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SHORT COMMUNICATION

Mulberry whitefly (*Pealius mori*) interference with silkworm (*Bombyx mori*) nymphal development

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Abstract

The mulberry whitefly (*Pealius mori* Takashashi) is increasingly causing concern in agriculture as the distribution of this pest expands. In Egypt, the recent invasion and population expansion of the mulberry whitefly has caused particular concern in the sericulture industry. The silkworm (*Bombyx mori* L.), also called mulberry silkworm or Chinese silkworm, is the most important source for natural silk production. A study was conducted to examine interference by the mulberry whitefly on the population development of the silkworm. White mulberry (*Morus alba* L.) leaves were obtained from trees maintained in the field which had different levels of natural infestation of whiteflies (*P. mori*), and these were fed to silkworms in the laboratory. Regardless of instar, all silkworm caterpillars that fed and developed on white mulberry leaves that were moderately whitefly-infested (350–450 nymphs per leaf) or heavily whitefly-infested (1650–1750 nymphs per leaf) failed to molt to the next instar or failed to pupate. By comparison, 88–95% of the caterpillars that were fed non-infested mulberry leaves molted and pupated. This study demonstrates that infestation of mulberry by *P. mori* can have a significant negative impact on the development of silkworms. These results have high economic implications for the sericulture industry.

Keywords Silkworm · Whitefly · Insect-interaction · Interference · Diet · Development · Sericulture · *Bombyx mori* · *Pealius mori*

Introduction

Whiteflies are widely distributed around the world. The mulberry whitefly (*Pealius mori* Takahashi) is among the important agricultural whitefly pests. Abd-Rabou and Evans (2013) reported on the first detection of *P. mori* in Egypt where they found this invasive whitefly feeding on *Euphorbia* sp. in Giza during February in 2013. Thereafter, populations of this pest have continued to increase and spread in Egypt. This invader has since been reported in Greece where it was found feeding on white mulberry, *Morus alba* L. (Wang et al. 2016). It has the potential to become a major pest of mulberry in the Mediterranean region. In addition to outbreaks in China where this whitefly was first reported (Takahashi 1932), *P. mori* experiences serious outbreaks on mulberry in India (David and Ragupathy 2004) and Thailand (Monchan et al. 2009). Known hosts of *P. mori* include members of the families Euphorbiaceae (*Glochidion philippicum* C.P. Robin), Moraceae (*Ficus* sp., *Morus alba* and *M. australis* Poiret), and Salicaceae (*Salix* sp.) (Evans 2007; Abd-Rabou and Evans 2013). Although another whitefly species (*Tetraleurodes mori* Quaintance) that occurs in the Americas shares the same common name and mulberry host with *P. mori*, the former has a much wider host range, is quite different taxonomically, and is not known in the Mediterranean region. These two species are among over 1500 described species of whiteflies in 161 genera around the world (Martin and Mound 2007).

The silkworm (*Bombyx mori* L.), also called mulberry silkworm or Chinese silkworm, is the most important arthropod species used in the global production of natural silk (Barber 1992). Mulberry leaves are essential in sericulture as food for the growth and development of *B. mori*. Nutrition is one of the basic requirements in sericulture, and insect quality, including

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Table 1 Survival of each larval instar of *Bombyx mori* when fed on different levels of whitefly (*Pealius mori*)-infested mulberry leaves

Variable	Experiment	Treatment	1st instar	2nd instar	3rd instar	4th instar	5th instar
Mean ± SEM % of larvae developing to next molt or pupation*	Moderate whitefly infestation	Infested	0	0	0	0	0
		Non-infested	90.4 ± 1.5	92.8 ± 2.2	90.0 ± 1.4	94.8 ± 1.4	95.2.6 ± 0.8
	Heavy whitefly infestation	Infested	0	0	0	0	0
		Non-infested	87.6 ± 2.9	87.6 ± 2.6	90.4 ± 2.1	93.2 ± 2.1	93.6 ± 1.7
Mean ± SEM % survival during 8 days (1st-4th instars) or 10 days (5th instar)*	Moderate whitefly infestation	Infested	64.4 ± 3.8	67.6 ± 3.4	58.0 ± 2.8	80.8 ± 2.1	80.8 ± 1.0
		Non-infested	90.4 ± 1.5	92.8 ± 2.2	90.0 ± 1.4	94.8 ± 1.4	95.2 ± 0.8
	Heavy whitefly infestation	Infested	9.6 ± 2.3	6.0 ± 1.9	7.2 ± 1.6	35.6 ± 3.0	46.0 ± 2.4
		Non-infested	87.6 ± 2.9	87.6 ± 2.6	90.4 ± 2.1	93.2 ± 2.1	93.6 ± 1.7

*All means within a column per instar per experiment are significantly different at $P < 0.001$ according to t-test

cocoon weight, is among the most important traits for sericulture economics (Hemmatabadi et al. 2016). It is well-known that diet is an essential factor that can affect animal growth and development. Namely, a good diet may result in good biological performance while an inferior diet may result in an inferior performance. The total protein level in 5th instar larvae of *B. mori* has been demonstrated to be reduced with a moderate decrease in caloric consumption (Li et al. 2009). When competing for the same resource, some arthropods may interfere with the populations of others (Grant and Grant 2006). The objective of this study was to determine any effect of mulberry whitefly-infected mulberry leaves on the development of *B. mori* caterpillars compared with whitefly-free mulberry leaves.

Materials and methods

The source of plant materials for this study was *M. alba* leaves (variety Canava 2) collected from the experimental farm of the Plant Protection Research Institute in El-Qanater El-Khariys, in Qalyubiya Governorate in Egypt. All silkworms in this experiment were from a laboratory colony from the abovementioned research farm. Leaves from the mulberry plants were arranged into three categories: leaves with no whitefly-infestation, slightly whitefly-infested leaves (about 350–450 *P. mori* nymphs per lower leaf surface), and heavily whitefly-infested leaves (about 1650–1750 *P. mori* nymphs per lower leaf surface). All categories of leaves were collected from multiple mulberry trees and were a result of natural whitefly infestation. Detached fresh leaves were held in a laboratory cage at 26 °C, 65–70% relative humidity, and a 16:8 h light:dark photoperiod. Treatments consisted of whitefly-infested leaves and the control consisted of non-infested leaves. The amount of leaves provided to *B. mori* varied based on the stage of the larval instar of the silkworm. Assays were set up consisting of 50 larvae of first, second, third, fourth and fifth instars that were provided 2, 3, 5, 16 and 45 kg of leaves, respectively. Paired trials of a control treatment and a treatment of heavily whitefly-infested leaves were set up. The leaves and silkworm larvae were confined in plastic cages (30 cm wide × 30 cm long × 20 cm high). There were five replicates for each of the five instars of *B. mori*. The experiment was repeated with paired trials of non-infested control leaves and moderately whitefly-infested leaves. The silkworm larvae on the leaves were observed daily for molting to the next instar or development to the pupal stage. First, second, third and fourth instar caterpillars were each observed for 8 days, while the fifth instar caterpillar was observed for

Table 2 Duration of each larval instar of *Bombyx mori* when fed on different levels of whitefly (*Pealius mori*)-infested mulberry leaves

Variable	Experiment	Treatment	1st instar	2nd instar	3rd instar	4th instar	5th instar
Mean ± SEM duration (days) to molt or pupation*	Moderate whitefly infestation	Infested	0	0	0	0	0
		Non-infested	4.5 ± 0	2.8 ± 0.12	4.8 ± 0.12	5.0 ± 0	8.9 ± 0.29
	Heavy whitefly infestation	Infested	0	0	0	0	0
		Non-infested	4.1 ± 0.19	2.8 ± 0.12	4.3 ± 0.12	4.7 ± 0.12	8.6 ± 0.33
Mean ± SEM duration (days) of feeding*	Moderate whitefly infestation	Infested	1.0 ± 0	1.0 ± 0	1.5 ± 0	2.2 ± 0.26	4.4 ± 0.18
		Non-infested	4.5 ± 0.19	2.8 ± 0.27	4.8 ± 0.12	5.0 ± 0.2	8.9 ± 0.29
	Heavy whitefly infestation	Infested	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
		Non-infested	4.1 ± 0.19	2.8 ± 0.12	4.3 ± 0.27	4.7 ± 0.27	8.6 ± 0.33

*Differences within a column between infested and non-infested are significantly different at $P < 0.001$ according to t-test

10 days. For each assay, fresh leaves were provided once to the silkworms. A total of 2500 larvae were set up in the experiment. The data were analyzed using procedures of SAS (SAS Institute 2012). Data for each whitefly-infested leaf treatment experiment were analyzed separately by instar and were subjected to the Student's t test procedure. Percentages were transformed using arcsine transformation before the analysis. Actual means and standard errors are presented in the text.

Results and discussion

No molting or pupation occurred for larvae of any instar of *B. mori* reared on either moderately or heavily infested mulberry leaves (Table 1). By contrast, the percentages of the larvae developing to the next instar or to pupation were high (88–95%) for those fed whitefly-free mulberry leaves (Table 1). Mortality was high for caterpillars fed whitefly-infested leaves (Table 1). For each instar, there was no replication effect on survival among larvae in the non-infested treatment. The duration of time to molt or pupation was affected by the treatment (Table 2). Excess food was available for all caterpillars in each assay. For caterpillars on the non-infested check leaves, no more than about 50% of the leaves were consumed. By contrast, no more than about 25% of the whitefly-infested leaves were consumed. One obvious reason for less consumption on the whitefly-infested leaves is due to caterpillar mortality. Also, although no behavior data were collected, larvae that were provided heavily whitefly-infested leaves appeared to be much less active in feeding, starting on the first day, as compared to larvae on the whitefly-free leaves. The effect of caloric restriction may be complex and not restricted to a single cause (Li et al. 2009). Moreover, the duration of the larval stage can be extended with a depletion of juvenile hormone esterase (Zhang et al. 2017). Results from the current study have direct implications on silkworm rearing and silk production. Notably, moderate or high levels of populations of whiteflies on mulberry leaves can lead to a direct loss of silkworms.

There is a high likelihood that *P. mori* will continue to spread to more countries through transportation or other modes of dispersal. Specimens of *P. mori* have been intercepted at the Republic of Korea port of entry, but it has not been reported to have established in that country (Shu and Ji 2014). Many insecticide options may be available to manage whiteflies on mulberry, but it is essential that the chemical or chemical residues do not affect the silkworm which needs quality mulberry leaves. In a recent laboratory and field study with 12 isolates of entomopathogenic fungi, researchers demonstrated that *Beauveria bassiana* (Balsamo-Crivelli) can be effective in controlling *P. mori* on mulberry without leaving any residue that could harm silkworms that would later feed on the leaves (Maketon et al. 2009).

This study was not designed to identify specifically what aspects of leaf quality affected by *P. mori* rendered mulberry leaves unsuitable for silkworms. There may be one or a combination of factors. Not only does the whitefly interact with the plant by removing sap from the pholem, but excretions from *P. mori* results in mold buildup on the leaves. Moreover, it is not known if the whiteflies that were consumed by the silkworms had negatively affected the performance of the silkworms. Worldwide, at least 24 species of whiteflies attack mulberry (Wang et al. 2016). These data document that among these species, at least *P. mori* can interfere with and out-compete silkworms on mulberry. We suspect that other whitefly species that feed on mulberry may also negatively impact the population of silkworms.

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