

# Wing Formation and Reproduction from Insecticide-Treated Cotton Aphids (Homoptera: Aphididae)<sup>1</sup>

Hugh E. Conway and Timothy J. Kring<sup>2</sup>

Department of Entomology, University of Arkansas, Fayetteville, AR 72701 USA

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**Abstract** Field observations of the cotton aphid, *Aphis gossypii* Glover, from 1999 to 2001 indicated an increase in percentage of winged aphids in cotton fields after an application of imidacloprid. Cotton aphid wing formation and fecundity of offspring were evaluated in a spray booth following five treatments: imidacloprid near LC<sub>50</sub> concentration on cotton, dicofol near LC<sub>50</sub> concentration on cotton, water spray on cotton, water spray on silk plants, imidacloprid spray near LC<sub>50</sub> concentration on silk plants. Silk plants were used to differentiate topical and systemic effects of imidacloprid. Prior to spraying, 40 aphids were transferred onto each plant. Aphids were moved from the silk plants onto unsprayed cotton plants 3 h post-spray. Offspring from imidacloprid-treated aphids on cotton produced twice as many winged offspring (8%) as did aphids from other treatments (3% to 4%) and only one-third as many offspring per founding relative to aphids in other treatments. In addition to increasing emigration by winged aphids, an increase in the proportion of winged aphids among survivors of an imidacloprid treatment may further reduce the number of aphids in a treated field because winged aphids require a longer developmental time, produced fewer offspring, and have an increased risk of mortality.

**Key Words** *Aphis gossypii*, imidacloprid, insecticide, fecundity, dicofol

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The cotton aphid, *Aphis gossypii* Glover, is considered one of the most important pests of vegetables and numerous field crops, including cotton, *Gossypium hirsutum* L. (Blackman and Eastop 2000). High aphid densities in cotton have resulted in yield reductions of 167 to 244 kg of lint per ha (Layton et al. 1996). In 2000, Arkansas reported losses of more than 9,500 bales of cotton (Williams 2001). Aphids infested 70% or 9.3 million hectares of US cotton in 2002 causing an estimated loss of 31,450 bales (Williams 2003).

Cotton aphids exhibit color changes, with a large green form that is twice as large as the yellow form. Cotton aphids also develop into either alate or apterous adults (Kring 1959). Alates are migratory and have a longer developmental time and decreased fecundity compared with apterous aphids (Dixon 1998). Crowding and nutritional factors are primarily responsible for the production of alates in most aphid species, including cotton aphids (Ebert and Cartwright 1997). There is evidence that the alate nymphs and adults of *A. gossypii* are more tolerant to insecticides than

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<sup>2</sup>Address all inquiries (email: tkring@uark.edu).

the apterous forms, possibly due to size difference, amount of sclerotization, and/or difference in behavior (Grafton-Cardwell 1991).

Insecticides that have proven most effective against cotton aphids during recent years include dicotophos (Bidrin® AMVAC Los Angeles, CA) and imidacloprid (Provado® and Trimax® Bayer CropScience, Kansas City, MO) (Layton et al. 2001). Imidacloprid is a neonicotinoid compound that acts as a contact and systemic stomach poison (Mullens 1993, Nauen et al. 1998). The mode of action of imidacloprid is similar to that of nicotine, acting as an agonist on the nicotinic acetylcholine receptor in the nerve's postsynaptic membranes, causing the insect to reduce or stop feeding and reduce mobility (Boiteau and Osborn 1997). Imidacloprid has a high degree of residual activity against cotton aphids although the compound is slow acting (Boiteau and Osborn 1997).

Dicotophos is an organophosphate compound that provides inexpensive control of susceptible aphid populations in both relatively early and mid-season cotton aphid populations (Kharboutli and Allen 2000). Dicotophos acts as a contact and systemic stomach poison, interfering with nerve transmission by depressing cholinesterase levels in the blood and nervous systems of vertebrate and invertebrate animals. Insects treated with dicotophos exhibit reduced mobility and feeding (Gianessi 1997).

Observations from a 3-yr field study conducted at Delta Branch Research Station in Clarkedale, AR, on cotton aphid thresholds revealed that the percentage of winged aphids in samples increased by 3 to 10% after each application of 0.044 kg ai/ha of imidacloprid compared to an untreated control (Conway et al. 2003). Although the observed increase in the proportion of winged aphids may have been a result of reduced susceptibility to the insecticide, the number of alatiform aphids prior to treatment in all plots was low. Preliminary laboratory tests on increased wing formation after imidacloprid treatment corroborated these field observations (Conway and Kring 2003). The objective of this study was to evaluate the extent of wing formation and fecundity in offspring from treated apterous adult cotton aphids with respect to mode of exposure to imidacloprid (topical vs systemic) and relative to another common aphicide (dicotophos).

## Materials and Methods

Wing formation data obtained from a 3-yr field study conducted at Delta Branch Research Station in Clarkedale, AR (Conway et al. 2003) were evaluated by comparing wing formation from the plots having two imidacloprid treatments per year with counts taken from untreated control plots. Insecticide applications were made on 18 and 28 June 1999, on 28 June and 3 July 2000, and on 7 and 12 July 2001. Methods for the field study involved bi-weekly sampling of aphids on two leaves from each of 20 plants/plot having four plots/treatment with each plot being approximately 0.3 ha in size. Total aphid counts were taken from each sampled leaf, and the numbers of nymphs, adults, mummies, and alate aphids were recorded.

Aphids used in laboratory experiments were apterous adult cotton aphids taken from a laboratory colony maintained on cotton (NuCOTN 33B, Delta and Pine Land Company, Scott, MS) at the University of Arkansas, Fayetteville. The culture was initiated from aphids taken from cotton at the Delta Branch Station near Clarkedale, AR. The colony was periodically refreshed with field-collected aphids to maintain vigor and aphid size. The aphids were raised on cotton in an environmentally controlled cabinet (Percival, Boone, IA) at  $18 \pm 2^\circ\text{C}$  and a photoperiod of 14:10 (L:D).

Probit analysis (SAS Institute, Inc. 1999) using five application rates from 0 to 0.54 mg/L of imidacloprid (Provado® 1.6F, Bayer Corporation, Kansas City, MO) and probit analysis using five application rates from 0 to 0.40 mg/L of dicrotophos (Bidrin® 8EC (AMVAC, Los Angeles, CA) were used in a spray booth (Research Track Sprayer SB6-079, DeVries Manufacturing, Hollandale, MN) to establish LC<sub>50</sub>'s values. The recommended application rate for aphids in Arkansas cotton is ~0.27 mg/L imidacloprid and ~0.175 mg/L dicrotophos (Johnson et al. 2002). The dicrotophos treatment was included to determine whether an insecticide-induced stress in the aphid survivors would cause increased wing formation in offspring. No prior observations have been made of increased wing formation following treatment with dicrotophos.

After the LC<sub>50</sub>'s were established, tests were conducted to evaluate the impact of insecticide on wing formation and on fecundity of offspring from founding aphids following five treatments: (1) imidacloprid near LC<sub>50</sub> concentration on cotton, (2) dicrotophos near LC<sub>50</sub> concentration on cotton, (3) water spray on cotton, (4) imidacloprid spray near LC<sub>50</sub> concentration on silk plants, and (5) water spray on silk plants. Silk plants were used to differentiate the topical vs the systemic effects of imidacloprid. Cotton was grown in 10-cm clay pots with standard potting soil in a greenhouse. Three silk plants or three randomly selected cotton plants were used for each treatment (completely randomized design with 10 replicates of the 5 treatments, each plant was an experimental unit). Prior to spraying, 10 adult apterous cotton aphids were transferred onto each true leaf (4/plant) of each plant (the experimental unit) with a fine camel hair brush. Prior to spraying, the aphids were allowed to settle and were recounted to insure all leaves still held 10 aphids.

After being sprayed, plants were allowed to dry for approximately 10 min and were then moved to a growth chamber (Convicon Controlled Environments Limited, Winnipeg, Manitoba Canada) [25:20 ± 1°C (D:N), 14:10 (L:D)]. Three hours post treatment, aphids were transferred from the sprayed silk plants onto untreated cotton plants in labeled pots. These plants were then moved into the growth chamber with the other nine plants. Based on dose-mortality values from probit analysis, live aphids were counted on each true leaf 48 h post-treatment to check mortality and to establish the number of live founding adults for subsequent fecundity estimation.

The life cycle of cotton aphids is extremely short, requiring only 5 d to complete one generation (Isely 1946), with a maximum of two generations in 10 d. Ten days post-treatment, the plants were taken into a laboratory where aphids on each leaf were counted and examined under a microscope for classification as either apterous or alate, based on the presence or absence of wings or wing pads. Because wing pads are not visible until late in the second nymphal stage, all first and early second stage nymphs were classified as apterous, producing an underestimate of the percentage of alate offspring. Each sample consisted of all the aphids from one cotton plant, with three plants (12 leaves) per treatment per test. Ten replicates of the test were conducted by the same protocol over time (120 leaves/treatment). Data on wing formation with log<sub>10</sub> transformed means and on fecundity were analyzed by analysis of variance with means separated by PROC GLM, ANOVA, and t-tests (SAS Institute 1997).

## Results

Mean peak density (1 July) for percentage wing formation in the conventional treatment in 1999 was 9.0% with insecticide applications on 18 and 28 June. Mean

peak density (20 July) for percentage wing formation in the conventional treatment in 2000 was 16.7% with insecticide applications on 28 June and 3 July. Mean peak density (18 July) for percentage wing formation in the conventional treatment in 2001 was 7.0% with insecticide applications on 7 and 12 July (Fig. 1). Mean peak density for percentage wing formation in the control was 8.3% on 1 July 1999, 7.2% on 29 June 2000, and 3.0% on 29 June 2001 (Fig. 1). Year-to-year variation in timing of peak densities is typical of aphids (Dixon 1998).

In the laboratory experiment to establish  $LC_{50}$ 's, control mortality 4 d post treatment was low in each replicate. There was a significant increased rate response in mortality from the  $0.54 \text{ mg L}^{-1}$  concentration of imidacloprid, which reached 87% aphid mortality at 4 d post-treatment. Imidacloprid  $LC_{50}$  was 0.12 ppm (Table 1), similar to a previously reported  $LC_{50}$  of 0.11 ppm (Nauen et al. 1998), and these measurements would be equivalent to a field rate of  $-0.014 \text{ kg ai/ha}$ . There was also a significant increased rate response in mortality on the  $0.40 \text{ mg L}^{-1}$  concentration of dicrotophos, which reached 79% mortality 4 d post-treatment. The  $LC_{50}$  for dicrotophos was 0.05 ppm (Table 1), and these measurements would be equivalent to a field rate of  $-0.092 \text{ kg ai/ha}$ .

As expected, aphid survivorship 48 h post-treatment was highest in water controls compared to other treatments (Table 2). The aphids on water-treated cotton plants had a significantly higher survival rate (11.4% higher) than aphids on water-treated silk plants 48 h post-treatment, possibly due to aphid restlessness on the silk plants from the spray and the lack of food during the 3-h post-spray period. Imidacloprid's contact effect was indicated by the imidacloprid-treated aphids on silk plants having a 19.5% decrease in the aphid survivorship relative to the water-treated aphids on silk plants (Table 2). The systemic effect of imidacloprid was indicated by the 6.9% decrease in survivorship of imidacloprid-treated aphids on cotton plants 48 h post-spray relative to imidacloprid treated aphids on silk plants (Table 2).

In the test evaluating wing formation and fecundity, highest aphid densities were observed on water-treated cotton plants and on water-sprayed silk plants (Table 2). By day 10 post-treatment, the imidacloprid-treated silk plants, which held only a mean of  $26.4 \pm 1.5$  founding adult aphids per plant on d 2, supported an aphid density of  $992.1 \pm 42.5$  per plant. On d 10 the insecticide-treated cotton plants held the lowest densities of aphids with dicrotophos-treated plants having approximately half as many aphids as on control plants. Similarly, imidacloprid-treated cotton plants had the fewest aphids at d 10, with one sixth the number of control plants (Table 2).

Significantly more winged aphids were produced on imidacloprid-treated cotton plants compared to other treatments ( $P \leq 0.0001$ ) (Fig. 2). There were no differences in wing formation in offspring from aphids in other treatments. There was a significant decrease in fecundity (offspring per founding aphid) from imidacloprid-treated aphids held on cotton ( $P \leq 0.0001$ ) (Fig. 3) compared to all other treatments.

## Discussion

Crowding and nutritional factors are the two main forces involved with the production of alates in most aphids, including *A. gossypii* (Tamaki and Allen 1969, Ebert and Cartwright 1997, Dixon 1998). Colonies with fewer than three aphids seldom produce alates, while colonies with more than three aphids often produce alate offspring (Reinhard 1927). Unfortunately, research has not identified the relative importance of nutrition versus crowding, and just two aphids can cause enough tactile stimulation

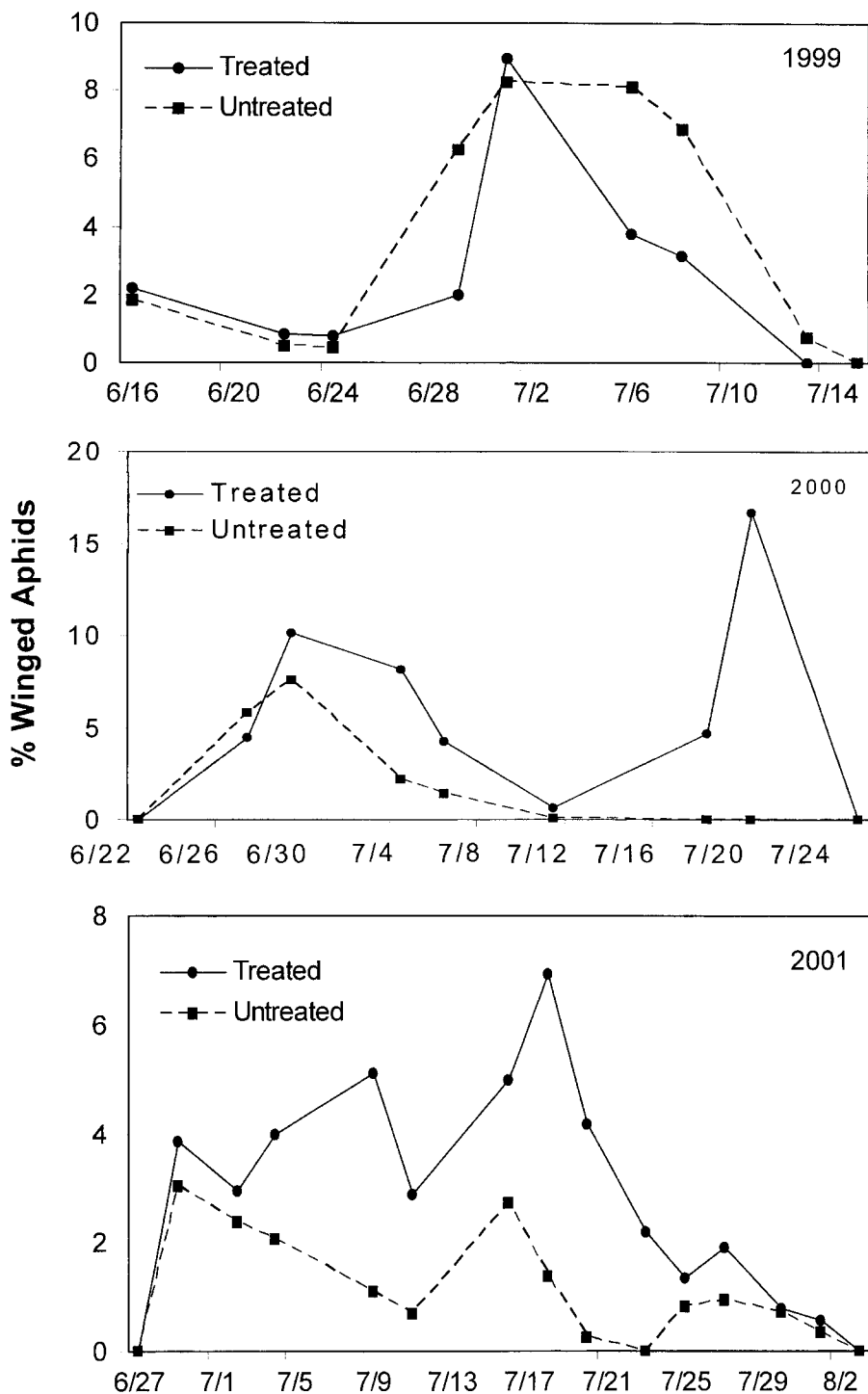


Fig. 1. Percentage of winged aphids in a 3-year field study at University of Arkansas Research Station in Clarkedale, AR. Treatments were made with imidacloprid 0.044kg ai/ha (0.04 Lb ai/A) applied by ground equipment.

**Table 1. Probit response of the cotton aphid, *Aphis gossypii*, from five concentrations of imidacloprid and of dicrotophos**

Insecticide	<i>n</i>	LC <sub>50</sub> (mg/L) (95% FL)	LC <sub>95</sub> (mg/L) (95% FL)	Slope (+SE)	Pearson Chi Sq
Imidacloprid*	800	0.12 (0.06-0.24)	3.43 (2.25-7.86)	1.24 (±0.11)	0.78
Dicrotophos**	800	0.05 (0.03-0.07)	1.90 (1.09-5.45)	1.08 (±0.15)	2.81

\* LC<sub>50</sub> imidacloprid solution 0.12 ppm (0.014 kg ai/ha).

\*\* LC<sub>50</sub> dicrotophos solution 0.05 ppm (0.092 kg ai/ha).

**Table 2. Cotton aphid laboratory survivorship on cotton two days post-treatment**

Treatment	Initial # aphids (No.)	Day 2 Survivorship ± SE (%)	Day 10 Mean # aphids/plant (No. ± SE)
Water control	1200	97.0 ± 0.7	1294.5 ± 108.3
Imidacloprid*	1200	59.1 ± 1.3	208.3 ± 29.4
Dicrotophos**	1200	51.4 ± 1.0	574.8 ± 53.9
Silk with water	1200	85.6 ± 1.2	1028.8 ± 74.5
Silk with imidacloprid*	1200	66.0 ± 1.6	992.1 ± 42.5

\* Imidacloprid solution 0.12 ppm (0.014 kg ai/ha).

\*\* Dicrotophos solution 0.05 ppm (0.092 kg ai/ha).

between them to promote wing induction (Muller et al. 2001). From imidacloprid treatment, we had hypothesized decreased aphid motility with less physical contact between aphids, yet imidacloprid use in this experiment resulted in increased wing formation. Given the extra handling of aphids treated on silk plants, we anticipated an increase in wing formation in these treatments, but no such effect was observed.

Aphids on a more nutritious host produce more offspring than those on poor hosts (Tamaki and Allen 1969). On poor quality hosts, aphids are more restless and more likely to contact other aphids, producing a crowding type response (Tamaki and Allen 1969). Most nutritional studies indicate that increases in the number of alates occur along with increases in the total number of aphids. In our studies with water-treated plants having over six times the number of aphids per plant as imidacloprid-treated cotton, a crowding effect may have been created and, thus, may have caused an underestimation of the actual percentage of wing formation in the imidacloprid-sprayed cotton. Aphid densities in the field study peaked at ~140, ~80, and ~30 aphids per leaf in untreated plots and in the treated plots at ~45, ~25, and ~15 in 1999, 2000, and 2001, respectively (Conway et al. 2003). In 1999, a crowding effect from the high aphid density in the untreated plots may have caused an increase in wing

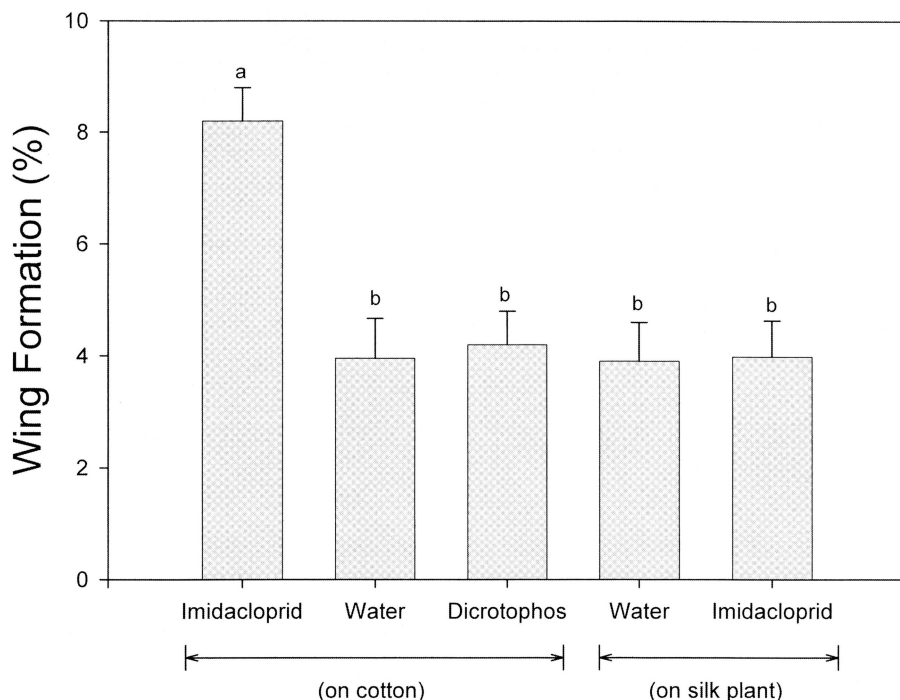


Fig. 2. Cotton aphid wing formation by treatment with imidacloprid (0.0138 kg ai/ha) and dicotophos (0.092 kg ai/ha). Bars with the same letter are not significantly different ( $P < 0.05$ ; ANOVA, LSD). Error bars = SE.

formation. In 2000 and 2001, wing formation increased markedly in the treated vs untreated plots, especially shortly after the imidacloprid treatment.

The only previously reported external force that has induced the production of alate aphids is tactile stimulation which mimics the effect of crowding (Lees 1967). The physical influence of spray likely induces movement and may have increased tactile stimulation in some aphids in our tests, but because we sprayed with a water control, this impact was controlled by design.

Our data demonstrate a relationship between imidacloprid application on cotton and wing formation in the offspring of treated adults that involves a systemic effect of the insecticide rather than a topical effect. This relationship suggests the presence of some factor or factors from the imidacloprid treatment on cotton that induces the cotton aphid to produce winged offspring independent of aphid crowding or plant senescence. Surviving imidacloprid-treated aphids may form wings because they are irritated and feed less, thus producing offspring which may disperse by flight.

The production of wings could be caused by the insecticide acting on the endocrine system in a way similar to that of precocenes (Hardie et al. 1996) or by the impact of the insecticide on the plant or a combination of the two. The precise process which caused imidacloprid to induce production of winged offspring remains unknown.

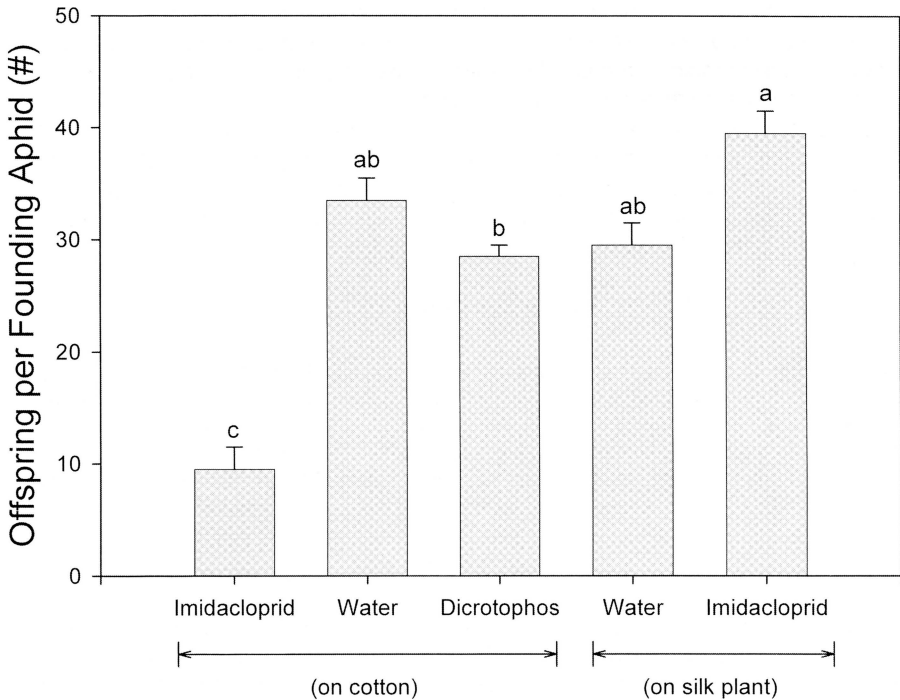


Fig. 3. Cotton aphid fecundity by treatment with imidacloprid (0.0138 kg ai/ha) and dicotophos (0.092 kg ai/ha). Bars with the same letter are not significantly different ( $P < 0.05$ ; ANOVA, LSD). Error bars = SE.

In our preliminary experiment, using a constant temperature of  $20 \pm 3^\circ\text{C}$ , (13:11, L:D), fecundity in the water-treated aphids was considerably lower ( $9.2 \pm 1.0$  offspring per adult) than we observed in this experiment (Conway and Kring 2003). This difference was likely due to increased and fluctuating (D:N) temperature and an hour longer daylight used in the current test. The cotton aphid's reproductive capacity increases with increasing temperature between 13 and  $21^\circ\text{C}$  but decreases with increasing temperatures between 22 and  $32^\circ\text{C}$ , while developmental time decreases with increasing temperature between 18 and  $28^\circ\text{C}$  (Isely 1946). Increasing day length from 6 h to 12 h to 18 h increases the intrinsic rate of increase, decreases population doubling time, and decreases generation time (Aldyhim and Khalil 1993). The increase in wing formation ( $-4\%$  to  $8\%$ ) in the imidacloprid-treated aphids on cotton relative to controls in this test was smaller ( $2\%$ - $12\%$ ) than in our previous test (Conway and Kring 2003), possibly due to the increased aphid density in control plants, thus leading to increased crowding.

When imidacloprid was applied to cotton plants, surviving cotton aphids produced a significantly higher percentage of winged offspring with significantly fewer offspring per founding adult. In addition to increasing emigration by winged aphids, an increase in the proportion of winged aphids among survivors of an imidacloprid treatment may result in a decrease in the number of aphids in the treated field since winged aphids



require a longer developmental time, produce fewer offspring (Noda 1959), and have an increased risk of mortality (Dixon 1998). Thus, an increase in the proportion of winged offspring would decrease the overall number of aphids in the field and increase the apparent efficiency of the insecticide.

Additional research is needed to determine the cause of wing formation induced by imidacloprid application. Additionally, the tendency for the insecticide-induced alate aphids to initiate flight (trivial or migratory) needs to be determined. Finally, the fitness of these induced dispersal forms needs to be compared with aphids induced to form wings under normal conditions.

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