Demographic Analysis of Induced Resistance Against Spider Mites (Acari: Tetranychidae) in Cotton

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ABSTRACT  Demographic analyses were conducted of twospotted spider mites, *Tetranychus urticae* Koch, raised on excised leaves of cotton, *Gossypium hirsutum* L., plants that had been previously damaged by mites, *Tetranychus turkestani* Ugarov and Nikolski, or on leaves of undamaged controls. There were no differences in survival or development of individuals reared on leaves of the two treatments. Fecundity of mites was reduced by approximately 50% on excised leaves from plants that had been damaged as cotyledons relative to mites grown on leaves from undamaged control plants. Other workers who have examined the effects of plant conditioning on spider mites also have found this striking effect on fecundity and no effect on survivorship and development. This demographic pattern is consistent with patterns noted when the nutritional quality of the host plant is diminished but inconsistent with patterns caused by changes in the "secondary chemistry" of the host. Adult female mites behaviorally preferred excised leaves of control plants compared with excised leaves of damaged plants. Further work is required before a reliable behavioral assay is available that can be used to determine the chemical nature of induced resistance in cotton.

KEY WORDS  Arachnida, *Tetranychus urticae*, *Gossypium hirsutum*, host plant resistance

Plants often respond to attack by herbivores by changing chemically or physically. These responses may influence subsequent populations of herbivores attempting to use the damaged host (reviewed by Fowler & Lawton 1985). The population growth of twospotted spider mites, *Tetranychus urticae* Koch, was reduced on cotton plants that had previously been damaged by mites compared with undamaged control seedlings (Karban & Carey 1984). This induced resistance was systemic; leaves not present at the time of the initial damage were nonetheless poor hosts for the population growth of mites. In addition to changes in their rate of population growth, mites detect and behaviorally respond to the subtle changes in host quality caused by damage. Adult female mites offered a choice between leaves of equal age on plants that either had been previously damaged by mites or were undamaged controls preferentially moved to undamaged plants, although immatures and adult males showed no such preference (Harrison & Karban 1986). All of the experiments outlined above were conducted on intact leaves attached to the plant.

However, these previous experiments did not provide information about the impact of induced resistance on stage-specific demographic parameters. Such parameters are difficult, if not impossible, to measure on whole plants; mites disappear if uncaged, and caging with clip cages or Tanglefoot causes severe, local leaf necrosis (unpublished data). Thus, after repeated, unsuccessful attempts at measuring demographic parameters on whole plants, we report here on experiments designed to measure stage-specific life history traits of mites raised on excised leaves.

**Materials and Methods**

We grew cotton seedlings (*Gossypium hirsutum* L., var. Acala SJ-2), one plant in each 7.5-cm plastic pot, in environmentally controlled chambers maintained at 28 ± 2°C with a photoperiod of 14:10 (L:D). Each plant was randomly assigned either to be damaged by feeding mites or to serve as an undamaged control. Ten adult female *Tetranychus turkestani* Ugarov and Nikolski were placed on the cotyledonary leaves of plants in the damage treatment as soon as these leaves had expanded. Control plants received no mites. Acetate cylinders were placed around each plant to reduce movement of mites between plants. After 7 d, each plant of both treatments was dipped in miticide (dicofol [Keltbane], 100 ppm), which killed all mites. No true leaves had expanded at the time the miticide was applied. Plants were then allowed to grow without disturbance for approximately 14 d until three true leaves had expanded. At this stage, one true leaf of each plant was excised at the base of the petiole with a razor blade. The petiole was placed in a CaCl solution (0.0001 M) while the leaf blade was supported, dry, on a platform. In this and all other experiments, each leaf was replaced every 4 d, or sooner if any signs of wilting or deterioration were apparent.

**Survival, Development, and Fecundity.** We examined survivorship and growth rates of immatures, survivorship of adults, and number of eggs
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![Figure 1](image1.png) | ![Figure 2](image2.png)

**Fig. 1.** Proportion of immature *T. urticae* surviving on excised leaves of previously damaged and undamaged plants.

**Fig. 2.** Proportion of adult *T. urticae* surviving on excised leaves of previously damaged and undamaged plants.

produced by mites on excised leaves from plants that had been damaged or had served as undamaged controls.

Tetranychid mites pass through five developmental stages: eggs, larvae, protonymphs, deutonymphs, and adults. Each stage is preceded by a period of quiescence. To assess immature survival and growth rate, three newly hatched *T. urticae* larvae were placed on one leaf from each of 80 plants. The presence and developmental stage of each mite were recorded daily. Because any mite that moved off the leaf drowned, persistence was the same as survival. The experiment was terminated when the mites reached the adult stage.

To assess adult survival and fecundity, quiescent deutonymphs (the stage immediately preceding the adult) were transferred to a large bouquet of cotyledons. On the following day, we transferred three newly eclosed adult *T. urticae* females to an excised leaf from each of 80 plants. The presence and developmental stage of each mite were recorded daily. Because any mite that moved off the leaf drowned, persistence was the same as survival. The experiment was terminated when the mites reached the adult stage.

For statistical analyses, the plant, rather than the mite, was considered as the independent sampling unit. Estimates of survival, growth rate, and fecundity for the several mites on each plant were averaged, and all statistics were conducted on the mean value for each plant. Data were first analyzed by two-way analysis of variance (ANOVA) with treatment and experimental replicate as main effects. Because in no case was there a significant interaction between treatment and experiment, we combined the experiments and used a *t* test to distinguish between treatment groups.

**Behavior.** In experiments using whole plants (Harrison & Karban 1986), female spider mites showed a preference for leaves of undamaged plants compared with previously damaged plants; therefore we tested the preference of adult females for excised leaves of the two treatments. Three adult female *T. urticae* were offered a choice between an excised leaf from a damaged plant and an excised leaf from an undamaged plant. The blades of these pairs of leaves were supported by a screen made of chicken wire, and the petioles were kept immersed in a CaCl solution. The leaves were selected such that both of each pair were of similar age and size. The two leaves were placed so that they just overlapped and a small piece of paper (1 by 0.5 cm) connected the leaves. Mites were placed onto this paper sequentially (only one mite was present on the paper at a time) and their choice of leaves was noted. After 24 h, the location of the mites was again noted. We thus have two measures, one of immediate choice (before direct contact with the leaf), and the second of choice after 24 h. As above, each pair of leaves (*n* = 64) rather than each mite was considered as the independent sampling unit. Only one leaf was used from any plant. The choice of the group of three mites was one entry in a binomial test (*e.g.*, if two of three mites chose the leaf from a previously damaged plant, the mites from that run were considered to have chosen the damaged leaf and a single entry in the analysis was made). If only two mites remained after 24 h, and each was on a separate leaf, that leaf couplet was removed from the 24-h choice analysis.

**Results**

**Survival.** Immature mites tended to have higher survival on leaves from damaged plants (Fig. 1), whereas adult mites tended to survive longer on leaves excised from undamaged control plants (Fig. 2). In neither case was the mean number of days surviving statistically different for mites raised on the two treatments, and the net effect over the entire lifetime was essentially zero. For immatures, mean number of days surviving ±SE on leaves of damaged plants was 6.6 ± 0.4, *n* = 40; on leaves of control plants, 5.5 ± 0.5, *n* = 40, *t* = 1.77, 0.05 < *P* < 0.10. For adults, mean number of days surviving ±SE on leaves of damaged plants was 7.1 ± 0.6, *n* = 58; on leaves of control plants, 8.5 ± 0.6, *n* = 59, *F* = 2.52, df = 1, 112, *P* = 0.12.
Developmental Time. Rate of development to the adult stage was unaffected by previous damage (Fig. 3; $t = 0.36$, df = 45, N.S.). Similarly, time to first reproduction was not different for mites raised on excised leaves from damaged plants versus leaves from controls (Fig. 3; $t = 0.88$, df = 32, N.S.).

Fecundity. The greatest difference in the variables measured between mites raised on previously damaged plants and controls was in fecundity (Fig. 4). This difference was apparent whether fecundity was measured as the total number of eggs per plant ($F = 8.18$, df = 1, 112; $P = 0.005$), as mean number of eggs produced per female ($F = 4.79$, df = 1, 112; $P = 0.03$), or as mean number of eggs produced per female excluding those females that produced no eggs ($F = 6.48$, df = 1, 112; $P = 0.01$). Females raised on excised leaves from control plants produced 40-60% more eggs than those on excised leaves from previously damaged plants. When all life stages are added together, total numbers of mites on leaves taken from plants that had been damaged at the cotyledon stage were approximately half those on undamaged controls.

Behavioral Preference. In 41 of 64 cases, at least two of three females moved immediately to the leaf from the control plant in choice experiments on 64 pairs of excised leaves ($P = 0.016$, binomial probability with $k = 0.5$). In 30 of 53 pairs of leaves, the majority of females were found on the control leaf after 24 h ($P = 0.205$, binomial probability with $k = 0.5$). After 24 h, 11 of the pairs were not suitable for inclusion in the analysis; in three cases one of the leaves had come out of the CaCl solution and shriveled, and in eight cases only two mites remained, and one was found on each of the leaves of the two treatments.

Discussion

The strength of induced resistance by cotton to spider mites is variable, ranging from no reduction in mite populations to a four-fold reduction on previously damaged whole plants (Karban 1987). Although we used excised leaves, we consistently found a reduction of approximately 50% in total numbers of mites (all stages) on leaves taken from plants that had been damaged by mites at the cotyledon stage compared with undamaged controls. Therefore, the effects of excising the cotton leaves did not negate the effects caused by previous damage.

Furthermore, the similarity between the behavioral preference we observed and that found for mites on leaves of whole plants (Harrison & Karban 1986) suggests that mites placed on excised leaves may provide a reliable bioassay for leaf quality. The immediate choice of females for leaves of previously undamaged plants is remarkable in that the females have not yet even physically contacted or tasted the leaf tissue. However, this choice was less apparent after 24 h. There may be changes in leaf chemistry that occur after a leaf has been removed from the plant that eliminate or mask the cues used by the mites. If such foliar changes did occur, they did not negate the differences caused by previous damage, as measured by mite fecundity. An accurate excised-leaf technique for assessing the level of induced resistance will greatly facilitate work to understand the chemical mechanisms involved. We will continue to attempt to perfect the accuracy and repeatability of this behavioral technique.

Our finding that plant conditioning affects fecundity primarily, not survivorship or development rate, is supported by other workers as well. *Panonychus ulmi* Koch fed on heavily damaged citrus leaves produced fewer eggs but did not differ in survival or growth compared with mites fed on lightly damaged leaves (Henderson & Holloway 1942). *Oligonychus punicae* Hirst had lower fecundity on damaged avocado foliage, although survival and growth were unaffected (McMurtry 1970). Differences in fecundity of *Tetranychus cinnabarinus* Boisduval were much larger than differences in survivorship and developmental rate for mites on damaged and fresh bean leaves (Wrensch & Young 1975, 1978). Finally, induced resistance in soybeans against *T. urticae* was primarily because of differences in fecundity (Hildebrand et al. 1986).

The demographic pattern we observed may provide a clue to the mechanism of induced resistance.
Induced resistance, as we use the term, does not imply that the plant is necessarily responding in an adaptive way. Rather, it may refer to a degradation of plant quality or to the synthesis of some novel chemicals or physical structures. Many workers have observed that a wide variety of manipulations of host plants that change their nutritional quality strongly affect fecundity of spider mites but have little effect on survival or development rate (reviewed by Karban & English-Loeb 1988). Manipulations that cause changes in a variety of "secondary chemicals" in many plants tend to affect survival and often development, as well as fecundity (Karban & English-Loeb 1988). While not conclusive, the demographic pattern reported in this study suggests that the induced resistance may involve degradation of nutritional quality of the host rather than the de novo synthesis of secondary chemicals.

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