

# Effect of foliar tomentosity on phenotypic plasticity in *Bemisia tabaci* (Hom., Aleyrodidae)

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**Abstract:** Some whitefly species, as well as members of other insect families, are known to develop different phenotypes under different environmental conditions. In this study, the identification of an environmental trigger and its sensitive stage for the development of the setose phenotype of *Bemisia tabaci* nymphs are reported. Quantification tests revealed phenotypic plasticity manifested by positive correlation between trichome density on tomentose leaves and the percentage of setose nymphs found upon them. Artificial simulation of leaf tomentosity provided sufficient stimulation to induce the setose nymph phenotype. The crawler was found to be the only sensitive stage for this induction. Polymorphism of the nymphal phenotypes correlated with additional biological features of both nymph and adult, which could be treated as proxies of fitness. Setose nymphs were smaller and developed (from eggs to adult) faster. Adults emerging from such nymphs were smaller and had shorter lives. Adaptivity of the detected phenotypic plasticity and of the correlating nymphal and adult features are discussed.

## 1 Introduction

Plant characteristics such as plant architecture, secondary metabolites and leaf features are commonly involved in phytophage–plant interactions (PRICE, 1997; ROSSI and STRONG, 1991). In whiteflies (Hom., Aleyrodidae), several relationships have been found between nymphal morphology and leaf features (BINK-MOENEN and MOUND, 1990). Various authors have long reported qualitative but circumstantial descriptions of *Bemisia tabaci* (Gennadius) nymphs on glabrous leaves exhibiting a smooth phenotype, whereas those found on tomentose leaves (i.e. leaves whose surface is covered by trichomes) are generally setose (MOUND, 1968; DUFFEY, 1986; BUTLER et al., 1988; BEDFORD et al., 1994; MCAUSLANE et al., 1995; NEAL and BENTZ, 1999). Despite the important role this feature may play in crop protection and biological control of this pest (GUERSHON and GERLING, 1999), few quantitative assessments of this phenomenon have been reported and explanations of the relationships between leaf properties and the nymphal phenotypes of *B. tabaci* remain mainly anecdotal and conjectural (NEAL and BENTZ, 1999).

Preliminary results (GUERSHON and GERLING, 1994) revealed that induction of nymphal phenotypes by the plant occurs at, or prior to, settlement of the whitefly crawler upon the leaf, thus excluding any influence of the plant through feeding stimuli, and reducing the candidate whitefly stages for induction to three: the laying female, the egg and the crawler. That preliminary report, as well as recent studies (NEAL, 1996, 1997; NEAL and BENTZ, 1999) presented circumstantial evidence for possible crawler ‘experience’ as the basis for nymphal setosity. However, neither conclusive

proof of such ‘experience’ nor demonstration of the stimulating factor have been presented to date. Our primary aim in the present study was to determine (a) the proximal causes by which nymphal phenotypes are induced on tomentose leaves, and (b) the whitefly stage on which they act.

In morphological studies, an ecomorphological approach may be a key factor for understanding interactions between the analysed individual and the ecological system as well as in the research of adaptivity and evolution of morphological variations (CARRASCAL et al., 1994; WAINWRIGHT and REILLY, 1994). Thus, our second aim was to examine additional associations between the differential morphology of nymphs and various aspects of the whitefly biology and life cycle. The determination and quantification of these associations are crucial for further understanding of any possible adaptivity of whitefly polymorphism.

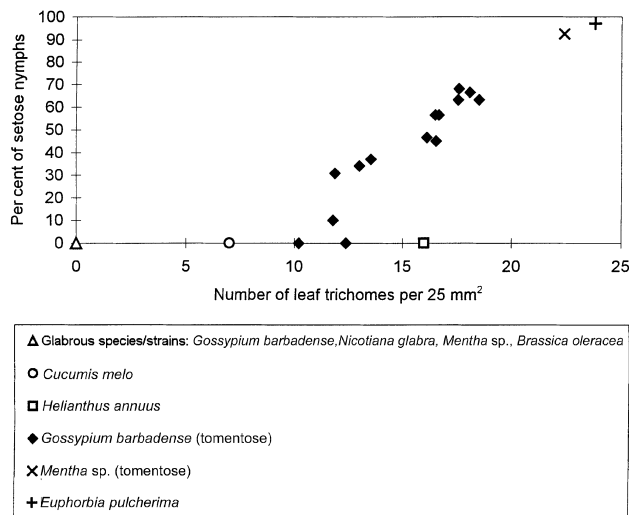
As stated by DROST et al. (1998), *B. tabaci* should be treated as a species complex in which basic terms such as ‘species’, ‘strain’ and ‘biotype’ are still under consideration. Following these authors’ classification, we refer to the insect under investigation as the ‘Old World biotype’ of *B. tabaci*.

## 2 Materials and methods

### 2.1 General conditions

#### 2.1.1 Plants

All plant species used during this work (see list in fig. 1) were grown in greenhouses and categorized according to the different degrees of trichome density on their leaves. Tomentosity was estimated by counting the number of



**Fig. 1.** Per cent of setose nymphs on leaves from diverse plant species and/or strains

trichomes present per 25 mm<sup>2</sup> in 10 random replicates per leaf. Cotton leaves differing in tomentosity came from plants belonging to two different strains: 'Acala SJ2', which is a mainly smooth-leaf variety, and from a hybrid (Michlo 195/86) variety characterized by highly tomentose leaves.

### 2.1.2 Whitefly

*B. tabaci* adults came from a greenhouse population reared on smooth leaf cotton (Acala SJ2). Two nymphal phenotype groups were classified: smooth, without any visible dorsal setae; and setose with four or more (usually six) dorsal setae visible to the naked eye. Rearing and experimental regimes were as follows: temperature of 26°C ( $\pm 1$ ), 50–60% relative humidity and a 14 : 10 light : dark photoperiod.

## 2.2 Quantitative assessment

### 2.2.1 Diverse plant species

Adults of *B. tabaci* were allowed to oviposit on plant species or strains categorized by differences in their degree of tomentosity. At 4th instar (approximately 12 days after oviposition), the number of nymphs on each leaf type was counted and the proportion of setose nymphs was plotted against the different plant species (sorted from low to high leaf trichome density).

### 2.2.2 Same plant species

In order to minimize the number of potentially different parameters among different plant species (additional to leaf tomentosity level), the same procedure was repeated for cotton plants, on which nymphs were left to develop on leaves differing in their degree of tomentosity. At 4th instar, measurements were taken as above.

## 2.3 Phenotype induction factor

In order to determine whether the physical presence of leaf trichomes acts as the trigger for the appearance of the setose phenotype upon the tomentose leaves, we produced an artificial layer of hairs on glabrous leaves. In this procedure, we attempted to simulate the tomentose surface by spreading

finely cut lint fibres on the surface of the leaves using a delicate brush (reaching a tomentosity level of *c.* 15 artificial hairs/25 mm<sup>2</sup>). Thus, we excluded possible differences in additional host plant characteristics (e.g. leaf physiological conditions), that could potentially influence nymph development. The simulated tomentosity leaves were then exposed to *B. tabaci* imagoes for 48 h, after which the adults were removed and the presence of eggs was verified. At 4th instar, we examined the nymph phenotype. The experiment was repeated comparing leaves from different or same plants and also with leaves of different or same 'ages' (as defined by its relative location on the stem).

Since no setose nymphs were expected on glabrous leaves, their presence, in any number, on glabrous leaves that had previously been treated by us, indicated a significant influence of the treatment, alleviating the need for additional statistical analysis.

## 2.4 Determination of the induction-sensitive stage

As noted, no plant influence on *B. tabaci* nymph phenotype was apparent following the settling of the 1st instar, as shown by previous experiments in which crawlers were transferred between leaf types (tomentose to glabrous and vice versa) immediately following egg hatch (GUERSHON and GERLING, 1994). However, those experiments involved the manipulation of eggs and crawlers and resulted in extremely high mortality of immatures. Consequently, we used the above-mentioned artificial trichome method to generate setose whitefly nymphs. We were thus able to expose each of the potentially sensitive stages of the whitefly to the presence or absence of the simulated hairs on the leaf surface.

The protocol for testing the possible sensitive stages was as follows:

### 2.4.1 Crawlers

Glabrous cotton leaves were offered to a large group of whitefly adults for 48 h. After this period, which is close to crawler eclosion, the adults were removed and half the leaves were treated with a layer of artificial hairs. The rest of the leaves remained untreated as controls. At whitefly 4th instar, nymphs were counted and the proportion of each phenotype was calculated.

### 2.4.2 Laying females and eggs

The influence of tomentosity upon the laying female (adult stage) and eggs was examined by exposing adults to 10 artificially tomentose leaves for 24 h. Adults were then removed from all plants and hairs were removed from five of them using a gentle air stream. In an additional group, eggs were left exposed to the artificial trichomes for another 24-h period, then removed before hatching. A parallel group of five leaves without simulated tomentosity was used as control.

## 2.5 Phenotype association with features of whitefly phenology

Following our preliminary results, which included determining adult size and duration of nymphal development (GUERSHON and GERLING, 1994), we measured two biological features: nymph size and adult longevity. Nymph size was assessed by measuring width and length of 100 4th instars for each phenotype. To calculate adult longevity, over 200 nymphs developing on tomentose leaves were isolated

according to phenotype. After eclosion, 100 adults from each group were left in rearing cages with cotton plants. The bottoms of the cages were covered with a black substrate to facilitate recognition of dead adults, which were collected and counted daily.

## 2.6 Statistical analysis

The relationship between tomentosity levels and setose nymphs on cotton strains was examined using Kendall's Rank-correlation test, excluding the value for the completely smooth cotton strain that has zero setose nymphs in order to avoid bias and maximize test reliability. Differences in nymphal size were examined using a *t*-test, and life span duration data were examined using the Mann-Whitney *U*-test (SOKAL and ROHLF, 1981).

## 3 Results

### 3.1 Quantitative assessment

As shown in fig. 1, the number of *B. tabaci* nymphs that developed setosity increased in direct proportion to leaf trichome density. However, in two plant species, *Cucumis melo* and *Helianthus annuus*, nymphal setosity was not induced. When these two plant species were excluded, a clear direct relationship was observed between proportions of the setose phenotype and tomentosity of the leaves.

A significant correlation was found between percentage of setose nymphs on the leaf and the tomentosity density of different tomentose cotton leaves (see data for tomentose cotton in fig. 1; Kendall's-Rank test coefficient = 0.625,  $P < 0.05$ ; as said, the analysis excluded glabrous leaves, scoring zero tomentosity and zero setose nymphs).

### 3.2 Induction factor

Figure 2 shows the results of the phenotypic display of nymphs developing on the simulated tomentosity leaves. As can be seen, spreading of artificial hairs on glabrous leaves sufficed to generate the appearance of setose nymphs in similar proportions to those found on the real tomentose leaf at an approximately equivalent trichome density. No significant differences were found between different leaves from the same plant or

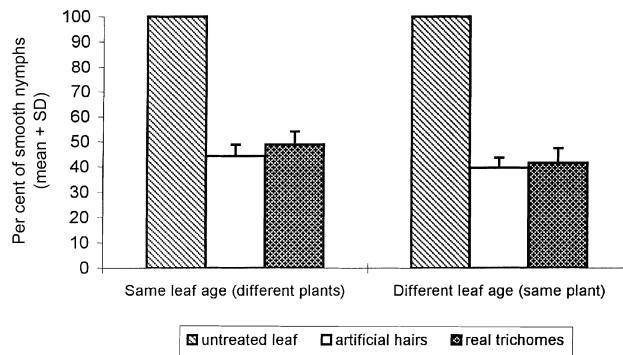


Fig. 2. Nymphal phenotypic response to simulated tomentosity.  $n = 5$  leaves per treatment

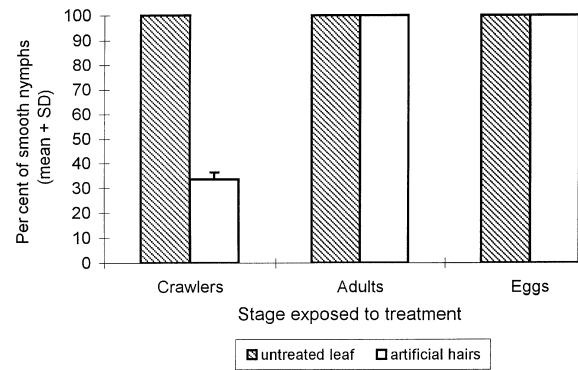


Fig. 3. Nymphal phenotypic response when crawlers, adults and eggs were exposed to simulated tomentosity.  $n = 5$  leaves per treatment

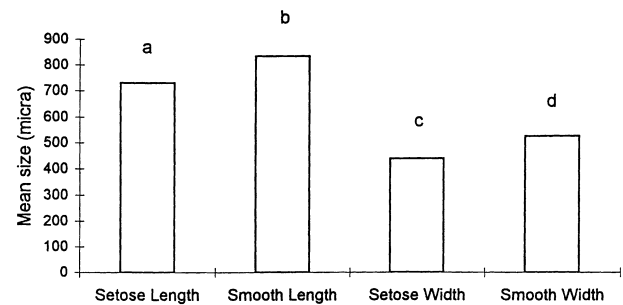


Fig. 4. Nymphal size (length and width) for the different nymph types. Different letters indicate differences at 0.05 level of significance

between leaves of the same age (position on stem) from different plants.

### 3.3 The sensitive stage

The exposure of crawlers to the artificial hairs on the leaf sufficed to induce the appearance of a certain percent of setose nymphs, whereas exposure of the ovipositing female or of the eggs had no effect on nymph phenotype (fig. 3).

### 3.4 Associations with further life-cycle attributes

The pupae of the setose phenotype were significantly narrower and shorter (*t*-test,  $P < 0.05$ , fig. 4) than the

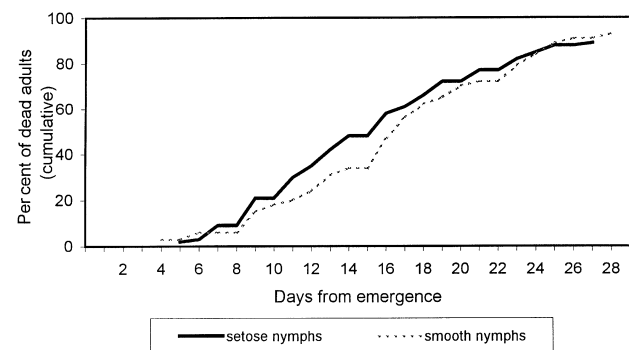


Fig. 5. Longevity of *B. tabaci* adults emerging from nymph with different phenotypes

smooth phenotype. Adults emerging from the former also had a shorter life span than those emerging from the latter (Mann–Whitney *U*-test,  $P < 0.05$ ; fig. 5).

#### 4 Discussion

Polymorphism and intraspecific variation have been reported for several whitefly species. In most cases, knowledge is limited to some descriptions of an observed relation between different morphs and a host plant character but little is known regarding the proximal causes and consequences of this phenomenon (e.g. BINK-MOENEN and MOUND, 1990; BEDFORD et al., 1994; MCAUSLANE et al., 1995). In the present study, we found that the difference in setosity of *B. tabaci* nymphs stems from a differential response to an environmental factor. Such a response may theoretically originate from chemical stimuli (usually introduced via feeding), or from physical stimuli such as sensitivity to microclimate or tactile stimulation. We have demonstrated here that for *B. tabaci*, the presence or absence of trichomes on the leaf surface was sufficient to generate the different phenotypes, and that, within the whitefly life cycle, the wandering crawler constitutes the sensitive stage to this environmental trigger. Our findings authenticate the hypotheses and circumstantial evidence previously presented by various authors and by ourselves (GUERSHON and GERLING, 1994; NEAL, 1996, 1997; NEAL and BENTZ, 1999), and corroborate that this response is environmentally induced and that the host plant leaf surface constitutes the main environmental stimulus generating the nymphal response. The influence of host trichomes is probably expressed through tactile stimulation of the crawlers wandering on the leaf. According to these results, other factors that provide similar mechanical stimuli for the crawler, such as extremely high egg/nymph density, may also be expected to induce the phenotypic response, as reported by NEAL (1997) and NEAL and BENTZ (1999). When comparing different plant species, the influence of plant trichomes was found in all species but two, *Cucumis melo* and *Helianthus annuus*. An absence of phenotypic response in these two cases might be related to the fact that their leaf trichomes differ from the rest of the tested species by being more erect and spiny, with a tendency to break during plant and leaf growth. In fact, by the time the nymphs were counted, the number of trichomes had frequently been reduced to a very low level.

It should be emphasized that our results indicate that the cited polymorphism should be treated as phenotypic plasticity not only in its classic definition as 'the capacity of a single genotype to produce a variety of phenotypes', but even according to a more demanding definition: 'a repeatable, directional change in the phenotype that is produced by a particular genotype in response to a directional change in a value of a specific environmental variable' (both citations from TRAVIS, 1994). However, further research concerning the genetic aspects of this phenomenon is needed.

Effects of the environment on a phenotype may represent an interaction between the genetic system and

the environment that has been selectively designed to produce different phenotypes under different conditions. Evidence for such adaptive plasticity can be found in the links between the phenotypic trait and certain features possessing ecological or fitness significance (TRAVIS, 1994). Employing fitness cost-benefit considerations, we may postulate that shorter development duration (GUERSHON and GERLING, 1994) and smaller size of immature setose nymphs (fig. 4) may be considered advantageous traits, since they reduce both spatial and temporal exposure to natural enemies. In possessing a sessile and continuously exposed nymph, whiteflies are particularly susceptible to predation and parasitism. Consequently, any abbreviation in their developmental duration can be considered beneficial by improving the chances for nymphal survival. The smaller size of nymphs may also be favourable as relevant natural enemies, such as parasitoids and coccinellid predators, scan the leaf area in a random pattern during their search for hosts or prey (VAN LENTEREN, 1991; VAN ROERMUND and VAN LENTEREN, 1995; GUERSHON and GERLING, 1999). Any reduction in size of the prospective host/prey will reduce its chances of being discovered, and is therefore a beneficial trait. On the other hand, the cost of being setose is probably expressed in reduced adult longevity (fig. 5) and body size (estimated by wing length measurements; GUERSHON and GERLING, 1994), which are commonly considered to play a role in determining female fecundity (PRICE, 1997). Reduced wing size may also affect the chances of migrating from one host plant to the next. This may play an important role in whitefly species like *B. tabaci* that need to migrate from one annual host plant to another (BYRNE and HOUCK, 1990).

In fact, additional comparative studies performed by us involving the analysis of predator–prey interactions, show an advantage for the setose phenotype when exposed to predation but only when phenotypic plasticity is manifested, i.e. when both phenotypes coexist on the same leaf (GUERSHON, 1997; M. GUERSHON and D. GERLING, unpublished data). Thus, we may hypothesize that the aforementioned costs and benefits are the ultimate causes and consequences of the observed phenotypic and physiologic plasticity. Clearly, such a hypothesis should be further examined through particulate studies on the phenology and dynamics of *B. tabaci* under field conditions.

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