Diapause and its regulation in the whitefly 

*Trialeurodes lauri*

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Abstract

This study focuses on the regulation of synchronization between the life cycle of the oligophagous whitefly, *Trialeurodes lauri* (Signoret), and its evergreen host tree *Arbutus andrachne* in Mediterranean chaparral. Whitefly infestations vary considerably among trees. The adults of the univoltine (one generation per year) whitefly emerge *en masse* during April and May and oviposit on the new spring foliage. Following approximately one month of development to the early fourth instar, the nymphs enter nine-month diapauses, terminating in February. This diapause is induced and maintained by the plant and can be experimentally avoided (in the case of developing young nymphs) or terminated (in the case of diapausing fourth instars), if whitefly-bearing branches are severed from the tree and placed in water under laboratory conditions. This study is the first report of a whitefly diapausing through both summer and winter seasons. The role of the host plant in the process is discussed.

Keywords: Aleurodidae, Mediterranean chaparral, diapause, tree phenology, plant-insect interaction, synchronization, univoltine, *Arbutus*

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Introduction

Whiteflies (Homoptera: Aleyrodidae) are small, sap-sucking insects. They have received considerable attention due to their including several extremely polyphagous pest species such as *Bemisia tabaci* (Gennadius). Host-plant specificity ranges from wide polyphagy to monophagy, e.g. *B. tabaci* with over 500 host plant species (Oliveira et al., 2001) vs *Aleurochiton aceris* (Modeer) with only a few *Acer* species known as hosts (Evans, 2009). Their life cycle comprises an egg, four nymphal instars and winged adults. Some species have an expedited (temperature-regulated) life cycle with several generations per year, while others are univoltine, usually with an extensive diapause period (Baehrman, 1979; Byrne & Bellows, 1991; Gerling, 1990).

*Trialeurodes lauri* (Signoret) is a common but poorly studied whitefly species. It was described by Signoret (1882) on *Laurus nobilis* trees in Greece, and its appearance has been recorded as causing heavy infestations in the Crimea (Korobitsin, 1964) and Turkey. The latter record is derived from the interception by British quarantine authorities of heavily infested branches of *Arbutus unedo* as components of imported Christmas wreaths from the Turkish highlands (Malumphy, personal communication). Russell (1947) and Mound & Halsey (1978) published taxonomic information on *T. lauri*, whereas its life cycle and that of its parasitoid *Encarsia scapeata* Rivnai were studied by Erel (2004), Gelman et al. (2005) and Gerling et al. (2009).

All known host plants of *T. lauri* (i.e. *A. andrachne*, *A. unedo* and *L. nobilis*) are evergreens, populating the hills of southern Europe and the Mediterranean woodlands (ca. 500 m above sea level and higher) (Ross, 1999; Celikel et al., 2008). Within Israel, *T. lauri* is mainly found on *A. andrachne* trees (Gelman et al., 2005) on Cretaceous and anaerobic chalky soils. The trees occur throughout the higher elevations (ca. 500 m and above) in the Mediterranean zone where the climate is typified by dry, hot summers (~ 15–30°C) and wet, cold winters (~ 2–22°C). They often form large stands of trees, easily distinguishable by their red bark. They foliate in the spring (mostly March–May).
and in May–June shed the leaves of the previous year, retaining the recently formed foliage until the following year. The whitefly eggs are positioned on the underside of young leaves with their longitudinal axis parallel to the leaf surface and are laid in high densities in circular batches. The extracuticular wax covering of the sessile nymph is surrounded by horizontally protruding wax filaments measuring half or more of the nymph's diameter. At times, T. lauri forms extreme infestations with hundreds of nymphs per leaf (Erel, 2004).

The current work was undertaken within the framework of elucidating the tritrophic interrelationships of native flora and fauna, with the following specific aims: (i) to reveal and describe the density and field distribution pattern of the whitefly; (ii) to describe its development; (iii) to characterize the whitefly's phenology in relation to the host tree; and (iv) to elucidate the factors that may control nymphal diapause. Two additional studies undertaken within the same framework are those by Gelring et al. (2009) and Gerling & Ben Ari (2010).

**Materials and methods**

**General**

The two-year study (2001–2003) was conducted in the Nahal Kafarav Nature Park (elevation 450–750 m above sea level; ca. 25 km southwest of Jerusalem). Trees were visited and monitored throughout the period of the study. Visits were conducted monthly, with four to five monthly visits during the plant foliation, whitefly emergence and oviposition periods (March–June). Additional observations and experiments were conducted in the laboratory and greenhouses at Tel Aviv University.

**Whitefly density and distribution pattern**

During November 2001, we scored the density of nymphs infesting leaves in 12 randomly chosen transects within the A. andrachne forest, each comprising 15 adjacent trees (total 180 trees). Visible foliage of each tree was examined visually for 2 min. and the numbers of whitefly nymphs per leaf were estimated and ranked as: 1 = 0–10; 2 = 11–50; 3 = 51–500; 4 = 501–1000; 5 ≥ 1001.

Whitefly distribution within a shoot was examined on each leaf on ten infested, randomly collected shoots. The number of whitefly nymphs on each leaf was scored, and their location upon the shoot was noted, with the oldest marked as no. 1 etc.

**Whitefly development**

**Natural development**

Several hundred adults collected in the field during April 2003 were introduced onto six A. andrachne saplings in the laboratory (25–26°C, 14:10 L:D regime). Fifteen cohorts of the eggs and hatching nymphs were followed daily until the nymphs entered diapause in the early fourth instar. This method was not suitable for the 4th stage nymphs, since the diapause lasted for 9–10 months (Gelman et al., 2005).

**Experimentally manipulated whiteflies**

Following the success of inducing continuous whitefly development of the fourth instar (see below), we also examined the developmental durations from the molt to fourth instar to adulthood in the laboratory.

For this purpose, we placed several field-infested branches with third instar nymphs in water under the same conditions (25–26°C) as above. Although this method resulted in high mortality, it allowed us to rear the whiteflies occurring on three leaves from third instar to adulthood.

**Phenology of the trees and whiteflies**

Development of new leaves and the shedding of old leaves took place between February and May. During the rest of the year, little change could be seen in the appearance of the foliage. Since both, whitefly development and emergence, occur during the same period, tree phenology was monitored on 22 whitefly-infested trees that were selected at random during each field visit from February to May. Each tree was visually scanned for 2 min. and stages of bud development were ranked on a scale of 0–5, with 0 representing trees with all buds still closed and 5 indicating that all leaves were fully expanded. Numbers 1–4 expressed the fraction of already expanded leaves to young folded leaves in the bud (1 = 0.2, 2 = 0.4, 3 = 0.6 and 4 = 0.8 of the total leaves being expanded).

Adult whitefly density was observed during both years of the study. Following general observations conducted during 2002, a more exact census was taken in 2003 using two methods as follows: (i) 2 min. direct counting of adult whiteflies visible while standing under each tree. The count was later ranked as described above for the nymphs in the transects. (ii) Counting of adults caught on six yellow, sticky traps (16 × 20 cm) hung vertically on infested trees at a height of 1–2 m. The traps were collected during each visit (i.e. monthly during most of the year and 4–5 times during the spring months) and examined in the laboratory.

**Control of whitefly phenology**

A qualitative estimate of the tritrophic interaction (tree-whitefly-parasitoid) was carried out by directly observing 10–20 infested leaves on intact branches (ca. 70 cm long) in the field. About 100 branches were collected monthly from October 2004 to November 2005 and placed immediately in water. In the laboratory, they were randomly divided into batches to simulate three sets of conditions: (i) outdoors, day length varying between 14:10 and 10:14 L:D (summer and winter), temperature on coldest nights ca. 5°C, by day ca. 10–17°C during November–March, and approximately 15–28°C during the rest of the year; (ii) in the unheated laboratory room under fluctuating temperatures on coldest nights ca. 10°C, by day ca. 15–25°C during November–March and approximately 20–25°C during the rest of the year; and (iii) in a temperature chamber at a constant temperature of 28 ± 2°C. All branches were kept in water in sleeve cages for about one month, until the leaves had dried up. Nymphal development was observed weekly and adult emergence of whiteflies and parasitoids was counted on a daily basis.

Immature whitefly stages were determined by direct observation of the leaf and through microscopic examination of slide-mounted nymphs. For this purpose, 3–5 leaves were removed each week from each of the three batches, starting on the day of collection and last until the leaves dried (3–4 weeks). All the whiteflies were removed from each leaf by dipping in Carnoy’s fixative (http://www.histosearch.com/histonet/Sep01A/Re.Carnoysfixative.html). Slides were
then made, as whole-mounts of the whiteflies and as sectioned material. The fixative was exchanged for absolute ethanol for about 2 h. The whiteflies were then run through two changes of Xylene (about 2 h); and, for whole mounts, they were placed on a microscope slide in a drop of Permount® (Fisher Scientific, Loughborough, UK). For histological observations, the material was prepared, sectioned, mounted, deparaffined and stained (see Blackburn et al., 2002). Whole mounts and sections were examined under a Wild M40 or a Nikon Eclipse 600 compound microscope. The latter was equipped with differential interference contrast optics, and photomicrographs were taken using a Nikon DMX 1200 CCD camera.

Whiteflies on the whole-mount slides were sorted according to their developmental stages: 1, diapausing nymphs: flat nymphs with undeveloped compound eyes (corresponding to stage 4.1 of T. vaporariorum (Gelman et al., 2002)); 2 on, developing nymphs: 2 = initial, developing wing buds; 3 = folded wing buds; 4 = initial or complete eye development. In addition, stereoscopic laboratory examination of whitefly development was conducted with approximately ten leaves from each site that were collected during every field trip.

The above observations revealed that cutting the branches had terminated the diapause of those whiteflies living on their leaves (see results). Thereafter, a series of experiments was conducted to determine whether the onset of diapause could also be prevented by detaching the leaf-bearing branches bearing pre-diapause whitefly nymphs in the spring. Branches with leaves bearing eggs and whitefly nymphs of all stages (1–4) were cut, taken to the laboratory and kept in water. The development of the whiteflies was followed until their emergence as adults or death was registered.

Data analysis

Comparison of duration to diapause break was made using two-way ANOVA following square root transformation. Non-parametric tests were carried out using Spearman-rank order correlations (P<0.05) using ‘Statistica 6.1’ (StatSoft Inc.) software.

Results

Whitefly density and distribution pattern

Infestation rate in the field was highly variable, ranging from ca. 25% of the trees on which no whiteflies were found on the leaves, to trees in which all of the observed leaves had >50 nymphs per leaf. High infestation rates caused extensive development of sooty mold. Most infested trees had low populations (ranks 1 or 2), whereas the two highest categories (trees with over 500 whitefly nymphs per leaf) amounted together to ca. 12% (fig. 1).

On shoots of highly infested trees (rank 3 and above), at least some nymphs could be found on most of the leaves, but leaves 4 and 5 were the most infested with over 120 whitefly nymphs per leaf. The very old and young leaves bore the least whiteflies (fig. 2).

Whitefly development

Under laboratory conditions (25–26°C), the duration of the different whitefly instars was the shortest in instars 1 and 2. The median duration of the fourth instar, which in nature diapauses for approximately nine months, lasted for eight days on the cut branches (table 1).

Fig. 1. Frequency distribution of tree infestation rates found during a single transect examination taken in the fall of 2001. (n = 180 trees). Numbers above columns designate percentage of the rank found in the sample.

Tree-whitefly association

Phenology of the whiteflies and trees

The phenology of A. andrachne trees varied during the study period of 2002–2003 (fig. 3a). Foliation peaked in mid April of 2002 and at the end of May in 2003. Leaf-shedding phase peaked at the end of May in 2002 and in early June in 2003. The observations conducted during 2002 and both yellow traps and direct field counts conducted during 2003 showed that adult emergence occurred en masse in the spring. Trialeurodes lauri adult emergence started in 2002 in early April and ended in early May; in 2003, it started late in April and ended late May (fig. 3b). Thus, although the data points did not suffice for testing the correlations between foliation and adult whitefly emergence, we found a similar trend in both during March and April.

First nymphal instars were observed in the field from early May (2003) on. The nymphs developed for six weeks till mid-June, when all had reached the early 4th instar in which they diapaused. In the field, diapause break (indicated by the appearance of wing buds) started in February and peaked in March (see below). Thus, T. lauri has one annual cycle, about nine months of which are spent as dormant early 4th instar nymphs (fig. 4). Whiteflies on saplings that were infested in the spring and kept in the greenhouse showed an identical pattern of diapause as those under natural conditions.

Examination of a total of 71,047 nymphs on 325 leaves (April–May 2003) yielded overall 20% mortality. This was caused both by parasitism (with E. scapeata being the only parasitoid) and other, undetermined sources, such as predation and desiccation. Mortality rates were inversely density-dependent (fig. 5); Spearman Rank Order Correlations, rs = −0.57, df = 324, P<0.05 (n = 325 leaves).

Control of whitefly phenology

Branch cutting induced diapause break from October on, nearly five months earlier than normal. The duration from
branch cutting to the first detection of wing buds (an indication of diapause break) decreased as the season progressed (table 2). On leaves that were kept in the room, wing buds started to develop after 15–18 days between October and December and after nine days in February. Faster development was observed in whiteflies that were kept in the temperature chamber (28±2°C), being 9–11 days throughout the observation period from October through February. The differences between the chamber and the room treatments were marginally insignificant (F=8.58, P=0.061).

Nymphs that were kept outdoors in November and December dried out together with their leaves after 30 days without showing any sign of diapause break (table 2). The outdoors treatment was, therefore, not included in the ANOVA analysis. The outdoor treatment did induce diapause break later in the season, in February. All material collected on 13th March had already developed wing buds.

### Discussion

The main goals of this work were to characterize the relationships between the host tree and the whitefly and to elucidate the factors that may control nymphal diapause. Our findings demonstrate a tight relationship between tree and whitefly phenology. This materialized through the induction of a nine-month-long diapause of the whitefly, enabling synchronization between tree foliation and *T. lauri* development. Diapause induction could be prevented by severing branches infested with developing whitefly nymphs during the spring.

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**Fig. 2.** Mean nymphal numbers of *T. lauri* on different leaves within shoots of highly infested *A. andrachne* trees. Leaf no. 1 is the oldest (proximal) and leaf no. 13 is the youngest (distal) on the shoot. Bars indicate standard errors of the mean (n=10 trees).

**Table 1.** Developmental duration of *T. lauri* immatures under laboratory conditions at 25–26°C. Eggs to 3rd instar nymphs were followed daily on *A. andrachne* saplings.

<table>
<thead>
<tr>
<th>Stage/instar</th>
<th>Egg</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>103</td>
<td>81</td>
<td>67</td>
<td>52</td>
<td>27</td>
</tr>
<tr>
<td>Range</td>
<td>6–12</td>
<td>2–7</td>
<td>2–7</td>
<td>3–8</td>
<td>8–18</td>
</tr>
<tr>
<td>Median</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Standard dev.</td>
<td>1.877</td>
<td>1.176</td>
<td>1.425</td>
<td>1.448</td>
<td>4.54</td>
</tr>
</tbody>
</table>

*, the development of the 4th instar nymphs on cut branches brought from the field as eggs or nymphs, was followed separately.
Distribution and abundance in the field

*Trialeurodes lauri* distribution corresponds well with its host trees, *A. andrachne* throughout its range in Israel and *A. unedo* and/or *L. nobilis* in other countries ranging from the Crimea (Korobitsin, 1964) to the Mediterranean basin. We detected considerable variation in the infestation rates of the trees. On the majority of the trees, only a few immature whiteflies were visible on a given tree, whereas 12% of the trees examined had high whitefly populations (fig. 1).

Intraspecific variation in the level of susceptibility of individual trees to phytophagous insects is a common phenomenon (Bernays & Chapman, 1994). Several genetically- and environmentally-based factors may influence such variation, including the level of defensive compounds, plant mechanical traits, phenological mismatching and the activity of natural enemies (e.g. Denno & McClure, 1983; Burstein & Wool, 1993; Bernays & Chapman, 1994; Hemming & Lindroth, 1995). Our study was not designed to explore which of these factors controls the whitefly, as such factors can only be revealed in a long-term study. Nevertheless, the data suggest that tree phenology (which was similar in all examined plants) and parasitoid activity (Erel, 2004; Gerling et al., 2009), which was not density-dependent, did not shape the observed variations in infestation levels. On the other hand, following the dynamics of infestations within the trees showed that the developmental sequence of leaves within each tree had a definite bearing on the distribution of infestation levels of individual leaves. Those leaves in the central part of each new branch that developed during the peaks of whitefly emergence were the most heavily infested.

Whitefly development

The whitefly has one generation per year. Adults emerge in April–May and oviposit on the new foliage. The eggs hatch...
within a few days, and the developing nymphs undergo three phases as follows: (i) development till their 4th instar, which is reached in late May and June; (ii) a diapause that lasts throughout the summer and winter; and (iii) development to adulthood that occurs during February and March (fig. 4). Phase 3, the post-diapause development, lasted for about one month, depending upon temperature. This cycle is tuned to the foliation that occurs once each year, starting about February and ending in May, allowing the new whitefly generation to settle on the young foliage.

Adults readily oviposited on young A. andrachne seedlings during the spring. According to field and laboratory observations, the fourth instar nymphs entered diapause on matured leaves. The onset of diapause was precluded when branches bearing young, pre-diapause, whitefly nymphs were experimentally removed from the trees. Diapause could also be terminated prematurely when the nymph-bearing branches were removed later on, after the onset of diapause. Thus, it appears that plant-borne cues control whitefly development.

Tree-whitefly association

Phenology of the whiteflies and trees

The development of phytophagous insects may be controlled by a number of factors, including the physiological cycle of the host plant and the climate (light and temperature). Typically, many insect species that encounter unfavorable developmental conditions during their life cycle may enter a period of reduced metabolic activity or diapause (Tauber et al., 1986; Koštál, 2005). The diapause may be facultative or obligatory and is induced by external factors referred to as token stimuli (Tauber et al., 1986). It may occur at any insect stage and often involves reproduction, directing the insect life cycle to be in harmony with available food and suitable climatic conditions. Diapause in whiteflies has so far been studied in Aleyrodites proletella (Linnaeus) and in Aleurochiton complanatus (Baerschprung). In the former, Adams (1985) reported the influence of photoperiod on ovarian development, regulating oviposition to the season in which growing host plants would be available. In the latter, the diapause materialized in the 4th instar nymph and entry into diapause appeared to be influenced by the effects of leaf age and light (day length and light intensity) (Baehrman, 1979). The age of the sycamore leaves affected entry into diapause of the 4th instar nymphs, whereas day length and light intensity affected the young, 1st and 2nd instars.

In T. lauri, diapause occurred during or following leaf maturation of A. andrachne and lasted through the summer and winter, indicating that the token stimulus had an overriding effect on both temperature and/or day length, which were permissive for development at least part of the time. Indication that the token stimuli were plant origin was demonstrated in the branch-cutting experiments, where we kept the branches alive but severed them from the natal tree and, therefore, disrupted the inflow of materials that are probably associated with whitefly diapause. Severing the branches either precluded diapause (when cut before the nymphs reached the fourth instar) or caused its break (when already diapausing nymphs occurred on the leaf between June and January). The duration between branch cutting and the break in diapause declined as the season progressed; outdoors, under natural conditions, it was the longest, lasting one month or more and was shortest when the branches were kept at 28 ±2°C in the temperature chamber. Since even whiteflies that had been taken from the field as 3rd instar nymphs reached adulthood in this experiment, it is apparent that the diapause-inducing token stimulus (or stimuli) might involve the continuous influence of a factor that originated in the tree. These dynamics match the description by Koštál (2005). "Unknown physiological process(es) lead to more or less gradual decrease of diapause intensity and increase of sensitivity to diapause terminating condition(s)." Such processes may be driven by the token stimuli, like phloem quality, as noted by Dixon (1997) for aphids. In our observations, the stimuli varied with the season, permitting or even encouraging normal, temperature-dependent development in February and early March. They might also be associated with the diapause stage, indicating a possible decline in their presence or the involvement of diapause-terminating stimuli or both.

Leaf suitability for whitefly development

The relationship between leaf age and success in oviposition and development in tree-insecting whiteflies was discussed by a number of workers. Bellows et al. (1998) found that Paraleurodes minei laccarino could only be grown on mature leaves, preferably with prior whitefly infestation either of their own or another species. Contrarily, Walker & Zareh (1990) showed that all three examined species, Aleurothrixus floccosus (Maskell), Dialeurodes citri (Ashmead) and Parabemisia myricae (Kuwana), preferred young leaves that had not yet obtained their full, dark color. Differences were observed among these three species; while P. myricae were able to develop only on very young leaves, the two other species were also able to develop on older leaves (Walker & Aitken, 1985). The need for young leaves for immature development might be associated with cuticular characteristics (Walker, 1988) and/or suboptimal amino acid nutrition (Walker & Aitken, 1985).

In the case of T. lauri, there is an apparent need for young foliage to enable immature development, a need that is furnished through the tight synchronization with the new flush of A. andrachne leaves. Moreover, this cycle allows the whiteflies to benefit from the only period during which new, non-dormant leaves exist on the plant. At the same time, daytime spring temperatures in the region rise to above 20°C, facilitating flight of the adults and development of the whitefly immatures.

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