves of its host plants followed by sepals, leaf neck and stem. Also, the apical part of the tested plants showed to be more attractive to the females' oviposition compared to the median and basal parts. The best time for mating started in the morning. The insect can discriminate between different host plants. It showed more preference to tomato followed by black nightshade, eggplant, potato and pepper. The larvae are sensitive to light and it prefers the dark zones. The larvae with its taste receptors are able to discriminate between host plants and other chemicals. For instance, it showed great sensitivity to various sugars in varying degrees. The flight range of the moth was determined and it showed the ability to fly for a distance of 0.4 km overnight.

Keywords: *Tuta absoluta*, host preference, behavior, mating, light reaction, flight range.

Introduction

The tomato leaf miner *Tuta absoluta* (Meyrick) became a serious pest to tomato cultivations in Egypt since 2009, where it causes great damage to the crop.Egypt has an appropriate climate for tomato cultivation and the annual production of the crop is 9 204 097 tons of tomato fruits from about 9 000 ha of the cultivated area (MOUSSA *et al*., 2013). So, it is considered as the fifth largest tomato producer in the world (WPTC, 2011).

This insect pest was recorded firstly in many countries of South America (GARICA & ESPUL, 1982). It then invaded Egypt in the nearest governorate to Libya (Mersa-Matrooh) in 2009. By 2010 it had reached Giza, coming well established in all governorates and reaching the border and north part of Sudan in June 2011 (TAMERAK *et al.*, 2011; GAFFAR, 2012). No tomato cultivars are entirely resistant to this insect, but not all cultivars are equally susceptible (BORGORNI *et al*., 2003; OLIVEIRA *et al*., 2009; DE OLIVEIRA *et al*., 2012). The larvae attack the tomato plants during all developmental stages causing great losses by attacking leaves, flowers, stems and especially fruits

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besides mining their leaves (ESTAY, 2000; TORRES *et al*., 2001)

Tomato (*Solanum lycopersicum*) is considered as the primary host of this pest. However, it can also attack other cultivated Solanaceae plants such as eggplant (*S. melongena*), potato (*S. tuberosum*), pepper (*Capsicum annuum*), sweet pepper (*S. muricatum* L.), tobacco (*Nicotiana tabacum*) and some other non-cultivated Solanaceae (*S. nigrum, S. eleagnifolium*). Also, it infests *Physalis angulata* and *Phaseolus vulgaris, Datura ferox* and some Chenopodiaceae plants such as *Chenopodium album* (VARGES, 1970; GARÎCA & ESPUL, 1982; FERNANDEZ & MONTAGNE, 1990; PORTAKALDALI *et al*., 2013).

In the present study, the behavior of the moth and the larvae of *Tuta absoluta* has been investigated under the laboratory and field conditions.

Materials and Methods

A laboratory culture was maintained on tomato plants as a natural food according to SALAMA *et al*. (2014).

Behavior of adult moths.

Behavior of oviposition. Tomato plants are infested during any developmental stage, with the females ovipositing preferentially on the leaves, sepals and stem.

To study the moth behavior in the field, ten tomato plants were collected randomly from an infested cultivated area of tomato, then transferred to the laboratory. Each plant was divided into three parts; apical, median and lower or basal part. In each part, five leaves were examined (upper and lower surface), leaf neck, stem, sepals, flowers and fruits for estimating the vertical distribution of the eggs on the plant. For studying the behavior in the laboratory investigations, ten potted tomato plants, (45 days old) were exposed to the moths in rearing cages. After egg deposition, the leaves (upper and lower surface), leaf neck and stem were examined to determine the vertical distribution of eggs on different parts of each pot.

Mating. To study the mating process of *T. absoluta*, newly emerged adults of both sexes (1 ♂ : 1 ♀) were kept in transparent glass Petri dishes (15 cm) and each dish contained a moistened filter paper and a cotton piece saturated with 10% honey solution. Morphologically the females are wider and more voluminous than the males; the abdomen is brown in the female and creamy in the male.

Flight range. An attempt has been carried out to determine the flight range of the moth aiming to throw some light on its ability to disperse and fly for long distances searching for nectar. For this purpose, an experiment was carried out in the field (locality of Bernucht, Giza governorate). In this experiment, groups of moths were used for release and were obtained from the laboratory colony. Before release, the moths were separated into two groups, and one group was sprayed with blue and the other one was sprayed with red color as a marking agent. All moths were provided with a diet of 10% honey and moist atmosphere to keep them healthy and active before release. Release was made at sunset (6.45 p.m.) from a fixed release point. After release, recapture of the marked moths from the field was adopted overnight for 2 hours and at different distances from the release point. In each recapture site, a lamp was hanged in the back of a white screen 50×50cm, that was stretched over a stalk, 1.5 meters in height so as to reflect the light of the lamp. Two release sites were determined; the group with blue moths was released 250 meters from the white screen, but 400 meters from the main light trap installed in the field. The group with red marked moths was released 250 meters from the second white screen, but 750 meters from the main light trap.

In each recapture site, a technician was standing so as to collect the moths that were attracted to the light source and rest on the white screen coated with sticky lubricant. Recapture of moths continued for 2 hours. The white screens with the trapped moths were transferred to the laboratory for examination and to sort the marked individuals.

Behavior of larvae.

Preference of different host plants. The orientation of the larvae of *T. absoluta* towards different host plants was studied in the laboratory. Leaves of tomato, potato, eggplant, pepper and black nightshade were tested. Small discs (2 cm diameter) were taken from the fresh leaves of these plants and placed in a cyclic form in Petri dishes (15 cm) lined with moistened filter paper. Three dishes were used as replicates. Twenty newly hatched larvae were released in the center of each Petri dish and the average number of larvae that settled on each disc was recorded after 1, 2, 3, 4 and 24 hours.

Orientation of the larvae to light. To determine the behavior of the larvae of *T. absoluta* towards light, a Petri dish (12 cm) was used. One half of this dish was covered with black paper to reflect the light, while the second half was exposed to room light. Three dishes were used as replicates. Thirty newly hatched larvae were released in the center of each Petri dish and the average number of larvae in both light and dark zones were recorded after 1, 5, 10, 15 and 30 minutes.

Taste sensitivity of the larvae to sugars. In this investigation, the stimulatory feeding effects of some carbohydrates that might occur in tomato or other host plants of *T. absoluta* have been evaluated to determine the ability of the larvae in discriminating between these compounds. For this purpose, 18 small discs of filter paper (0.5 cm in diameter) were placed in a circular form in a Petri-dish (15 cm in diameter). These discs were impregnated with 0.1 M of 8 tested sugars (2 discs for each sugar and 2 pure discs as a control). These discs were left to dry and sixty newly hatched larvae were released within the Petri-dish containing the discs impregnated with the tested sugars. Counts of larvae that settled on these discs were made after 1,2,3,4 and 24 hours. The experiment was repeated three times.

In all experiments, data obtained were subjected to analysis of variance, one way ANOVA was used (F-test) to compare the results and then differences were considered significant at $P < 0.05$ level using SPSS program.

Results

Behavior of the moth.

Behavior of oviposition. In field investigations, data presented in table (1) indicated that the eggs were recorded on all parts of the plant except flowers and fruits where no eggs were detected. The leaves were more attractive to the females oviposition followed by sepals, leaf neck and stem where the percentages of deposited eggs were 96.45, 2.3, 0.7 and 0.61 %, respectively. The lower leaf surface showed to be more infested than the upper surface and the percentage of deposited eggs was 52.06 and 47.94 %, respectively.

When the plant was divided into three parts, the apical parts showed to be more attractive to the female oviposition compared to the median and basal parts, where the average number of deposited eggs on the apical parts was 49.8 eggs (43.12%) distributed on leaves (96.6 %), leaves neck (1.2 %), stem (1 %) and sepals (1.2 %).

Meanwhile, the average number of deposited eggs on the median parts was 38.9 eggs (33.67 %) distributed on leaves (96.14 %), leaves neck (0.52 %), stem (0.52 %) and sepals (2.82 %). On the other hand, the basal part of the tomato plant was less attractive where the average number of deposited eggs on it was 26.8 eggs (23.2 %) distributed on leaves (96.64 %) and sepals (3.36 %) with no record of eggs on both stem and leaf neck. Statistical analysis show high significant differences ($P<0.05$) in the vertical distribution of the eggs on the plant parts under laboratory conditions.

The laboratory observations (Table 2) showed that the leaves of the apical part of plant were more attractive for moth oviposition than the basal part, where the average number of deposited eggs on the leaves was 88.9 and 57.8 egg, respectively. Also, the upper leaf surface was more attractive than the lower surface. The number of deposited eggs on the upper leaf surface were 54.4±15.29 (61.2%) and 32.5±3.98 (56.23%) and for the lower leaf surface they were 34.5±9.28 (38.8%) and 25.3±2.13 (43.77%) for the apical and basal plant leaves, respectively. The percentage of total deposited eggs on all plant parts (leaves of apical part and basal part, leaves neck and stem) were 54.9, 35.7, 2.77 and 6.66%, respectively. Statistical analysis show high significant differences ($P<0.05$, $F= 19.3$) in the vertical distribution of the eggs on the plant leaves under laboratory conditions.

Table 1. Vertical distribution of *T. absoluta* eggs on tomato plants in the field

<table>
<tr><th rowspan="2">Plant part</th><th rowspan="2">Average number of deposited eggs</th><th colspan="2">Leaves</th><th rowspan="2">Leaf neck</th><th rowspan="2">Stem</th><th rowspan="2">Sepals</th><th rowspan="2">Fruits & Flowers</th></tr>
<tr><th>Lower surface</th><th>Upper surface</th></tr>
<tr><td rowspan="2">Whole plant</td><td rowspan="2">115.5</td><td>52.06 %</td><td>47.94 %</td><td rowspan="2">0.7 %</td><td rowspan="2">0.61 %</td><td rowspan="2">2.3 %</td><td rowspan="2">Zero %</td></tr>
<tr><td colspan="2">96.45 %</td></tr>
<tr><td rowspan="2">Apical part</td><td>49.8</td><td>51.98 %</td><td>48.02 %</td><td rowspan="2">1.2 %</td><td rowspan="2">1 %</td><td rowspan="2">1.2 %</td><td rowspan="2">Zero %</td></tr>
<tr><td>(43.12%)[a]</td><td colspan="2">96.6 %</td></tr>
<tr><td rowspan="2">Median part</td><td>38.9</td><td>52.4 %</td><td>47.6 %</td><td rowspan="2">0.52 %</td><td rowspan="2">0.52 %</td><td rowspan="2">2.82 %</td><td rowspan="2">Zero %</td></tr>
<tr><td>(33.67%)[b]</td><td colspan="2">96.14 %</td></tr>
<tr><td rowspan="2">Basal part</td><td>26.8</td><td>52.1 %</td><td>47.9 %</td><td rowspan="2">Zero %</td><td rowspan="2">Zero %</td><td rowspan="2">3.36 %</td><td rowspan="2">Zero %</td></tr>
<tr><td>(23.2 %)[c]</td><td colspan="2">96.64 %</td></tr>
</table>

The values followed by letters mean that the differences were significant at $P<0.05$.

Table 2. Pattern of oviposition of *T. absoluta* on the tomato plants under laboratory conditions

<table>
<tr><th rowspan="3">Deposited eggs / Plant parts</th><th colspan="4">Leaves</th><th rowspan="3">Leaf neck</th><th rowspan="3">Stem</th></tr>
<tr><th colspan="2">Apical part</th><th colspan="2">Basal part</th></tr>
<tr><th>Upper surface</th><th>Lower surface</th><th>Upper surface</th><th>Lower surface</th></tr>
<tr><td rowspan="2">Average number of deposited eggs / plant</td><td>54.4 ± 15.29</td><td>34.5± 9.28</td><td>32.5± 3.98</td><td>25.3± 2.13</td><td rowspan="2">4.5 ± 2.28 [c]</td><td rowspan="2">10.8 ± 6.06 [c]</td></tr>
<tr><td colspan="2">88.9 [a]</td><td colspan="2">57.8 [b]</td></tr>
<tr><td rowspan="2">% of total deposited eggs on plant parts</td><td>61.2 %</td><td>38.8 %</td><td>56.23 %</td><td>43.77%</td><td rowspan="2">2.77 %</td><td rowspan="2">6.66 %</td></tr>
<tr><td colspan="2">54.9 %</td><td colspan="2">35.7 %</td></tr>
</table>

The values followed by the same letters mean that the differences were not significant at $P< 0.05$.

Mating. Follow up of the mating process showed that the best time for mating started at 7.30 a.m. in the morning. At start of mating, the lower extremity of the male body gets in contact with that of the female. A single mating lasted for 5.14 ±1.15 hours.

Flight range. Counts of recaptured released moths (blue or red) showed a flight range of 250 meters during a period of 2 hours. In the blue marked moths, 3 moths were recaptured after an hour and 42 moths after 2 hours. In the red marked moths, 6 individuals were recaptured after one hour and 24 after 2 hours. In addition, examination of the collected moths by the main light trap (400 meters from the release point) one day after release, showed that 67 of the blue marked moths were recaptured. So, it appears that the moths of *T. absoluta* have a flight range up to 250 meters during a period of 2 hours after release and 0.4 kilometers overnight. The ability of the moth to fly for a distance of 0.4 kilometers overnight is of great importance in regulating its dispersion and oviposition on different host plants during its life span. Accordingly, the moths after emergence will be able to detect the source of nectar, either from the field where they emerge or from flowering weeds on the borders of cultivations or from other host plants within the limit of their activity in the neighboring areas.

Behavior of larvae.

Preference of different host plants. The orientation of the larvae of *T. absoluta* towards different host plants was studied in the laboratory. Observations (table 3) indicate that hatched larvae were attracted in a descending order to leaves of tomato >black nightshade>eggplant> potato where the average number of oriented larvae was 4.33, 2.33, 2 and 1.33 larvae, respectively, after one hour of hatching. Meanwhile, no larvae were oriented to the discs of pepper leaves. With increase of the exposure time to 24 hours the tomato leaves appeared to be the most attractive host to *T. absoluta* larvae followed by black nightshade, eggplant, potato and pepper, where the percentages of the oriented larvae were 46.65, 20, 18.3, 11.65, and 3.3 %, respectively. Statistical analysis show high significant differences ($P<0.05$, $F= 36.73$) in tomato leaves as a host preferred by the larvae compared to the other hosts under laboratory conditions.

Table 3. Preference of larvae of *T. absoluta* to host plant leaves.

Exposure time / hour	No. of larvae that settled on				
	Tomato	black nightshade	Pepper	Potato	Egg plant
One	4.33±0.47	2.33±0.47	0±0	1.33±0.47	2±0
2	7±0.82	3.66±0.47	0.7±0.47	2.66±0.94	3.66±0.47
3	7.7±0.47	4.33±0.47	1±0.82	2.66±0.94	4.33±0.47
4	9±0.82	4.33±0.47	1± 0	2.33±0.94	4.33±0.47
24	9.33±1.3	4±0.82	0.66±0.47	2.33±0.47	3.66±0.82
% of oriented larvae at 24 hrs.	46.65 % [a]	20 % [b]	3.3 % [d]	11.65 % [c]	18.3 % [bc]

Values followed by the same letters mean that the differences were not significant at P< 0.05.

Orientation of the larvae to light. Our observations showed that the hatched larvae of *T. absoluta* were sensitive to light and it preferred the dark zone. The data in table (4) showed that, immediately after hatching the larvae were scattered but after 5 minutes the average number of oriented larvae to the dark zone was 8.7±1.7 larvae compared to 4 ± 0.82 larvae in the light zone. The increase of exposure time led to increase the number of larvae attracted to the dark zone, where the average number of larvae attracted to the dark zone was 13.33±2.63 and 18.3±0.47 larvae compared to 6.33±0.95 and 9±0.82 larvae in the light zone after 10 and 15 minutes, respectively. After 30 minutes, the highest number of larvae settled in the dark being 22.7±1.47 (75.66%) compared to 7.33±1.25 (24.43%) in the light zone. Statistical analysis show high significant differences between dark and light zones ($P<0.05$, $F=16.94$).

An experiment was carried out to determine the time required for the newly hatched larvae to penetrate or bore the tomato leaves. Immediately after hatching, the neonate larvae behave like a borer where it feeds on leaf surface then penetrate within the leaf tissues. The required time for the penetration was about 30 - 37 minutes, where the average number of the penetrating larvae was 26±0.92 (86.66 %) larva after 30 minutes, while all the hatched larvae disappeared completely in the tunnel after 37 minutes.

Taste sensitivity of the larvae to sugars. The stimulatory effects of some carbohydrates that might occur in tomato or other host plants of *T. absoluta* have been investigated to determine the ability of the larvae in discriminating between the tastes of these sugars. The data obtained (table 5) clearly show that the tested sugars can be arranged according to the stimulating effectiveness as follows: monosaccharides: D-fructose = D-glucose > D-galactose > D-mannose, disaccharides: sucrose > D-maltose = D-lactose, polysaccharides: glycerol.

So, the structure of the sugar molecules shows to be effective in stimulation (table 5). The statistical analysis show high significant differences in the number of larvae that settled on sucrose ($P<0.05$, $F=60.9$) compared to the other sugars under laboratory conditions.

Table 4. Orientations of the larvae of *T. absoluta* towards light

Exposure time in minutes	On filter paper		On tomato leaf
	Dark zone	Light zone	
	Mean No. of oriented larvae ± SD		Mean No. of penetrating larvae ± SD
One	All hatched larvae were scattered		0
5	8.7 ± 1.7	4 ± 0.82	0
10	13.33 ± 2.63	6.33 ± 0.95	0
15	18.3 ± 0.47	9 ± 0.82	0
30	22.7 ± 1.47	7.33 ± 1.25	26 ± 0.94
% of oriented larvae at 30 min.	75.66 % [a]	24.43 % [b]	86.66 %

The values followed by letters mean that the differences were significant at $P< 0.05$.

Table 5. Orientation of larvae of *T. absoluta* to sugars

Exposure time/hour	No. of larvae that settled on								
	Sucrose	Fructose	Glucose	Galactose	Mannose	Maltose	Lactose	Glycerol	Control
One	0±0	0±0	0.33 ±0.05	0±0	0±0	0±0	0±0	0±0	0±0
2	3.33 ±0.47	3 ±2.1	4.33 ±0.94	2.33 ±0.47	1.33 ±0.47	0±0	0.33 ±0.04	0±0	0±0
3	3.33 ±0.47	4 ±0.8	5 ±1.4	3.33 ±0.4	2 ±0.82	1±0	1±0	1±0	2 ±0.82
4	8 ±1.4	6.7 ±1.24	8.33 ±0.94	5 ±1.4	5.33 ±0.94	3±0	2.33 ±0.47	2.33 ±0.04	0±0
24	12.33 ±0.47	9 ±1.6	9 ±0.8	5.7 ±0.94	4.33 ±0.47	3±0	3±0.8	0±0	0±0
% of oriented larvae at 24 hrs	20.55% [a]	15% [b]	15% [b]	9.5% [c]	7.22% [cd]	5% [d]	5% [d]	0% [e]	0% [e]

The values followed by the same letters mean that the differences were not significant at $P< 0.05$.

Discussion

Studies on the behavior of oviposition of *T. absoluta* moth in the field indicated that the tomato plants were infested during any developmental stage and the females deposited their eggs on all parts of host plants; leaves, leaf neck, stem and sepals. The leaves were more attractive to females and the lower leaf surface appeared to be more infested than the upper surface. After egg hatching, the larvae of *T. absoluta* penetrated tomato leaves, fed on leaf parenchyma tissues forming irregular leaf mines that got longer and wider as the larvae continued to feed. The apical part of the plant was more attractive to the females compared to the median and basal parts. These observations coincide with those recorded by previous authors (ESTAY, 2000; TORRES *et al.*, 2001; LEITE *et al.*, 2004).

The variation in oviposition site selection and in vertical distribution of eggs on plant parts may be correlated to various factors. TORRES *et al*. (2001) stated that the female lay eggs uniformly on the under and upper side of leaves that are covered with trichomes that provide chemical and mechanical stimuli. Also, PROFFIT *et al.* (2011) demonstrated the essential role of plant volatiles in *T. absoluta* females' host-finding behavior and suggested that the leaf contact significantly increased the number of eggs laid and leaf surface morphology and contact chemicals are important factors in oviposition site selection. Additionally, in agreement with our findings, LEITE *et al.* (1999) found that *T. absoluta* oviposited more on leaves of the apical and median portions than in the basal parts of the tomato plant. The same authors stated that this oviposition behavior could be linked to the lower calcium content of apical leaves, which are tender, compared to middle or basal leaves of the host plant. Furthermore, LEITE *et al*. (2004) demonstrated that there was a preferential deposition of *T. absoluta* eggs on the apical leaves of the tomato plant.

Our observations on the mating behavior showed that the best time for mating was in the early morning at 7.30 a.m. and the mating process lasted for 5.14± 1.15 hours. These observations agree with those of IMENES *et al*. (1990) who studied the biology and behavior of *T. absoluta* in the laboratory and observed that the mating activities started at 7 a.m. and duration of mating took about 4.49 hours. In this concern, HIKEL *et al*. (1991) found that the duration of copula of this insect was variable (2-6 hours). MIRANDA-IBARRA (1999) reported that the greatest number of males was captured in pheromone traps during the period 7 to 11 a.m. suggesting that this is the best time when males are searching for calling females. MARINA *et al.* (2014) stated that mating always began during the first hour of the photophase, and mating pairs took from a few minutes to 6 h 40 min. and 76 % of females laid eggs on the same days they mated. The variation in copulation time may be proportional to complete transfer of the spermatophore from male to the female (OUYE *et al.*, 1965; SETH *et al.*, 2002) or may be to spermatophore size (FRANCO *et al.*, 2011).

The flight range of the moth was determined to shed light on its ability for dispersion and the field observations clearly indicated that it was able to fly up to 250 meters during 2 hours and 400 meters from the release point overnight after release.

As already mentioned, the moth is nocturnal in habits. The newly hatched larvae tend to orient itself and settle in the dark zones. They behave like a borer when it feeds on the leaf surface and then penetrate within the leaf tissues within 30-37 minutes. This coincides with observations of CUTHBERTSON *et al*. (2013) who showed that the first instar larvae of the same species fed on the leaf surface for approximately 82 minutes before becoming fully submerged inside the leaf.

T. absoluta is polyphagous, but it prefers some of its hosts such as tomato. The senses of taste and smell in lepidopterous larvae are important in host plant selection and the acceptance of the larva of *T. absoluta* to a host plant is due probably to chemical volatiles rather than purely physical factors as reported by TORRES *et al.* (2001) and PROFFET *et al*. (2011).

In this concern, BECK (1956) stated that among the numerous compounds that can be extracted from plants are the sugars which occur in appreciable quantities in plant tissues and are known to induce feeding in some phytophagous insects. The larvae of *T. absoluta* with taste receptors located on the mouth parts were able to discriminate between sugars showing more sensitivity to sucrose as compared with D-fructose, D-glucose and D-galactose. A great difference in taste sensitivity to sugars was observed such as D-glucose and D-galactose and also between D-glucose and D-mannose despite slight differences in their configuration. D-mannose and D-galactose are almost equal in stimulating effects although they differ in their structural configuration. Sucrose with α-linkage is superior feeding stimuli, while lactose which lacks α- linkage is weakly stimulating. This shows that the difference in the structure of sugar molecules results in different physiological effects. In this concern, HASSETT *et al.* (1950); DETHIER (1955); KHALIFA *et al.* (1974) reported that sugars with α- linkage were more stimulating than those lacking it and that the response of various insects to carbohydrates shows a spectrum of activity from unresponsiveness to extreme sensitivity. Glycerol from polysaccharides is not stimulating to the larvae. Our preliminary microscopic examination of the larval mouth parts shows the existence of various sensilla or chemoreceptors.

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