Video Article

Methodology for Developing Life Tables for Sessile Insects in the Field Using the Whitefly, *Bemisia tabaci*, in Cotton As a Model System

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URL: https://www.jove.com/video/56150
DOI: doi:10.3791/56150

Keywords: Environmental Sciences, Issue 129, Life table, population dynamics, marginal mortality, irreplaceable mortality, direct observation, predation, parasitism, dislodgement, key-factor

Date Published: 11/1/2017

Citation: Naranjo, S.E., Ellsworth, P.C. Methodology for Developing Life Tables for Sessile Insects in the Field Using the Whitefly, *Bemisia tabaci*, in Cotton As a Model System. *J. Vis. Exp.* (129), e56150, doi:10.3791/56150 (2017).

Abstract

Life tables provide a means of measuring the schedules of birth and death from populations over time. They also can be used to quantify the sources and rates of mortality in populations, which has a variety of applications in ecology, including agricultural ecosystems. Horizontal, or cohort-based, life tables provide for the most direct and accurate method of quantifying vital population rates because they follow a group of individuals in a population from birth to death. Here, protocols are presented for conducting and analyzing cohort-based life tables in the field that takes advantage of the sessile nature of the immature life stages of a global insect pest, *Bemisia tabaci*. Individual insects are located on the underside of cotton leaves and are marked by drawing a small circle around the insect with a non-toxic pen. This insect can then be observed repeatedly over time with the aid of hand lenses to measure development from one stage to the next and to identify stage-specific causes of death associated with natural and introduced mortality forces. Analyses explain how to correctly measure multiple mortality forces that act contemporaneously within each stage and how to use such data to provide meaningful population dynamic metrics. The method does not directly account for adult survival and reproduction, which limits inference to dynamics of immature stages. An example is presented that focused on measuring the impact of bottom-up (plant quality) and top-down (natural enemies) effects on the mortality dynamics of *B. tabaci* in the cotton system.

Video Link

The video component of this article can be found at https://www.jove.com/video/56150/

Introduction

Life tables are a common tool with a long history in ecology[1,2]. Life tables are essentially a schedule of the births and deaths in a population over time and such data can be used to quantify a number of parameters important to understanding and predicting population dynamics. Life tables may also provide information on causes of death that are important to understanding trophic interactions and in developing control strategies for managing pests in agricultural and natural systems. Numerous field-based life tables have been constructed for insects[3,4,5], and analyses have provided important insights into the dynamics, regulation and prediction of insect populations in many managed and natural systems[6,7,8,9,10,11,12,13,14]. The term life table is also often used to describe laboratory based studies that largely examine schedules of births and deaths but under artificial conditions that do not expose the insect to natural mortality forces and realistic environmental variables. Generally, the goal of laboratory studies is to estimate the comparative biotic potential of a species. The focus of the methods described here is for field based investigations that define realized potential relative to the environment.

Life tables can be characterized as horizontal, in which a real cohort of equal aged individuals are followed from the beginning of their lives until death, or vertical, where frequent samples are taken through time of a population with an assumed stable age structure and then vital rates are inferred from mathematically constructed cohorts[2,15]. The type of life table that can be deployed depends on the nature of the insect. Horizontal life tables can often be developed for univoltine (one generation per year) insects, while such an approach can be very challenging for a multivoltine insect with multiple and widely overlapping generations each year. A host of analytical methods have been proposed and used to develop vertical life tables for insect populations (see Southwood[2] for examples). The methodology demonstrated here allows for the development of cohort-based, horizontal life tables in the field for multivoltine insects with specific life history characteristics, notably, the presence of sessile life stages. The method is demonstrated for a key pest in cotton as a model system.

The whitefly, *Bemisia tabaci* biotype B (= *Bemisia argentifolii*, Middle East-Asia Minor 1[16]) is a global pest of agriculture that negatively impacts yield and quality in many agronomic and horticultural crops, including protected agricultural systems in temperate regions[17]. Impacts occur due to phloem feeding that disrupts nutrient flow, disorders of unknown etiology caused by nymphal feeding, transmission of numerous plant viruses and crop quality effects due to the deposition of honeydew[18,19]. The insect has a broad host range and is multivoltine, having as many as 12-13 generations per year depending on region and available food resources[20]. Management challenges also are exacerbated by its high reproductive

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potential, its ability to disperse and migrate within and between agricultural systems, its lack of a quiescent stage (diapause or estivation) and its disposition to rapidly develop resistance to insecticides used for suppression[21,22].

Considerable progress has been made in developing integrated pest management (IPM) strategies to effectively and economically manage populations of this pest in affected crops[23,24,25]. These management systems were predicated on a sound fundamental understanding of the population dynamics of *B. tabaci* and life tables have been a key technique that have enabled this understanding. In Arizona, life tables have allowed the estimation and identification of important mortality forces for *B. tabaci* in multiple crop systems[13,26], have enabled the measurement of mortality dynamics relative to management strategies including non-target effects of insecticides[14], have provided a means of estimating potential functional non-target effects of transgenic cotton producing insecticidal proteins[27], have supported rigorous assessment of a classical biological control program[28] (Naranjo, unpublished data) and helped to explore the comparative effects of top-down and bottom-up effects on pest dynamics[29]. All of these applications have deployed the methodology described here. The approach could be useful for the study of insect population ecology in a number of natural and managed systems.

Protocol

NOTE: The techniques described below are considered partial life tables because they do not explicitly include reproduction or mortality of the adult stages. The term cohort is equivalent to generation because it examines mortality from the egg to the adult stage.

1. Establish Field Sites

1. Conduct life tables at any time during the growth of the crop once insects are present. The choice of when to initiate studies will depend on the goals and objectives of the research.
2. Select two rows of crop near the center of the plot to minimize edge effects from surrounding plots or uncultivated areas. Mark the head of each row with a wire flag or a wooden stake to facilitate easy relocation once the crop gets large.
3. Move 3-4 m in from edge of plot parallel to the row. Use one row to establish egg cohorts and the second to establish nymph cohorts

2. Establish Egg Cohorts

1. **Use an 8X hand lens to search for newly laid eggs on the underside of leaves near the top of the main stem of the cotton plant (generally the second or third node from the top).**
   NOTE: Life stages of *B. tabaci* are generally distributed vertically in the plant canopy with eggs near the top of the plant and progressively older nymphal stages below. This results because 1st instar nymphs settle on the same leaf as the egg and the plant adds leaves above the initial oviposition site as it grows.
   1. Use a higher power 15X lens to observe fresh eggs and verify identification before using the 8x lens to mark the insect. Fresh eggs have a bright white coloration under the lens and stand out from eggs that are older (**Figures 1A** and **1B**). Eggs darken to a tannish color as they mature.

2. **Use a non-toxic, ultra-fine-point black permanent marker to draw a small circle around the egg. Draw the circle small enough to minimize the chance of a female laying another egg within the circle later on.**
   1. Cut a hole or slot in the side of the 8X hand lens with a hacksaw or drill bit so that the pen can be inserted and viewed through the lens when drawing (**Figure 2A**).

3. Repeat this process on the same leaf, if possible, to mark other eggs, marking a total of no more than four eggs on a single leaf and no more than one egg per leaf sector. For cotton, the leaf is subdivided into four sectors by three major leaf veins (**Figure 3**).
4. Tie a small lightweight cardboard tag around the petiole of the leaf containing marked egg(s). Number the tag and include notation for the plot or treatment number depending on the experimental design (**Figure 2B**).
5. Tie a 1 m long length of flagging tape around the main stem near the top of the plant. Use a bow-style tie so that the tape can be easily relocated as necessary to keep it visible for repeated visits to this location in the field.
6. Record leaf number and positional information on a portable analog or electronic notebook (**Table 1**). Positional information uses the sectors of the leaf to more finely note location (*e.g.*, 1-1, 1-2, 1-4 denote eggs in sectors 1, 2 and 4 on leaf #1).
7. **Establish each cohort on a single day.**
   NOTE: A cohort in a given plot is comprised of a minimum of 50 eggs total.
   1. Use no more than one leaf per plant to better distribute the members of the cohort.
      NOTE: Depending on insect density, this may comprise anywhere from 13 leaves with 3-4 eggs each or 50 leaves with one egg each. The individual plants are selected to distribute the marked leaves along as much of the row as possible.

3. Establish Nymph Cohorts

1. Use an 8X hand lens to search for newly settled 1st instar nymphs on the underside of leaves about 3-5 leaves down from the top of the main stem of the cotton plant. Use a 15X lens to verify identification before marking using the 8X lens.
2. **Use a non-toxic, ultra-fine-point black permanent marker to draw a small circle around the nymph. Draw the circle small enough to minimize the chance of a crawler settling within the circle later on.**
   NOTE: Newly hatched 1st instar nymphs are called crawlers, which can move several cm during the first few hours after egg hatch. It then "settles" onto a site where it will feed and molt without ever moving again until the adult emerges. These settled 1st instar nymphs (**Figure 1C**) are distinctive from the crawlers. First, they are immobile and second, they are more 2-dimensional and lay tight and flat on the leaf and have a slightly more translucent amber color.

1. Cut a hole or slot in the side of the 8X hand lens with a hacksaw or drill bit so that the pen can be inserted and viewed through the lens when drawing (**Figure 2A**).

3. Repeat this process on the same leaf, if possible, to mark other nymphs, marking no more than four nymphs on a single leaf and no more than one nymph per leaf sector. For cotton, the leaf is subdivided into four sectors by three major leaf veins (**Figure 3**).
4. Tie a small lightweight cardboard tag around the petiole of the leaf containing marked nymph. Number the tag and include notation for the plot or treatment number depending on the experimental design (**Figure 2B**).
5. Tie a 1 m long length of flagging tape around the main stem near the top of the plant. Use a bow-style tie so that the tape can be easily relocated as necessary to keep it visible for repeated visits to this location in the field.
6. Record leaf number and positional information on a portable analog or electronic notebook (**Table 1**). Positional information uses the sectors of the leaf to more finely note location (*e.g.*, 1-1, 1-2, 1-4 denote nymphs in sectors 1, 2 and 4 on leaf #1).
7. To ensure that settled 1st instar nymphs and not crawlers are marked, go back to each marked leaf and observe the marked insect about 1-2 h after the initial set-up. Remarking of settled nymphs may be necessary.
8. Establish each cohort on a single day.
   NOTE: A cohort in a given plot is comprised of a minimum of 50 nymphs total. No more than one leaf is used per plant to better distribute the members of the cohort. Depending on insect density, this may comprise anywhere from 13 leaves with 3-4 nymphs each or 50 leaves with one nymph each. The individual plants are selected to distribute the marked leaves along as much of the row as possible. Because of the 1st instar crawler stage, the eggs marked in protocol 2 are not the same insects that are then followed as nymphs. Thus, no crawler mortality is measured and the life table is slightly disjointed in time because egg and nymph cohorts are typically established on the same day. Research has shown the crawler mortality is negligible and can be essentially ignored[30].

4. Observation and Recording of Egg Hatch and Mortality

1. After 8-10 d (28-32 °C; average Arizona summer conditions) after establishment of egg cohorts, collect leaves containing marked eggs and return to the laboratory for observation under a dissecting microscope. Eggs are too small to clearly evaluate mortality and causes of mortality in the field.
2. Determine causes of death for eggs and record in the notebook initiated at cohort establishment (**Table 1**).
   NOTE: Death is characterized as dislodgement, predation or inviability. Dislodgement: the egg is missing due to weather events (wind, blowing dust, rain) or chewing predation. Predation: sucking predators leave behind a collapsed chorion (**Figure 4K**). Hatched eggs can appear collapsed, but there will be a vertical slit in the egg chorion. Use a minuten pin to tease the chorion on the leaf under the microscope to look for this slit. Inviability: eggs fail to hatch after the 8-10 d period and are a dark tan color. Under Arizona summer conditions (28-32 °C) eggs would normally hatch in 5-7 days. This may differ in other regions and adjustments may be necessary in collection time from the field.

5. Observation and Recording of Nymphal Development and Mortality

1. **One to two days after cohort establishment, use a 15X lens to assess the development of nymphs and to assign a cause of mortality if dead. Make observations at least three times per week (every other day).**
   1. Use relative size (**Figure 1C-G**) and time after establishment to assess instar.
      NOTE: There are four nymphal instars and development is rapid under Arizona summer conditions (28-32 °C) with each of the first three stages lasting around 2 d and the final stage lasting 3-5 d (total nymphal development 2 wk or less). New observers should learn instar sizes by observing insects reared in the laboratory or greenhouse on the host plant of interest. The relative volume in the abdomen of the bacteriosomes (symbiont harboring organs of the whitefly) relative to overall body size is a helpful indicator of nymphal instar (**Figure 1C-G**). Newly molted nymphs are very flat and translucent. Nymphs ready to molt are more turgid, domed in profile, and opaque in appearance.
   2. Determine causes of death for nymphs and record in the notebook initiated at cohort establishment (**Table 1**).
      NOTE: Death is characterized as dislodgement, parasitism, predation or unknown depending on instar (**Figure 4**). Dislodgement: nymphs of any stage are missing due to weather events (wind, blowing dust, rain) or chewing predation. Estimate the stage of dislodged nymphs as the average stage of dead and live nymphs on a given observation date. Parasitism: only observable in 4th instar nymphs. The paired yellowish bacterisomes are displaced by the developing parasitoid larva (**Figure 4A**); the larval stage is sometimes visible (**Figure 4C**). The pupal stage of the parasitoid is distinctive and genera specific (**Figure 4B, 4D**). Predation: sucking predators will evacuate the contents of the nymph and leave behind a collapsed cadaver (**Figure 4G-4I**). Rarely, a chewing predator may leave evidence (**Figure 4J**). Unknown: death that cannot be attributed to one of the above causes. In humid environments, fungal disease may be an additional cause of death. This category might also include nymphs killed by parasitoid host-feeding. Nymphs that survive emerge as adults leaving a distinctive t-shaped slot in the exuviae (**Figure 1H**)

2. Record development stage (if alive) and cause of death and stage in the notebook initiated at cohort establishment (**Table 1**).
3. Once all the nymphs being observed on a single leaf have either died or emerged as an adult whitefly, collect the leaf and return to the laboratory. Use the higher magnification of a dissecting microscope to confirm that the cause of death noted in the field is accurate and make any corrections.
   NOTE: Not every non-dislodged dead insect will remain on the leaf over the typical two-week observation period and so some verifications will not be possible.

6. Data Summary and Analyses

1. **Consult resources available to help in constructing life tables from the data collected[2,8,11,31]. An example life table is presented as Table 2.**
   NOTE: Robust life table analyses require multiple independent life tables conducted over time and/or different sites. For a multivoltine insect like *B. tabaci* this could be multiple life tables over the course of a single season and/or multiple seasons and sites.

1. Estimate real mortality (d_x/l_0) based on the number of insects established at the beginning of the generation.
   Real mortality=(d_x/l_0)
   Where d_x is the number dying during stage x and l_0 is the number of insects at the beginning of the generation. These mortality rates are additive and the sum of d_x over stages estimates the total mortality rate for the generation (**Table 2**).
2. Estimate apparent mortality within a stage (q_x) based on the number of insect alive at the beginning of a specific stage (Table 2).
   Estimate stage-specific q_x or factor within stage-specific q_x. These rates are additive only within a stage.
3. Determine marginal mortality using the formula:
   $M_B = d_B/(1-d_A)$
   Where M_B is the marginal rate of mortality factor B, d_B is the apparent rate of mortality from factor B and d_A is the apparent rate of mortality summed for all mortality factors that can outcompete factor B[13,32] (**Table 3**).
   NOTE: For sessile insects like whiteflies and many other insects, the multiple causes of death within a particular life stage are not sequential. Instead they act contemporaneously and so estimation of marginal rates is required to accurately estimate stage-specific rates of mortality from any one cause[32,34]. For example, a parasitoid may attack a whitefly nymph. The parasitoid egg might hatch and the larvae may develop in the host. This activity, initially asymptomatic to the observer, does, or would most likely, kill the host insect and should be credited as the cause of death. But in some cases, a predator may attack this same nymph or the nymph may be dislodged from the leaf leading the observer to note the cause of death as predation or dislodgement. Marginal mortality corrects for this.
   1. Convert marginal stage-specific rates to k-values[35] as $k = -ln(1-M)$, where ln is the natural logarithm and M is the marginal mortality rate of interest. k-values are additive and this simplifies further analyses. k-values can be back-converted to proportional mortality rates by $1-e^{[-k]}$.
4. Estimate irreplaceable mortality as $[1-e^{(-TotalK)}] - [1-e^{(-\{TotalK - kvalue_i\})}]$.
   NOTE: This gives the portion of total generational mortality that would not be realized if a particular mortality factor was removed. For example, how much generational mortality might be lost if predation or parasitism were removed because of an insecticide spray? Irreplaceable mortality estimated in this way assumes that there is no density-dependence in mortality.
5. Key factors
   1. Use a simple graphical analysis to plot the k-value for any one stage, or any one mortality factor (or one mortality factor within one stage) against the Total-K value for the entire generation (Total K = sum of all individual k-values).
      NOTE: The mortality factor that most closely mimics the pattern of Total-K the best is the key factor, the factor that contribute the most to changes in generational mortality[35]. A more quantitative method regresses individual k-values on Total-K and identifies the key factor as the one with the greatest slope value[3].
6. Test density dependence by regressing k-values for factors of interest on the natural log of insect population density measured independently (*e.g.*[13]). A significant positive slope suggests direct density dependence and a negative slope inverse dependence.
   NOTE: With additional life table information on adult survival and reproduction, many additional parameters (*e.g.*, generation time, net reproductive rate, instantaneous rates of increase, life expectancy at a given stage, *etc.*) and analyses (matrix models and elasticity analyses[36,37] can be conducted.

Representative Results

An example cohort is presented in **Table 2** to show a typical presentation and calculation of life table results. The most useful data is captured in the marginal mortality rates for each factor within each stage. By converting these rates to k-values (protocol section 6), stage-specific mortality over all factors and factor-specific mortality over all stages can be easily estimated, as can total generational mortality. This also facilitates irreplaceable mortality, key-factor and density-dependence analyses.

Representative results are presented from the study of Asiimwe *et al.*[29]. This study used life tables to measure the comparative consequences of bottom-up (plant quality) and top-down (natural enemy impacts) effects on mortality of *B. tabaci* populations in the cotton system. A three-year replicated (n = 4) split-plot study used three levels of irrigation frequency (20, 40 and 60% soil water depletion representing wet, normal and dry conditions) as main plots and two levels of natural enemy manipulation (undisrupted and disrupted by the repeated application of broad-spectrum insecticides) as split plots. The insecticides used were selected because they have no effect on the pest insect but broad negative effects on the natural enemy community in cotton. Life tables were conducted on three generations in each experimental plot each year for a total of 36 over the three-year study. Neither marginal or irreplaceable mortalities for any factor differed significantly from year to year and so results were pooled over the three years. In addition, there were no differences in mortality rates and patterns due to plant quality manipulations and so as an example of the output, the results of only the top-down manipulations pooled over all plant quality treatments are shown in **Figure 5**. Marginal rates of predation by sucking predators declined significantly when insecticides were applied indicating the impact of top down control in this system. Rates of parasitism declined numerically but the change was not statistically significant. Rates of egg inviability increased slightly with insecticide applications.

Patterns of irreplaceable mortality were similar to those for marginal rates (**Figure 6**). When natural enemies were not disrupted by insecticides, predation supplied the highest level of irreplaceable mortality and this level declined significantly with disruption. Parasitism supplied low levels of irreplaceable mortality and again there was a numerical decline due to insecticides but the change was not statistically significant. Dislodgement and egg inviability increased in response to insecticide disruption.

An example of key factor analyses using the graphical method of Varley and Gradwell (1960)[35] is shown in **Figure 7**. Here the results from the four replicate plots were combined into a total of 9 cohorts over the three-year study. Comparison of the individual k-values for various mortality factors summed over all life stages to total mortality for the generation indicated that predation most closely matched the pattern for total mortality, followed by dislodgement and parasitism. The key factor regression method[3] quantitatively confirmed these visual observations with the highest slope value associated with predation. Thus, predation was most closely associated with changes in generational mortality.

Finally, an example of density dependence analysis is shown in **Figure 8** for the two main sources of mortality associated with natural enemies. Again, the four replicate plots were combined into a total of 9 generations over the three years. Both relationships showed a pattern of direct density dependence, indicating that rates of mortality increased with increasing density, but the relationship was only statistically significant for parasitoid-induced mortality.

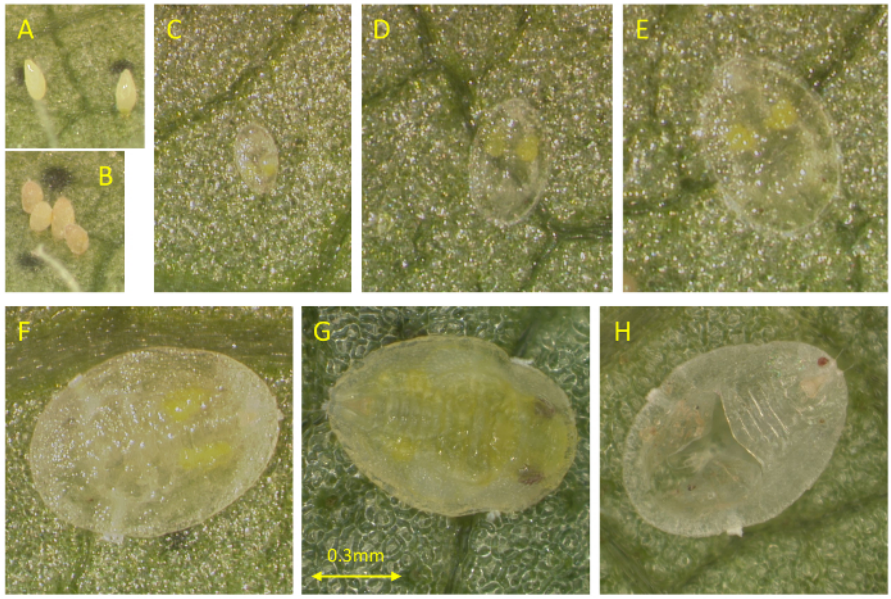

Figure 1: Examples of live immature *B. tabaci* life stages. (**A**) Newly laid eggs. (**B**) Older eggs are more amber colored. (**C**) 1st instar nymph. (**D**) 2nd instar nymph. (**E**) 3rd instar nymph. (**F**) 4th instar nymph. (**G**) Late 4th instar nymphs sometimes referred to as "pupa" or "red-eyed nymph". (**H**) Exuviae after the emergence of the adult. Please click here to view a larger version of this figure.

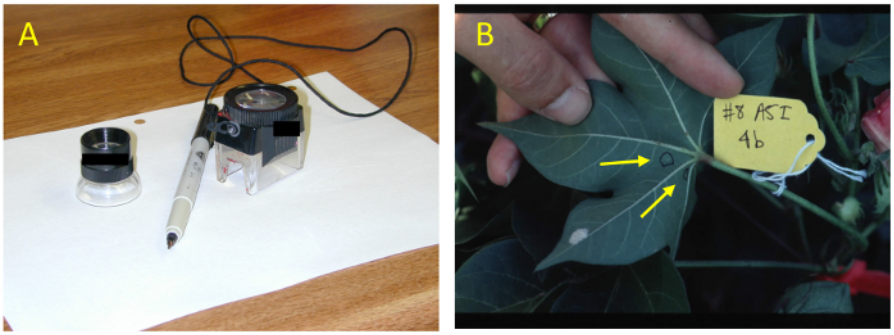

Figure 2: Examples of the tools used in setting up life table cohorts. (**A**) The larger 8X lens is used to locate and mark newly hatched eggs or newly settled 1st instar nymphs. Note the slot in the side of the 8x lens that allow the pen to be inserted so that the observer can draw the small circle around insect while simultaneously viewing through the lens. (**B**) An example of a tagged leaf showing marked nymphs (indicated by arrows). Please click here to view a larger version of this figure.

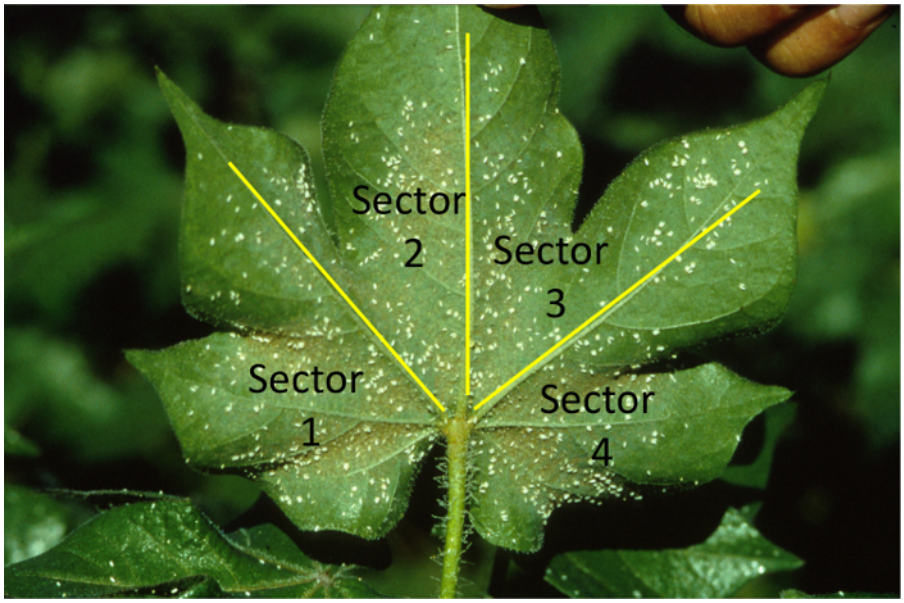

Figure 3: Underside of cotton leaf. Three major leaf veins were used to delineate four sectors on the leaf to facilitate re-locating insects during repeated observation intervals to assess development and mortality of egg and nymph cohorts. Please click here to view a larger version of this figure.

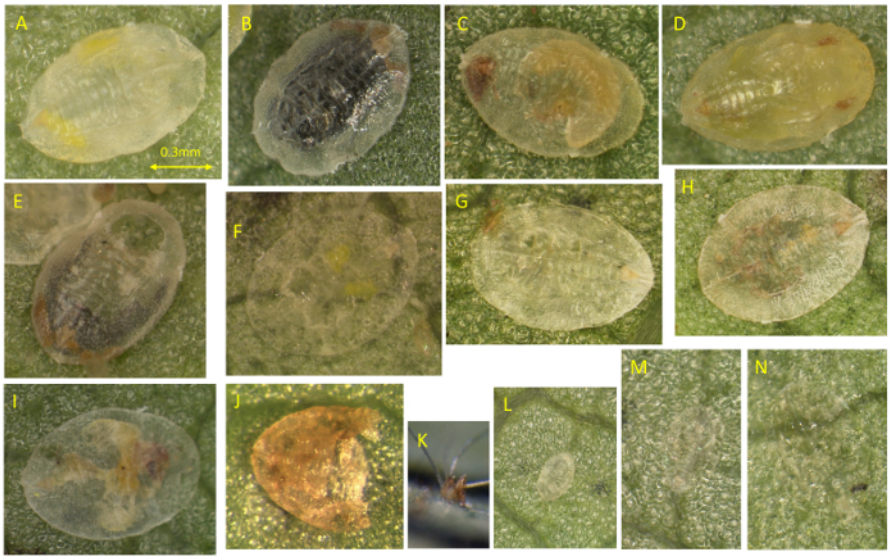

Figure 4: Examples of immature *B. tabaci* killed by various causes. (**A**) Evidence of parasitism in fourth instar nymphs. Note how the paired yellowish bacteriosomes have been displaced to the perimeter indicating the presence of a developing parasitoid larva. (**B**) Pupal stage of *Encarsia sophia*, an introduced parasitoid of *B. tabaci,* in a 4th instar cadaver. Note the brown-colored meconia (fecal pellets) at the periphery of the cadaver that are characteristic for this parasitoid genus. (**C**) Larva of *En. sophia* developing inside a 4th instar whitefly nymph. (**D**) Pupal stage of introduced *Eretmocerus* sp. (Ethiopia) in a 4th instar cadaver. (**E**) Emergence hole from *En. sophia* in 4th instar whitefly cadaver. This would never be seen in a cohort given that parasitoid development is longer than whitefly development and the cohort would be concluded before parasitoid emergence. (**F**) Parasitoid host-feeding in a 4th instar nymph in which host organs are still largely intact but the cadaver is slightly collapsed and sometimes discolored. Note the retention of the bacteriosomes and the faint eye spots in the cadaver. (**G-I**) Fourth instar nymphs preyed upon by sucking predators. The partially or completely evacuated cadaver remains on the leaf. In G, the entry wounds from a predatory green lacewing larva are visible. (**J**) Rare example of a 4th instar nymph partially consumed by a chewing predator. Most often the entire nymph is removed from the leaf. (**I**) Eggs that have been preyed on by a sucking predator (adjacent to leaf trichomes). (**L-N)** Predation on 1st, 2nd and 3rd instar nymphs, respectively. Please click here to view a larger version of this figure.

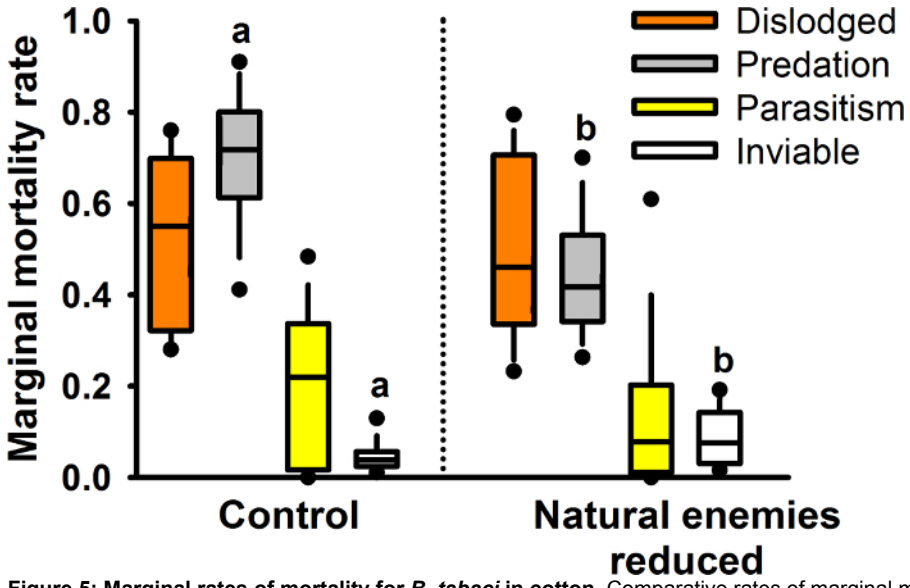

Figure 5: Marginal rates of mortality for *B. tabaci* in cotton. Comparative rates of marginal mortality from multiple factors when top-down control by natural enemies is disrupted by the application of broad-spectrum insecticides[29]. For plots (n = 36; 9 cohorts replicated 4 times), the line within the box is the median, the box denotes the 25th and 75th percentiles, whiskers denote the 10th and 90th percentiles and points depict the 5th and 95th percentiles. Marginal rates of predation by sucking predators declined significantly when insecticides were applied. Rates of parasitism declined numerically but the change was not statistically significant. Rates of egg inviability increased slightly with insecticide applications. Modified from Asiimwe *et al.* 2016[29] Please click here to view a larger version of this figure.

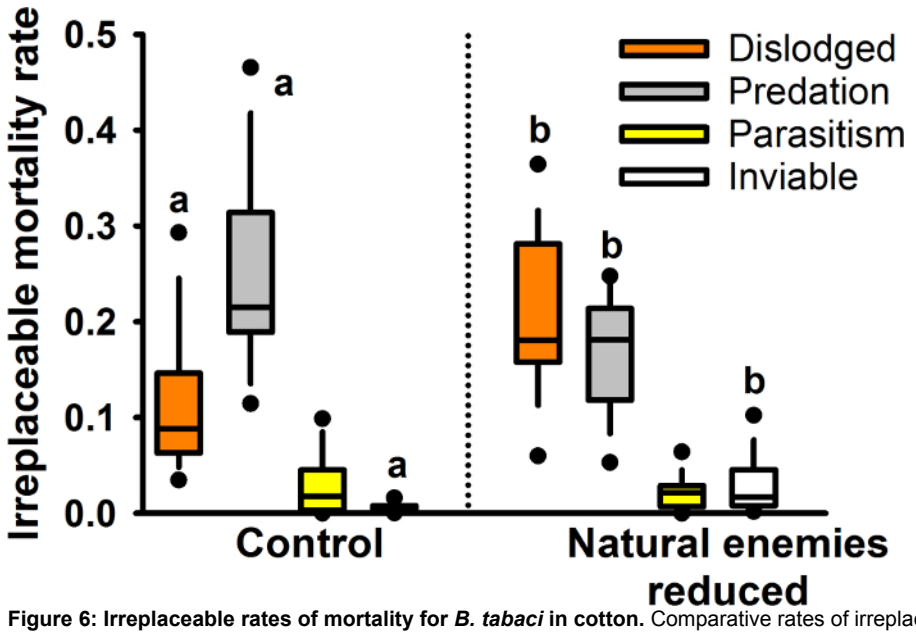

Figure 6: Irreplaceable rates of mortality for *B. tabaci* in cotton. Comparative rates of irreplaceable mortality from multiple factors when top-down control by natural enemies was disrupted by the application of broad-spectrum insecticides[29]. Irreplaceable mortality estimates that portion of generational mortality that would not occur if the factor in question is absent. For plots, the line within the box is the median, the box denotes the 25th and 75th percentiles, whiskers denote the 10th and 90th percentiles and points depict the 5th and 95th percentiles. When natural enemies were not disrupted by insecticides, predation supplies the highest level of irreplaceable mortality but this level declined significantly with sprays. Low levels of irreplaceable mortality were supplied by parasitism and they did not change with insecticide use. Modified from Asiimwe *et al.* 2016[29] Please click here to view a larger version of this figure.

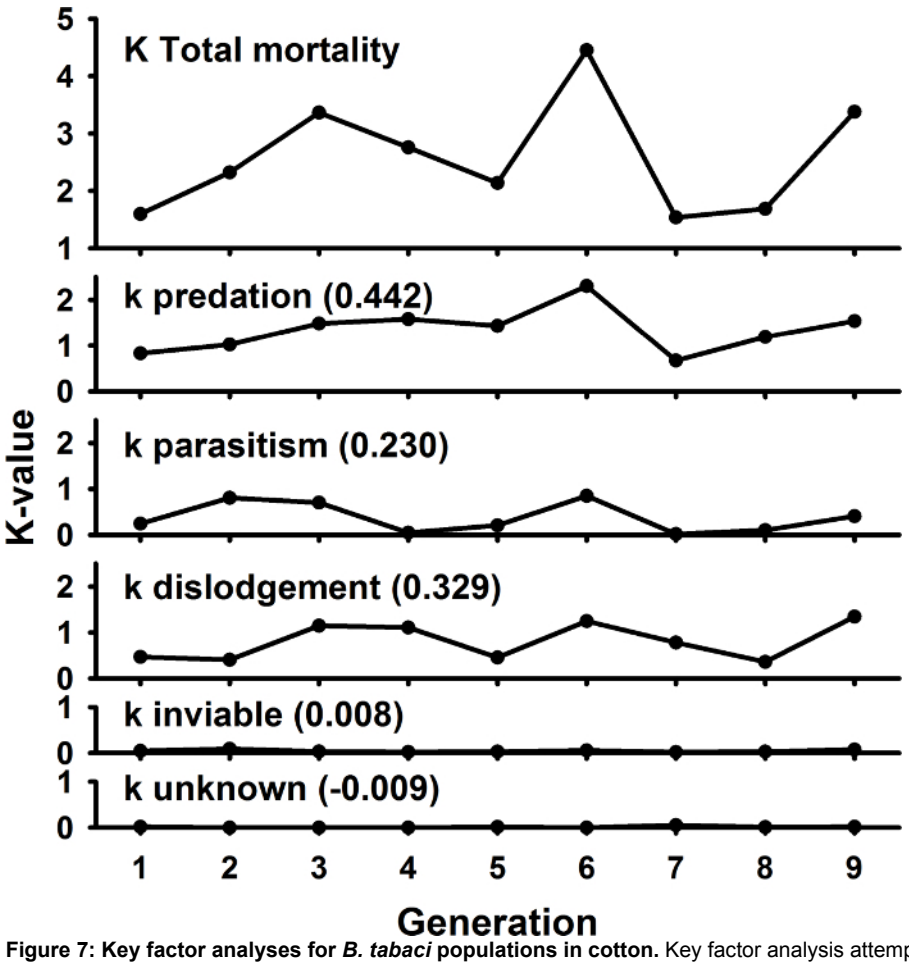

Figure 7: Key factor analyses for *B. tabaci* populations in cotton. Key factor analysis attempts to identify the factors that are most closely associated with changes in generational mortality. The method uses k-values, which are estimated as $-ln(1-M_i)$, where *ln* is the natural log, and M_i is the marginal mortality rate for factor *i*. K-total is the sum of all mortality factor k-values and represents total mortality for the generation. The pattern displayed by K-total over 9 generations is compared to those of specific mortality factors (here summed over all life stages). The factors that most closely resembles K-total is the key factor. A more quantitative approach is to regress individual k-values on K-total[3]. The factor with the highest slope value (in parentheses) is the key factor. Here, predation was identified as the key factor. Please click here to view a larger version of this figure.

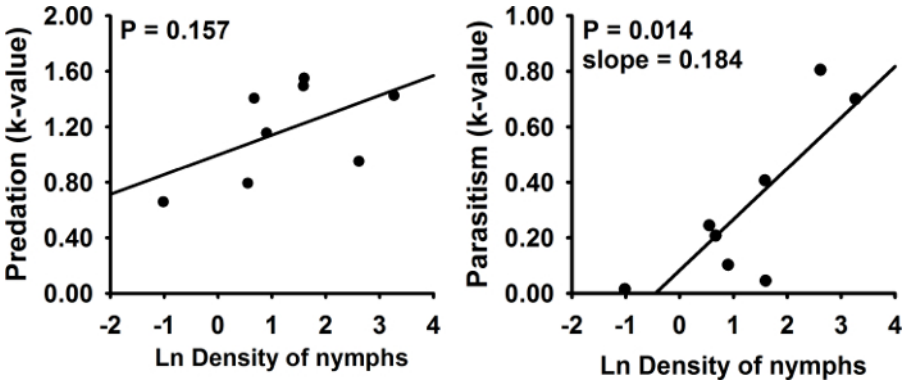

Figure 8: Testing for density-dependence in mortality factors. Temporal density dependence can be tested by regressing the k-value of a specific mortality factor on the ln density of the life stage affected by this mortality. A statistically significant positive slope would indicate direct density-dependence or an increased rate of mortality with increasing insect density. A negative slope would indicate inverse density-dependence. In the example here, direct density-dependence is supported for parasitism of nymphs but not predation of nymphs. Please click here to view a larger version of this figure.

Table 1: Example of data sheet taken to the field to record life table observations. This table shows an example of the way that data was recorded in the field. Each insect being followed is either alive at the time of observation or is dead from one of several causes. As observations are completed it is convenient to black out the lines of previously dead insects. Data from two hypothetical observation dates are given. Please click here to download this Table.

				Real mortality		Apparent mortality		Marginal mortality	k-value
Stage/Factor	**Factor (l_x)**	**Stage (d_x)**	**Factor(d_x)**	**Stage(d_x/l_0)**	**Factor(d_x/l_0)**	**Stage(q_x)**	**Factor(q_x)**		
Egg	1000	748		0.748		0.748			
Dislodgement			421		0.421		0.421	0.421	0.546
Inviability			57		0.057		0.057	0.184	0.204
Predation			270		0.270		0.270	0.466	0.628
1st Instar[a]	252	37		0.037		0.147			
Dislodgement			18		0.018		0.071	0.071	0.074
Predation			20		0.020		0.079	0.085	0.089
Unknown			0		0.000		0.000	0.000	0.000
2nd Instar	215	49		0.049		0.228			
Dislodgement			8		0.008		0.037	0.037	0.038
Predation			41		0.041		0.191	0.198	0.221
Unknown			0		0.000		0.000	0.000	0.000
3rd Instar	166	31		0.031		0.187			
Dislodgement			16		0.016		0.096	0.096	0.101
Predation			16		0.016		0.096	0.107	0.113
Unknown			0		0.000		0.000	0.000	0.000
4th Instar	135	88		0.088		0.652			
Dislodgement			37		0.037		0.274	0.274	0.320
Parasitism			14		0.014		0.104	0.443	0.585
Predation			37		0.037		0.274	0.378	0.474
Unknown			0		0.000		0.000	0.000	0.000
Adult	47								
Generational Mortality					0.955			0.966	3.394
[a]1st instar does not include the brief crawler stage									

Table 2: Example life table for a *Bemisia tabaci* population in cotton at Maricopa, Arizona, USA. This table shows the standard values typically estimated in life tables. l_x is the number of insects alive at the beginning of each life stage (by convention results are normalized to start with 1000), stage d_x is the number dying during each stage interval, and the factor d_x indicates the number dying by a given cause within each stage. Stage or factor q_x estimates the rate of mortality occurring within a specific stage and is based on the number of insects alive at the beginning of that stage. The apparent factor q_x values are used to estimate rates of marginal mortality due to each factor (see Protocol 6.2.3 and Table 3). Real mortality gives the rate of mortality in each stage and by each factor relative to the number of insects alive at the beginning of the cohort (here 1000). Generational mortality can be estimated by the sum of real mortalities or the sum of k-values for marginal mortality. The difference is due to the fact that marginal rates are approximate[32]. Generally, average error rates are 0.07%[13].

Marginal rate of interest (M_B)	Apparent rate (d_B)	Apparent rate (d_A)	Stage
Inviability	Inviability	Predation + dislodgement	Egg
Parasitism	Parasitism	Predation + dislodgement	4th stage nymphs[a]
Predation	Predation	Dislodgement	Egg and all nymphal stages
Insecticide	Insecticide	Predation + dislodgement	Eggs and all nymphal stages
Unknown	Unknown	Predation + dislodgement	All nymphal stages
Dislodgement	Dislodgement	No competing factors	Eggs and all nymphal stages

[a] Aphelinid parasitoids can successfully attack all nymphal stages of B. tabaci[38,39,40], but parasitism can only be observed in 4th stage nymphs in the field; thus d_A is the sum of predation and dislodgment from all nymphal stages combined. Adapted from[13,14] .

Table 3: Matrix for estimating marginal rates of mortality for *Bemisia tabaci* populations. Mortality factors in this system do not act sequentially but contemporaneously and so special techniques are required to estimate stage-specific rates of mortality as marginal rates. The formula is $M_B = d_B/(1-d_A)$, where M_B is the marginal rate of mortality factor *B*, d_B is the observed rate of mortality from factor *B* and d_A is the observed rated of mortality summed for all mortality factors that can outcompete factor *B*. The table shows which apparent (observed) rates of mortality are necessary to estimate the marginal rate for a given mortality factor. Aphelinid parasitoids are capable of attacking all nymphal stages of *B. tabaci*[38,39,40], but evidence of parasitism in the field can only be reliably seen in 4th instar nymphs. To fully account for all simultaneous competition from predation and dislodgement during all nymphal stages, the d_A for marginal parasitism is estimated as the sum of apparent predation and dislodgment for all nymphal stages combined.

Discussion

Typically, the development of life tables for multivoltine insects with broadly overlapping generations are constrained to a vertical approach where a population is sampled repeatedly over time and various graphical and mathematical techniques are then used to estimate recruitment to the various stages and infer rates of mortality from changing densities of the various life stages[2]. The strength of the approach here is that it navigates this limitation by isolating a group of immobile equal-aged insects from a population and then following their fate over time. Rates of mortality can be directly estimated, and equally important, the agents of this mortality can be identified, at least within broad categories (*e.g.*, sucking predation, dislodgement).

These broad categories of mortality are relatively easy to distinguish in the field with a 15X lens, but the specific causes of death are less certain. Further delineation of specific sucking predator species or specific causes of dislodgement is possible. Naranjo and Ellsworth[13] used multiple regression to identify predator species associated with measured rates of stage-specific predation and the association of various chewing predator species and weather parameters (rainfall, wind speed) to rates of stage-specific dislodgement. The unknown category likely captures several potential sources of mortality. For example, many species of aphelinid parasitoids are known to host-feed[41,42]. This feeding results in the death of the host but does not appear the same as predation (compare **Figure 4F** to **4G-4I**). During many years of conducting life tables we have never observed nymphs that have been definitively preyed upon by parasitoids, but this may differ in other systems and may be a separate source of mortality that can be quantified.

Critical steps in the protocol include the accurate identification of newly laid eggs and newly settled 1st instar nymphs. If older individuals of either of these stages were marked, then the resulting mortality rates would be censored, and thus, less accurate. The accuracy and consistency of the repeated observations following cohort establishment are also important. Sometimes the scale of the study require that multiple observers are needed to complete the study. In the studies of Naranjo and Ellsworth[13,14] there were four main observers and they were each responsible for one replicate block of the experiment. Differences between observers was then accounted for through block variation in the statistical analyses. The observers also conferred on a regular basis to reduce individual differences in interpretation of stage development and causes of death. In other studies, a single individual did all the observations[29], thus reducing observer-based inconsistencies. It is also important to establish the cohorts within a fairly narrow window of time so that a given identified population could be followed on subsequent observations dates. Depending on the scope of the study, it would be possible to stagger cohort initiation, but then careful planning would be needed to ensure that the ensuing observations for development and mortality be timed at similar intervals, especially if development is rapid, as it is for the species studied here.

An obvious limitation of the method is that it does not include reproduction and mortality of the mobile adult stage. Several predators can potentially prey on adult *B. tabaci*[43,44,45] and may be an important source of mortality not captured by this method. Reproduction is also vitally important to understanding the overall population dynamics of a species. It is possible to combine laboratory generated information on temperature-dependent adult reproduction and survival with field-based life table data from the immature stages[13], but it is unclear how well such laboratory data represent the reproductive process under variable field environments. With contemporaneous measurement of population dynamics of whiteflies along with models, these life table results can be used to draw inferences about adult immigration and emigration[13]. Another limitation is that mortality during the crawler stage of the insect is not measured. Supporting research suggests that the crawler stage is very short in duration[46,47] and that rates of mortality are negligible[30]. A third limitation is that the insects in the cohort are all located near the top of the plant. Certain mortality factors (predation, parasitism, dislodgement) might vary depending on location with the canopy. For example, certain predators or parasitoids may have specific micro-climate preferences and dislodgement forces such as wind and rain may be less severe lower in the canopy. This limitation can be easily overcome by simply altering the distribution of the marked insects in the cohort. The other limitations deserve further research and development towards a more complete life table. Similar limitations are likely to affect other insect species with similar life styles and behaviors.

Additional limitations involve some of the analytical methods described here. While key factor analysis has been widely used in life table analyses[12], it has been criticized as an inadequate method for defining the casual mechanisms that drive population dynamics[48]. However,

in conjunction with other analyses it can shed light on the important life stages and mortality forces impacting insect populations[13]. Density-dependent analysis has also been questioned on both methodological and ecological grounds and while direct density dependence is sometimes associated with population regulation, debate continues on how best to measure and demonstrate the effect[4,31,49,50,51]. Finally, irreplaceable mortality analyses is a mathematical construct and it is difficult to know exactly how contemporaneous mortality forces will interact and compensate for any factor that might be eliminated[2,11]. The method presented here assumes that there is no density-dependence in mortality.

The field protocols are flexible and can be applied in a number of circumstances and to a number of different crops beyond cotton so long as the insect stages of interest are sessile[26]. It can be applied to simply describe the sources and rates of mortality for an insect population or can be used in an experimental context to assess the influence of a broad number of factors on the mortality dynamics of a populations[31,36]. The general analytical methods describe here have broad application, in spite of the limitations of key factor, density-dependence and irreplaceable mortality analyses already noted. The inclusion of adult reproduction and survival would open up additional avenues of analyses and understanding through the application of matrix models and the rich suite of interpretive tools they permit. For example, a complete life table would enable the application of elasticity analysis, a robust method for identifying which life stages contribute most to population growth[36,52]. This can allow a more fundamental understand of the population dynamics of a species and also facilitates identification of which life stages might be most profitably targeted by control measures such as biological control[37]. Application of such analyses to *B. tabaci* could contribute to even more robust management strategies in affected cropping systems.

Disclosures

The authors have nothing to disclose.

Acknowledgements

We thank D. Ashton, V. Barkley, K. Beimfohr, F. Bojorquez, J. Cantrell, G. Castro, R. Christensen, J. Fearn, C. Jara, D. Meade, G. Owens, L. Rodarte, D. Sieglaff, A. Sonoqui, M. Stefanek, B. Stuart, J. Trejo, A. Slade and E. Yescas for technical assistance. Partial support was provided by USDA-Agricultural Research Service, USDA-National Institute for Food and Agriculture Extension IPM Program and Pest Management Alternatives Special Projects, Cotton Incorporated, Arizona Cotton Growers Association, Cotton Foundations, USDA-CREES, NAPIAP (Western Region), and Western Region IPM Special Projects.

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