ASSESSMENT OF COTTON AS AN ALTERNATIVE HOST PLANT FOR THE BROWN CITRUS APHID, *TOXOPTERA CITRICIDA* (HOMOPTERA: APHIDIDAE)

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ABSTRACT

Seven populations of *Toxoptera citricida* (Kirkaldy) were sampled in central Florida sweet orange groves in 2001. All populations contained individuals that accepted cotton seedlings as a host in a no-choice situation; many of these matured and deposited nymphs that also developed and became reproductive on the same plant. Significant differences were noted among populations with respect to the proportion of nymphs accepting, maturing, and ultimately reproducing on cotton. Differences in aphid survival were largely a function of differences in host plant acceptance, rather than differential mortality on the plant. A significant proportion of the apterous adults maturing on cotton abandoned the plant without reproducing. Second and third instars transferred from laboratory colonies maintained on sweet orange were more accepting of cotton than were either first or fourth instars. Apterous adults accepted cotton at rates similar to second and third instars. Alate adults settled on cotton seedlings in greenhouse choice experiments and probed the plants, but none deposited nymphs. Alatae that matured on cotton readily accepted citrus for feeding and reproduction. It is concluded that cotton may be useful as a factitious host plant for rearing *T. citricida* in the laboratory, but field planted cotton is unlikely to serve as a reservoir of the aphid in regions where citrus is grown.

Key Words: *Gossypium hirsutum*, host plants, reproduction, survival, *Toxoptera citricida*.

RESUMEN

Siete poblaciones de *Toxoptera citricida* (Kirkaldy) fueron muestreadas en huertos de naranjas dulces en Florida central en 2001. Todas las poblaciones tenian individuos que aceptaron plantulas de algodón como una hospedera en una situación de una sola opción; muchas de estas maduraron y depositaron ninfas que tambien se desarrollaron y se reproducieron en la misma planta. Diferencias significativas fueron notadas entre las poblaciones con respeto a la proporción de las ninfas que aceptaron, maduraron, y finalmente se reproducieron en el algodón. Las diferencias en la sobrevivencia de los áfidos fueron mayormente en función de las diferencias en aceptar la planta como una hospedera, y no debido a la mortalidad diferential en la planta. Una proporción significante de los adultos ápteros maduraron en el algodón y abandonaron la planta sin reproducirse. Las ninfas en el segundo y tercero estadio transferidos de las colonias de laboratorio mantenidos en naranjas dulces fueron más receptivas al algodón que las ninfas en el primero o cuatro estadio. Los adultos ápteros aceptaron el algodón en las proporciones similares de las ninfas en el segundo y tercero estadio. Los adultos alados posaron sobre las plantulas de algodón en experimentos de selección en el invernadero y probaron las plantas, pero ninguno depositó ninfas. Los adultos alados que maduraron sobre el algodón aceptaron con rapidez el cítrico para alimentarse y reproducirse. Se concluye que el algodón puede ser útil como una planta hospedera facticiosa para criar *T. citricida* en el laboratorio, pero es poco posible que el algodón sembrado en el campo servirá como un refugio del áfido en regiones donde se siembra los cítricos.

The brown citrus aphid, *Toxoptera citricida* (Kirkaldy) (BCA), is the primary vector of citrus tristeza virus (CTV), one of the important diseases of citrus world-wide (Meneghini 1946). Its importance as a pest of citrus derives from its high efficiency in transmitting this virus, rather than from any direct damage (Michaud 1998). The BCA has been present in Florida since 1995, but remains absent from other citrus-growing regions of the United States including Louisiana, Texas, Arizona and California. Although it has been present in Belize, Central America, since 1996 (Halbert 1996), the Yucatan Peninsula was not infested until 1999 (Michaud & Alvarez 2000). Northerly movement of the BCA has been also slow along the eastern seaboard of Mexico, and the major citrus-growing states of Tabasco and Veracruz remain uninfested to date. If and when further northerly movement

occurs, citrus plantings as far north as Texas could be heavily impacted as most citrus in the region, both north and south of the border, is planted on sour orange rootstock. Various strains of CTV cause "quick decline" of trees on sour orange and this rootstock must be abandoned wherever the virus and its vector are present together. An area-wide effort to identify and eliminate CTV-infected trees prior to the arrival of BCA is the best strategy for ameliorating the inevitable impact on the citrus industry.

The performance of BCA has been compared on various citrus varieties (Komazaki 1989) and related species of Rutaceae (Tang et al. 1999), but its ability to utilize non-rutaceous plants has not previously been explored. Although a substantial number of plants have been recorded as potential hosts for BCA (Michaud 1998), the actual role of these plants in supporting BCA populations in the field is unknown. It is suspected that many plants listed as hosts may represent mis-identifications of the aphid due to its similarity to the black citrus aphid, *Toxoptera aurantii* (Boyer de Fonscolombe), a related species with a very broad host range (Halbert & Brown 1996). Observations of BCA behavior suggest that anomalous host plant associations may arise when high-density populations 'overflow' from heavily infested citrus trees. Crowding in BCA colonies stimulates alate production (Michaud 2001), and large numbers of reproductive apterae also emigrate from crowded colonies (Michaud & Belliure 2000). These dispersing apterae ascend almost any other green plant adjacent to the source tree and often settle to feed. Residual nutrition acquired from the original host plant may then permit some limited reproduction to continue on the colonized plant, creating the semblance of host suitability. Thus, discrete field observations of host plant associations can be misleading and careful laboratory studies are required to determine whether a particular plant is truly a potential or suitable host.

The cotton plant, *Gossypium hirsutum* L., was first reported as an occasional host plant of BCA in southern Africa (Symes 1924) and later in Australia (Carver 1978). The present study was undertaken to evaluate the potential suitability of cotton as a host plant for BCA for two reasons. First, laboratory studies of the BCA and its biological control agents are hampered by the continuous requirement for citrus trees with new growth suitable for aphid colony growth and development. These are expensive to acquire and maintain, demand warm temperatures and intense supplementary lighting in order to produce new growth, and are susceptible to many other pests in a greenhouse environment. If the BCA could be reared effectively on a herbaceous host plant that could be planted from seed as required, laboratory studies of BCA biology and ecology would be greatly facilitated. Second, the close

proximity of cotton plantings to citrus groves in many regions of Texas and California raises the question of whether or not cotton fields could potentially support reservoir BCA populations that could reinfest citrus, just as they now serve as a reservoir for *Aphis gossypii* Glover, another vector of CTV (Cisneros & Godfrey 2001).

The present study had three objectives: (1) to assess the general acceptability and suitability of cotton for various BCA populations in central Florida, (2) to test whether acceptance of cotton, and subsequent developmental performance, varies with the growth stage of the aphid colonizing the novel host, and (3) to determine whether alate aphids developing on citrus would colonize cotton and vise versa.

MATERIALS AND METHODS

Variation among Populations in Acceptance of Cotton in No-Choice Experiments

Preliminary work conducted by Dr. A. Chow in Immokalee, FL indicated that BCA could be induced to feed on cotton provided that very young plants were provided and that relatively cool temperatures were maintained. Seeds of cotton, *Gossypium hirsutum* L., var "Suregrow", were planted individually in plastic cones (20 cm ht × 4 cm diam) filled with Metromix 500® potting soil. The cones were held at 24 ± 2°C in a climate-controlled greenhouse under natural light until germination. Following germination of the cotton, and before expansion of the first pair of true leaves, cones were individually labeled and a coating of Tanglefoot® (The Tanglefoot Company, Grand Rapids, MI 40504) was placed around the inner rim of each.

Seven populations of BCA were sampled in sweet orange groves in seven distinct locations in Polk County, FL between 25-IX-2001 and 4-XI-2001 by collecting a single, heavily-infested citrus terminal from each grove and transporting it to the laboratory in a 500-ml ventilated plastic container. A series of 60 apterous, BCA 4th instars were selected from each sample under a low power stereo microscope and transferred with a sable hair brush in groups of 5 to each of 12 cotton seedlings. The seedlings were then placed in a growth chamber set to 16:8, L:D period, 75% RH, and a constant temperature of 20.0 ± 1°C. Each replicate was examined once every 24 h and the number of nymphs remaining on the seedling was recorded, as was the number dying in the Tanglefoot barrier. In addition, data were recorded on the number of nymphs maturing to the adult stage, the number of adults that reproduced, and the number of second generation nymphs that matured. The data were analyzed by one-way ANOVA (SPSS 1998) followed by an LSD test for separation of means ($\alpha = 0.05$).

Variation among Instars in Acceptance of Cotton
in No-Choice Experiments

A stock colony of BCA was initiated from material field-collected in Polk County, FL, in March, 2002 and maintained on potted sweet orange, *Citrus sinensis* L., var. "Pineapple" at 24 ± 2°C in a climate-controlled greenhouse under natural light. Cotton seeds were planted individually in plastic cones, germinated in the greenhouse, and Tanglefoot was applied to the rim of the cone as above. Colonies of BCA were removed from the stock laboratory culture and the aphids separated according to stage (n1-n4 and apterous adults) under a 10× stereo microscope. For each growth stage, five aphids were transferred individually with a sable hair brush to a single cotton seedling in each of 20 replicates. Any aphid suspected to have sustained injury in the process of transfer was immediately replaced. Since adults are not reproductive for least 24 h following their last molt (Michaud 2001), pre-reproductive apterous adults were obtained by removing all adults from a stock BCA colony and, the next morning, harvesting all those that molted to adult overnight. All experimental replicates were maintained in a climate-controlled growth chamber under the same conditions as described above. Replicates were examined daily and the following information was recorded: the number of nymphs that settled and remained feeding on the plant after 24 h, the number maturing to the adult stage, and the number of adults that became reproductive. For transferred adults, only the number reproducing on the cotton was recorded. The data were analyzed by one-way ANOVA (SPSS, 1998) followed by an LSD test for separation of means (α = 0.05). Survival of first instars was compared between experiments with a Chi-square, Goodness-of-fit test.

Alate Acceptance of Host Plants in Choice Experiments

Alate aphids were produced in high-density BCA colonies grown on potted sweet orange trees in the greenhouse (as above). Sweet orange seedlings and cotton seedlings were planted individually in plastic cones as above. Orange seedlings ca. 6 mo old with a single growing terminal were used in experiments; cotton seedlings were 2-4 days old. The experiments were performed in the greenhouse in wood frame cages (120 cm long by 65 cm wide by 80 cm high). Each cage was screened with white muslin on the side panels and had a clear plexiglass roof. In each trial (n = 12) the alate source consisted of a single 15-cm diameter pot containing a sweet orange plant with a mature BCA colony producing alatae. This alate source was placed in the center of a cage with 4 trap plants in plastic cones (2 cotton seedlings and 2 sweet orange seedlings) arranged equidistant (40 cm) in an alternating sequence around the source plant. After 24 h, the numbers of alatae settling and feeding on each of the trap plants were counted and the plants were replaced with cotton and citrus in reversed positions in the cage. In cases where alatae settled on a seedling, the seedling was isolated in another cage and examined on subsequent days to determine whether or not reproduction occurred.

Alate BCA were produced on cotton by transferring large numbers of reproductive apterous adults from the stock colony to the potted cotton seedlings and then moving them into a climate-controlled growth chamber under the same conditions as described above. The adult aphids were left to reproduce for a period of 48 h whereupon all adults were removed and first instar nymphs were left *in situ* to complete development. A total of 25 alatae produced on potted cotton seedlings were caged individually on flushed sweet orange terminals in the greenhouse in a muslin bag fastened with a twist-tie at the base of the twig. Observations were then made at 24 and 48 h to determine whether or not alates accepted the terminal and deposited nymphs.

RESULTS

Variation among Populations in Acceptance of Cotton
in No-Choice Experiments

All seven populations of BCA sampled contained some apterous fourth instars that accepted cotton as a host plant (Fig. 1), but there was significant variation among populations in the proportion of aphids that accepted the cotton seedling within the first 24 h ($F = 3.708$; 6,76 df; $P < 0.01$). There were also significant differences among populations in the number of individuals molting to adult ($F = 4.868$; 6,76 df; $P < 0.001$) and the number of adults reproducing ($F = 3.706$; 6,76 df; $P < 0.01$). Overall, a mean ± SEM of 21.4 ± 1.8% of aphids accepted cotton, 16.4 ± 1.6% molted to adult, and 10.2 ± 1.6% became reproductive. Population 2 had the highest proportion of individuals accepting, maturing and reproducing on cotton; only populations 1 and 6 had as many individuals accepting cotton, but their success in maturing and reproducing was significantly lower than population 2. A total of 69 aphids matured to the adult stage and 43 of these (63.2%) deposited at least one nymph before abandoning the plant. Apterous adults that became reproductive produced a mean (±SEM) of 13.9 ± 1.82 progeny (Fig. 2a) with no significant difference among populations in adult fecundity ($F = 0.904$; 6,26 df; NS) or in the number of progeny maturing ($F = 1.109$; 6,26 df; NS). The mean reproductive rate ranged from 0.4-2.2 nymphs/adult/day of reproduction (mean = 0.94 ± 0.23) and the overall maturation rate of 2nd generation nymphs was 33.8% (Fig. 2b).
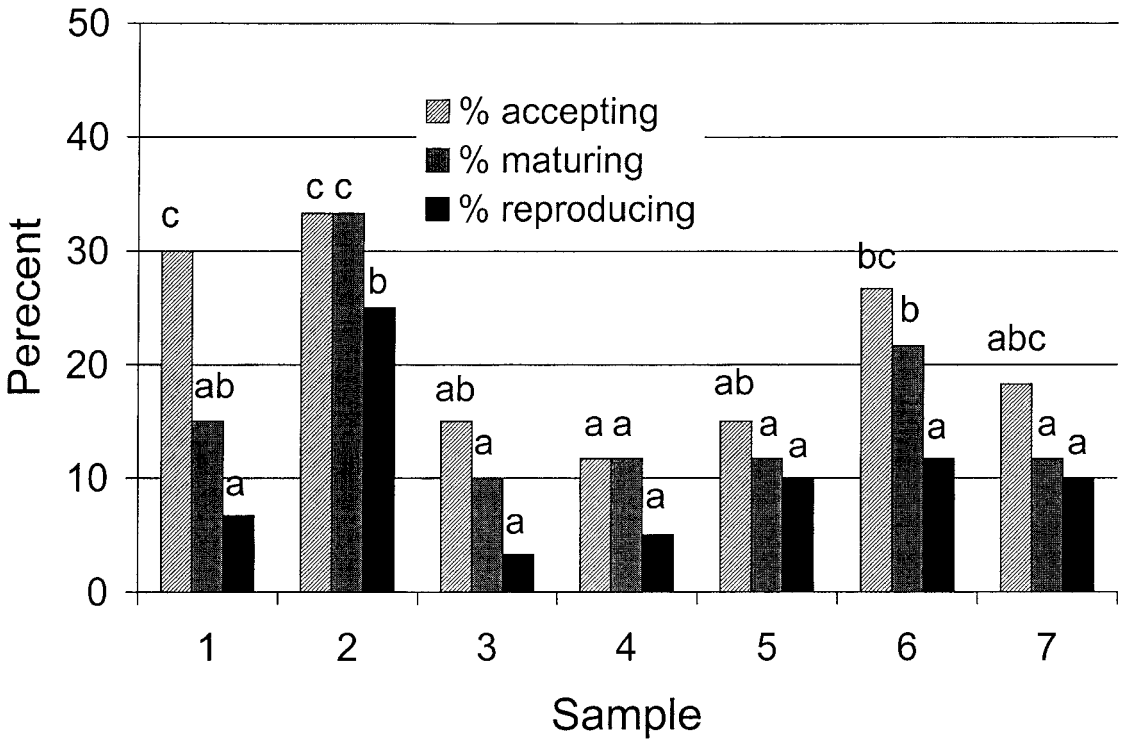
Fig. 1. Performance data for 4th instar *Toxoptera citricida* obtained from seven different populations in central Florida and transferred to cotton seedlings (N = 12), five per plant. "% accepting" = percentage of aphids feeding on the cotton seedling after 24 h, "% maturing" = percentage molting to adult, "% reproducing" = percentage depositing at least one nymph following molt to adulthood. Means in columns bearing the same letter are not significantly different among populations in a one-way ANOVA followed by LSD (α = 0.05).

Variation among Instars in Acceptance of Cotton
in No-Choice Experiments

There were significant differences among instars in the proportion maturing to adulthood when transferred from citrus to cotton seedlings (F = 8.622; 5,72 *df*; P < 0.001) and also significant differences in the proportion becoming reproductive (F = 5.755; 5,92 *df*; P < 0.001). Second and third instars were more likely to remain on a cotton seedling, survive to adulthood, and become reproductive than were either first or fourth instars (Fig. 3). Experiment-wide, a total of 266 of the aphids transferred as immatures molted to adulthood, and 190 of these (71.4%) deposited nymphs before abandoning the plant. Pre-reproductive adults transferred to cotton seedlings remained to reproduce with a frequency similar to second and third instars (Fig. 3).

Alate Acceptance of Host Plants in Choice Experiments

The proportion of nymphs maturing into alatae versus apterae on the source plants varied considerably under the conditions of these experiments, largely due to variation in both the number of apterous adults accepting the cotton, their distribution among the plants, and their reproduction during the 48-h period. High aphid density within colonies is the primary environmental factor influencing wing development in BCA (Michaud 2001), but high density colonies were difficult to achieve on cotton seedlings, leading to much lower rates of alate production than were achieved on citrus. A total of 186 alate aphids settled on plants and began feeding in the 12 replications of this experiment. Of these, 181 settled and fed on a sweet orange seedling and 5 settled and fed on a cotton seedling (Chi-square = 83.269, P < 0.001). Since observations were made only once every 24 h, it is possible that additional alates settled on cotton seedlings for shorter periods without remaining to feed. Whereas 98.3% of alates remained on orange seedlings long enough to initiate reproduction, all five that settled on cotton abandoned the plant within the following 24 h without depositing any nymphs. All 25 alatae that were reared on cotton and then caged individually on sweet orange terminals accepted the plant and began reproduction within 48-72 h.
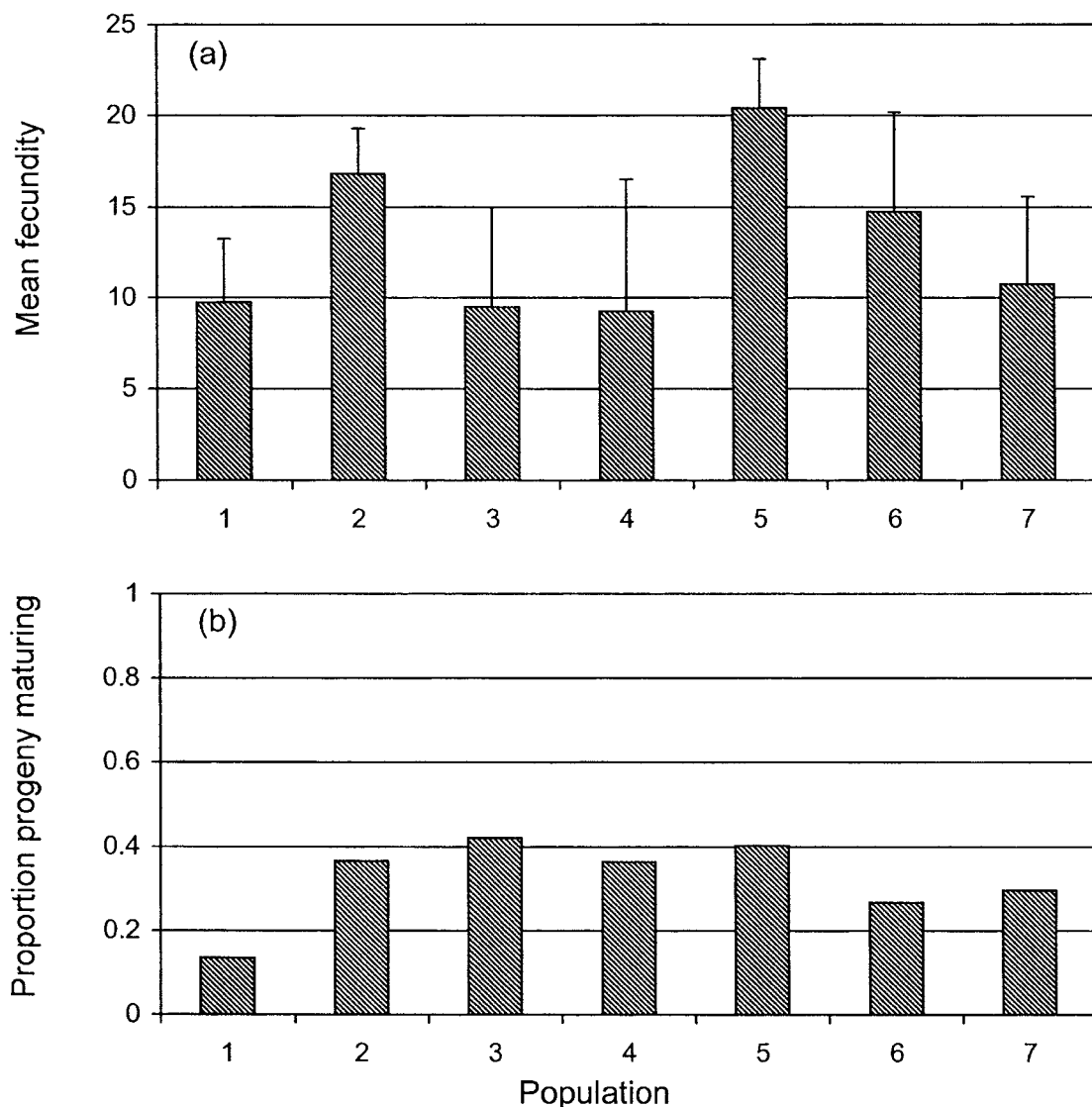
Fig. 2. Mean fecundities (+SEM) of *Toxoptera citricida* from seven field population that matured on cotton seedlings following transfer from citrus in the 4th instar (a), and proportions of the second generation nymphs that matured (b). There were no significant differences among populations (ANOVA, $P > 0.05$).

DISCUSSION

The fact that all sampled BCA populations contained fourth instars able to feed, and ultimately reproduce, on cotton suggests that the physiological ability to utilize cotton as a host plant is probably a general characteristic of *T. citricida* populations. While it is not surprising that considerable variation exists among populations with respect to the acceptance of cotton, the potential significance of this variation remains obscure, given that reports of BCA attacking cotton in the field are evidently rare (Symes 1924;

Carver 1978). However, BCA will also readily colonize *Murraya paniculata* (L.) Jack and *Malpighia punicifolia* L. under laboratory conditions (J. P. Michaud, unpublished) but rarely, if ever, utilizes these plants in nature.

If alatae are more selective of their host than are apterae, this could provide a partial explanation of why potential host plants such as cotton are rarely, if ever, utilized in the field. Alatae are physiologically very different from apterae in many ways. Their lower reproductive rate and longer lifespan (Takanashi 1989) may afford them more opportunity to be selective among host
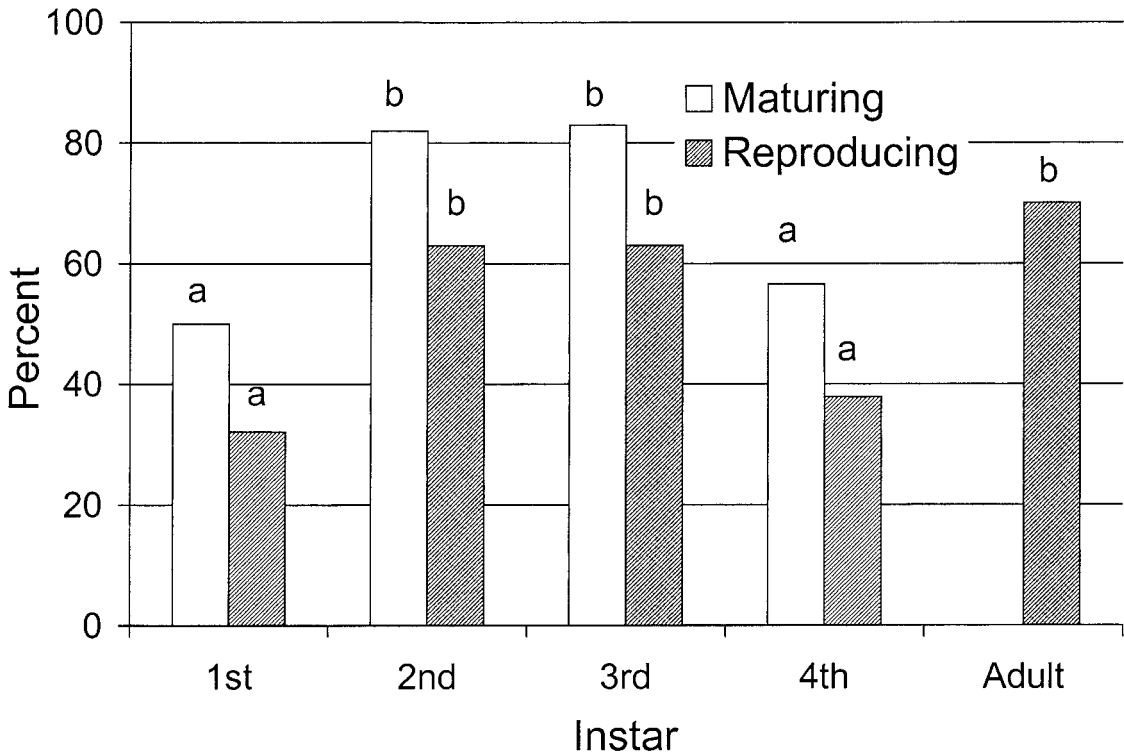
Fig. 3. Performance data for *Toxoptera citricida* transferred from sweet orange to cotton seedlings at various life stages. "% maturing" = percentage of aphids molting to adults, "% maturing" = percentage molting to adult, "% reproducing" = percentage depositing at least one nymph as an adult. Means in columns bearing the same letter are not significantly different among life stages in a one-way ANOVA followed by LSD ($\alpha = 0.05$).

plants. Apterae may often be constrained to accepting sub-optimal plants when dislodged from their primary host. Therefore, when reporting unusual host records for aphids it might be useful to distinguish between alate-founded versus apterous-founded colonies. Alate aphids are known to settle and probe on many non-host plants. For example, BCA alates probing soybean can contribute to transmission of soybean mosaic virus without ever colonizing the plant (Halbert et al. 1986). Similarly, many apterous-founded colonies on anomalous host plants may be chance events without ecological significance for the aphid population. An alate-founded colony (foundress with nymphs) is likely the best indicator of recurrent host plant utilization in nature.

It is important to note that aphid death in the first two experiments was almost invariably the result of aphids leaving the cotton seedling and dying in the Tanglefoot barrier, rather than simply expiring on the plant. Thus the differences observed in 'survival' and 'maturation' are largely a function of differential host plant acceptance, rather than differential mortality on the plant. Of all nymphs remaining on the cotton seedling for the first 24 h in

the first experiment, more than three quarters matured and almost half became reproductive.

The variation in acceptance of cotton among different BCA instars was neither positively nor negatively correlated with aphid growth stage. If aphid nymphs increasingly 'acclimated' to cotton over the course of their development, one might expect early instars to perform better than later instars, but this was clearly not the case. Intermediate instars had higher acceptance and better performance on cotton than did either first or fourth instars. Better acceptance and survival of later instars was initially predicted on the assumption that more time spent feeding on the high quality host would yield better nutritional status and greater survival when transferred to a lower quality host. This would seem to adequately explain the results for early instars, but not for later instars. It is also possible that migration tendency is age- or size-dependent to some degree, since size and nutritional status could strongly influence survival during migration. Therefore, the pattern of acceptance observed in Fig. 3 is likely a function of various factors acting at different stages of development.

The foraging decisions of adult aphids necessarily concern the placement of their offspring, rather than being exclusively concerned with food consumption. A large proportion of the apterae maturing on cotton left the plant immediately upon molting to the adult stage (37.7% in the first experiment, and 28.6% in the second). Migration of reproductive apterae from BCA colonies has been documented in response to crowding (Michaud & Belliure 2000, 2001), but pre-reproductive apterae were not observed to emigrate under these conditions. In the present experiments, the emigration of many apterae immediately following the adult molt might reflect a decision to seek a more suitable host plant for progeny while adequate resources are still available. Although the majority of maturing apterae opted to allocate a fraction of their (potential) offspring to the cotton seedling before emigration, virtually all ultimately opted to abandon the plant. In the first experiment, only three reproductive apterae died on the seedling and remained hanging by their stylets; the remaining individuals were all recovered from the Tanglefoot barrier. Thus the estimate of fecundity is more reflective of the length of time apterous adults tolerated feeding on the cotton, rather than their intrinsic reproductive potential on the plant. Furthermore, the observed reproductive rate was only a fraction of that typically observed on citrus at a comparable temperature (Takanashi 1989) and is indicative of the relatively low suitability of cotton as a host for BCA.

Alate BCA frequently landed on cotton seedlings in the greenhouse but never remained on them long enough to deposit nymphs under the conditions of these experiments. While alatae placed directly on cotton seedlings and maintained at 20°C in a growth chamber will ultimately deposit some nymphs (J. P. Michaud, unpublished), this is not a meaningful observation since alatae seldom fly at this temperature, if they are able to fly at all. Thus colonization of cotton seedlings in the field by BCA alatae seems unlikely even under cool temperature conditions.

These experiments demonstrate that cotton seedlings may be colonized by apterous morphs of BCA, that BCA can develop and reproduce successfully on cotton under certain conditions, and that alatae developing on cotton will readily return to citrus. Cotton may, therefore, be useful as a factitious host plant for rearing BCA for purposes of scientific study, although colony growth rates are slower than on citrus. However, given that cotton is only acceptable during the seedling stage, and only to apterae under conditions of relatively low ambient temperature, it seems unlikely that there is much risk of cotton serving as a pest reservoir for BCA under field conditions.

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