

## Survival and Movement of *Bemisia tabaci* (Homoptera: Aleyrodidae) Crawlers on Cotton

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**Abstract.** Greenhouse and field studies were conducted to estimate survival rates of crawler stage *Bemisia tabaci* (Gennadius) Biotype B (= *B. argentifolii* Perring & Bellows) on cotton (*Gossypium hirsutum* L.) plants to enable more accurate life tables to be constructed for this important insect pest. The survival of crawlers did not differ significantly between still-air and artificial wind treatments in the greenhouse and averaged 90%. Overall, 99.5% of the crawlers settled on the abaxial surface of leaves, and 99.9% settled on the leaf where eggs were oviposited. Out of 4,125 nymphs, only five settled on leaves other than the leaf of origin, and they all moved to leaves lower on the plant. Assuming that these individuals crawled rather than fell, the mean distance moved was at least 200 mm. Using a combination of cohort-based life table studies to measure egg mortality and recruitment studies to measure egg to settled first instar mortality in the field, crawler survival was estimated to average 89.2% over three study sites in Phoenix and Maricopa, AZ. Overall results suggest that crawlers move very little on cotton and that survival of the crawler stage is relatively high under both greenhouse and field conditions. Generational mortality rates estimated from previous life table studies in the field that excluded crawler mortality would increase by only 0.8% with the addition of this minor mortality component.

### Introduction

*Bemisia tabaci* (Gennadius) Biotype B (= *B. argentifolii* Bellows & Perring) is a major pest of field and horticultural crops throughout the world (Oliveira et al. 2001). This insect remains a key pest of cotton and vegetable crops in the southern U.S. where it can impact yield through direct feeding on plant sap and transmission of various viruses, and reduce the quality of harvested products through the excretion of honeydew. The B biotype of *B. tabaci* became problematic in Arizona and California in the early 1990's after which time considerable effort has been focused on gaining a better understanding of the biology and ecology of the pest as well as developing the basic tools needed to effectively manage it in agricultural systems (Ellsworth and Martinez-Carrillo 2001, Naranjo 2001, Palumbo et al. 2001). A highly successful management plan has been developed for *B. tabaci* in cotton in Arizona based on a foundation of avoidance tactics such as cultivar selection, irrigation management and biological control, along with the judicious use of effective and selective insecticides (Ellsworth and Jones 2001, Ellsworth and Martinez-Carrillo 2001, Naranjo et al. 2004b).

The success of this management plan can partly be attributed to a thorough and more basic understanding of the population and mortality dynamics of this pest under unmanaged conditions. Recent field life table studies in cotton have demonstrated that immature stages of *B. tabaci* are subject to significant natural mortality, with survival to the adult stage averaging just over 6% (Naranjo and Ellsworth 2005). Natural enemies, particularly arthropod predators, impose high levels of mortality to the egg and nymphal stages of this insect with predation during the fourth nymphal stadium representing the key factor. Mortality from predators and other sources such as dislodgement from the plant surface also contribute significant mortality to *B. tabaci* on a suite of other host crops and plants (Naranjo et al. 2004a). The in situ technique used to construct these life tables in cotton and other crops took advantage of the sessile nature of most of the immature stages by separately following cohorts of eggs and settled first-instar nymphs to estimate sources and rates of mortality. However, the method explicitly ignored the crawler stage, the mobile form of the first instar that is characteristic of Aleyrodidae.

The crawler has well developed legs and uses its mobility to select suitable feeding sites on the host leaf before the insect settles and becomes sessile for the remainder of its immature life (Lopez-Avila 1986). Existing research suggests that the mobility and duration of this specialized stage vary somewhat, mainly as a function of the host plant. Price and Taborsky (1992) reported that the net movement of *B. tabaci* crawlers on poinsettias was less than 2 mm from the egg and that most crawlers settled within 6 h following eclosion. Summers et al. (1996) found that *B. tabaci* crawlers were capable of moving at least 50 mm on broccoli and various weedy plants and may even move between plants if host conditions are unsuitable on the plant of origin. Simmons (1999) demonstrated that crawlers readily moved from the upper surface to the lower surface of various vegetable leaves, and that the net distance of movement may exceed 60 mm for some individuals on certain hosts (Simmons 2002). While some information is available on duration and extent of movement by *B. tabaci* crawlers, data on survival rates of this mobile stage are generally lacking, especially under field conditions. Life table studies by Horowitz et al. (1984) suggest that mortality between egg hatch and the second nymphal instar can be significant, and it is reasonable to hypothesize that this small and fragile crawler stage may be vulnerable to many mortality forces over the short duration of its existence. Measurement of mortality during this stage will help to refine existing estimates of stage-specific and generational mortality of *B. tabaci* populations.

The objectives of this study were to estimate crawler mortality and to further determine the extent of movement by this mobile stage on cotton plants. Controlled greenhouse studies were used to examine movement and to determine preliminary estimates of mortality, largely in the absence of natural forces. Subsequent field studies were then conducted to estimate rates of crawler mortality under variable field conditions.

## Materials and Methods

Cotton plants 'Deltapine 33B' were grown in individual pots (15.2-cm dia.) in an insect-free greenhouse until they reached the 6-8 true leaf stage. Plants were then carefully examined with an 8X hand lens, and any incidental whiteflies (eggs, nymphs or adults) and other insects (e.g. thrips, mites) were removed from all stems and leaf surfaces. Plants were then moved into an adjoining, insect-free greenhouse and placed inside an organandy mesh cage. This greenhouse was maintained at approximately  $28\pm 3^{\circ}\text{C}$ . A single clip-cage, fashioned from a vented, 3-dram plastic

vial and a hairpin, containing ten mating pairs of adult *B. tabaci* was attached to the second leaf down from the terminal so that adults could deposit eggs on the abaxial surface of the leaf. A non-toxic, ultra-fine point, black permanent marker (Sanford, Bellwood, IL) was used to mark the location of the clip-cage by placing six to eight dots around the perimeter of the cage. A small tag was placed around the petiole of the leaf containing the clip-cage so that its location could be determined at a later time. Once the clip-cage containing insects was in place, the plant was inspected for any escaped adults and then moved into a second organdy mesh cage to exclude colonization by whiteflies or other insects and mites. After 24 h, the clip-cage and all adult whiteflies were removed. The entire plant was inspected a final time for adult whiteflies and moved into a third organdy mesh cage (60 x 60 x 60 cm). This process was repeated over time for a total of 30 replicates. After approximately five days, a 15x lens was used to check daily for eclosion. Egg hatch was very synchronous, and all eggs hatched within a week of oviposition. Plants remained in the cages for two additional days to allow crawlers to settle.

To simulate the effects of a moderate breeze on crawler movement and mortality, this entire experiment was repeated (30 plants total) with the addition of an artificial wind source from an oscillating table-top fan. The air velocity was measured at approximately 2.2 m/sec and was sufficient to significantly agitate the leaves on plants within the cages.

Following exposure in the greenhouse, the plants were moved to the laboratory for further processing. First, the marked area where the eggs were deposited on each plant was inspected with a dissecting microscope and the number of eclosed eggs was recorded. Then, both the upper and lower surface of all leaves was examined under a microscope and the number of settled first-instar nymphs was recorded. The nodal position of each leaf also was noted. The petiole length of each leaf and the internode distances between leaves were measured. This information was used to determine the minimum distance that nymphs moving from the leaf of origin traveled. All greenhouse studies were conducted during the spring.

Three study sites of cotton 'Deltapine 449BR' were established at a single site in Phoenix, AZ, adjacent to the USDA-ARS, Western Cotton Research Laboratory and at two sites at the University of Arizona, Maricopa Agricultural Center, Maricopa, AZ, in 2005. The site in Phoenix was about 1.2 ha in size. The first and second sites in Maricopa were separate fields about 0.6 ha and 1.3 ha in size, respectively. No insecticides were applied to any of these fields during the study period and all studies were initiated between 11 and 28 July.

A recruitment approach was used to estimate crawler survival. At each site, a total of 50 leaves from the second mainstem node below the terminal were randomly collected from plants within the center two rows of each field. At the same time, an additional 50 leaves at the same nodal position were marked by tying a small tag around the leaf petiole so that these leaves could later be relocated. The collected leaves were taken to the laboratory and the total number of newly deposited eggs (<1-d old) was counted and recorded. These eggs were readily identifiable by their creamy-white color (Naranjo and Ellsworth 2005). After five days, a 15x lens was used to check plants daily for eclosion of egg from the life table cohorts described below. These cohorts were established on the same day as the initial collection of the second mainstem node leaves noted above. As in the greenhouse studies, egg hatch was very synchronous and all eggs eclosed after about 6 days. On day seven, the 50 tagged leaves were collected, and the total number of settled first instar nymphs was counted and recorded.

During each study period at each site, cohorts of 200 eggs were followed using the methods described in Naranjo and Ellsworth (2005) to estimate the rate of egg

mortality. Briefly, newly deposited eggs on leaves were identified in the field, and a small circle was drawn around these individual eggs with a non-toxic, ultra-fine-point, black permanent marker. A small numbered tag was placed around the petiole of the marked leaf, and flagging tape was tied around the mainstem of the plant to facilitate relocation. No more than five eggs were marked on a single leaf, and only one leaf was used per plant. Tagged leaves were collected after eight days and taken to the laboratory where the fate of each egg was determined with the aid of a dissecting microscope (see Naranjo and Ellsworth 2005). For purposes herein, only total mortality was of interest and not the causes of that mortality.

In the greenhouse study, the total number of eclosed eggs and settled nymphs on each plant were used to determine survival rates for the crawler stage. For nymphs that were found on leaves other than those on which the eggs were deposited the minimum distance moved was estimated by summing the attending internode and petiole length. This assumed that nymphs found on other leaves below the leaf of origin crawled rather than dropped from upper leaves. One-way ANOVA for a completely randomized design was used to test for differences between still conditions and artificial wind in crawler survival and distribution of eggs. Proportional survival and proportions of nymphs on the abaxial surface were transformed by arcsine (proportion)<sup>0.5</sup> before analysis. The potential correlation between egg density and nymphal survival was evaluated using Pearson's correlation coefficient. In the field studies, the total number of eggs (corrected for mortality from cohort studies) and the total number of settled nymphs on leaves from that same nodal position after one week were used to estimate survival rates for the crawler stage at each study site.

## Results and Discussion

The mean ( $\pm$ SE) number of eggs deposited within each clip-cage in the greenhouse studies was  $76 \pm 10$  over all plants and treatments. The application of artificial wind had no effect on crawler survival ( $F=1.44$ ,  $df=1$ ,  $58$ ,  $P=0.23$ ) or the distribution ( $F=1.43$ ,  $df=1$ ,  $58$ ,  $P=0.24$ ) of settled first-instar nymphs between the adaxial and abaxial surfaces of the leaf (Fig. 1). Mean ( $\pm$ SE) survival over both treatments was  $90.0 \pm 0.0\%$ , and  $99.5 \pm 0.40\%$  of all nymphs settled on the abaxial leaf surface. Survival on individual plants ranged from 50 to 100%. There was no correlation ( $P=0.15$ ) between initial egg density and crawler survival. Out of a total of 4,125 nymphs observed, only five (0.12%; three in wind and two in no-wind treatments) settled on leaves other than those on which the eggs were initially deposited. In all cases, these nymphs colonized the abaxial surface of leaves two nodal positions below the leaf on which eggs were deposited, and the average distance that these nymphs would have had to crawl was 199 mm. Although the movement of nymphs on the leaf of origin was not explicitly measured, they tended to spread randomly beyond the small area of oviposition on the abaxial surface.

The patterns of movement and colonization observed here are largely consistent with other findings. Summers et al. (1996) found that crawlers released on the stems of four host plants readily moved and settled on the abaxial leaf surfaces of these hosts, and Simmons (1999) reported that the majority of crawlers hatching from eggs on the adaxial surface of pepper, cantaloupe and cowpea eventually settled on the abaxial surface. When searching for a suitable host leaf, crawlers appear to behave phototactically, moving up the plant to colonize younger leaves (Summers 1997). However, once on the leaf, crawlers don't appear to respond to light or gravity when determining upon which side of the leaf to settle (Simmons 1999, Summers 2002). Instead, they appear to respond more to host plant cues that

will increase their chances of locating a suitable feeding site (Simmons 1999, Cohen et al. 1996). The average estimated distance that crawlers in this study moved was about 200 mm, and this is consistent with a maximum distance of 204 mm observed on sowthistle by Summers et al. (1996). The five nymphs out of 4,125 that moved from the leaf of origin in this study all moved to leaves two nodes lower on the mainstem. Because existing data would suggest that crawlers are more likely to move up the plant, it is probable that these rare individuals fell from leaves above and then moved to the abaxial surface before settling. This observation is further supported by the fact that cotton plants have an alternate leaf pattern that places every other leaf in the same vertical plane.

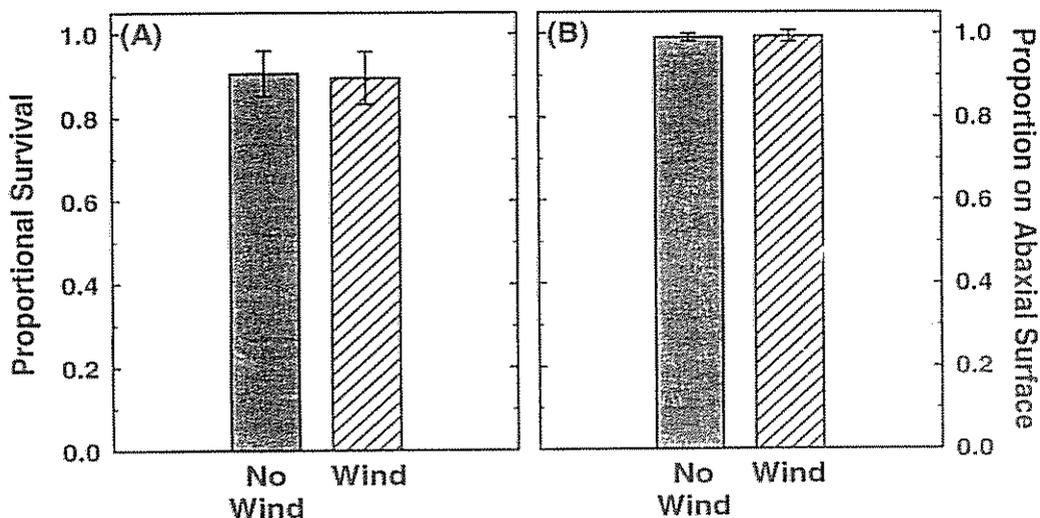


FIG. 1. Survival (A) and distribution (B) of settled first-instar nymphs of *Bemisia tabaci* on cotton in greenhouse studies with and without artificial wind, Phoenix, AZ. Error bars are 95% confidence intervals, n=30.

Survival of *B. tabaci* eggs from life table studies at sites in Phoenix and Maricopa, AZ, ranged from 59.0 to 90.7% and averaged 79.3% (Table 1). Based on these egg survival estimates and differences in densities of newly-laid eggs and settled first-instar nymphs from leaf samples at these same sites, crawler survival ranged from 76.9% to 96.2% and averaged 89.2% over all three study sites. Overall survival from oviposition to settled nymphs averaged approximately 70.7%. The close agreement between average greenhouse and field estimates of crawler survival suggests that natural forces in the field (e.g., natural enemies, dislodgement) do not appear to play a consistent role in crawler mortality. The highest mortality for egg (41%) and crawler (23.1%) stages was observed at the second Maricopa site that was initiated on 28 July. Six nights later and one day before leaves were collected to count settled first instars, there was a thunderstorm that deposited 8.3 mm of rain and sustained maximum wind speeds of 21.5 m/sec. Such storms have been shown to be associated with increased levels of dislodgement of both eggs and nymphal stages from the plant surface (Naranjo and Ellsworth 2005), and storm activity may have played a role in increased levels of

egg and crawler mortality in my study. Weather during the other two field observation periods was seasonably hot, but free of storms.

Table 1. Survival of Eggs and First-Instar Crawlers of *Bemisia tabaci* at Three Field Sites in Central Arizona, 2005.

Population Parameter	Phoenix Site	Maricopa Site 1	Maricopa Site 2
Number eggs/50 leaves	5,144	721	5,620
Number settled 1 <sup>st</sup> -instar nymphs/50 leaves	4,286	629	2,552
Egg survival <sup>a</sup>	0.883	0.907	0.590
Crawler survival <sup>b</sup>	0.944	0.962	0.769

<sup>a</sup>Estimated from egg cohorts established at the time of egg sampling (n = 200 per site)

<sup>b</sup>Estimated from the number of eggs and settled nymphs from sampled leaves corrected for egg mortality

As noted previously, relatively little is known about survival during the crawler stage. Summers et al. (1996) noted that less than 25% of the crawlers placed on stems below a leaf managed to reach the leaf and settle; even fewer (7-12%) successfully moved between host plants even when adjacent leaves were allowed to touch, creating a bridge. From field-based life table studies in Israeli cotton, Horowitz et al. (1984) reported high levels of mortality during the first-nymphal stadium of *B. tabaci* and further determined that mortality during this stage was a key factor. It is not clear from their methodology how this mortality was apportioned between crawlers and settled first-instar nymphs. Naranjo and Ellsworth (2005) found that mortality during the settled first-instar stadium averaged less than 20% over multiple generations.

The low average rates of mortality in the crawler stage observed in this study is consistent with the short duration of the stage and the relatively small distances that most crawlers move before settling on the plant surface. Both of these factors provide little time for natural forces of mortality such as predators and weather to act. Adding an average field estimate of 10.8% mortality to the known stage-specific rates of mortality for the remaining immature stages of *B. tabaci* (Naranjo and Ellsworth 2005) would increase average generational mortality by only 0.8%. Placed within the context of overall generation mortality, this insignificant amount of crawler mortality is unlikely to be important in affecting the dynamics of *B. tabaci* populations in the field or the interpretation of key factors associated with variations in population size. Nonetheless, the field methods outlined here would be a relatively simple addition to future life table studies.

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