EFFECTS OF DEFOLIANTS ALONE AND IN COMBINATION WITH INSECTICIDES ON BOLL WEEVIL AND WHITEFLY IN COTTON. B. BOLL WEEVIL FLIGHT BEHAVIOR

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Abstract

Boll weevil flight behavior was examined by tethering individuals to 16 computer-interfaced flight mills. Measured parameters included total flight distance, total flight duration, number of flights, flight speed, average flight distance, and average flight duration. Weevils were reared from infested squares and their flight behavior tested at different ages after eclosion. There were no significant differences between males and females for any of the flight parameters measured at any of the ages tested. For the first 5 days post-eclosion, flights were short in distance and duration, then increased to a plateau by age 9 d that remained relatively constant through age 28 d. Average flight speed varied little with age, ranging from 1.2 to 2.1 km/h, with the exception of 3 day old weevils, which flew significantly slower (0.5 km/h). Maximum recorded flight speed was 5.0 km/h. Flight behavior was tested using weevils exposed to the defoliant Def, the insecticides Guthion and Karate, and to combinations of Def and the insecticides. Statistical differences were not detected among treatment groups from any of the trials or combinations of trials for any of the boll weevil flight parameters calculated. However, because of high variation in flight behavior among individuals, much larger sample sizes will be necessary to fairly evaluate this question.

Introduction

Boll weevils (*Anthonomus grandis*) disperse out of cotton fields at the end of the growing season, probably because of a deteriorating food supply and lack of suitable oviposition sites (Fenton and Dunnam 1928, Dunnam 1929, Gaines 1932, Jones and Sterling 1979, Guerra 1986). However, the extent, distance, and timing of weevil dispersal, and the effects of intrinsic factors such as age, sex, and reproductive development on such movement are largely unknown. Chemical defoliation of the crop may induce weevils to disperse out of a field either indirectly through accelerated deterioration of the host crop, or directly through effects on weevil flight behavior. Experiments on the effects of chemical defoliants alone or mixed with the organophosphate Guthion or the synthetic pyrethroid Karate (Greenberg et al. 2001a,b) provided an opportunity to investigate their influence on boll weevil dispersal in the field (Sappington et al. 2001) and their direct effects on weevil flight behavior.

Laboratory assays are essential to identifying sources of variation in flight behavior among individual insects (Sappington and Showers 1991). Flight mills are an invaluable tool for characterizing behavioral variation generated by age, sex, nutritional history, crowding, reproductive development, mating status, and genetic predisposition (Sappington and Showers 1991, 1992a,b, 1993, Sappington et al. 1995, Taylor et al. 1992, Cooter and Armes 1993, Stewart and Gaylor 1994). Previous work with boll weevils on flight mills was preliminary in nature and limited to assessing flight speed (McKibben et al. 1988, 1991). We employed an ultralow-friction flight-mill system, previously designed and constructed for use with noctuid moths (Beerwinkle et al. 1995), to examine boll weevil flight behavior. Our objectives in this study were to determine the effects of boll weevil age and sex on several flight parameters measured on flight mills. Having that data as a foundation allowed us to make comparisons in flight parameters among weevils exposed to various chemical defoliants, insecticides, and combinations.

Materials and Methods

Flight Mill System

Sixteen flight mills were placed on shaded shelves in a greenhouse. Temperature in the greenhouse could not be controlled, but high temperatures were moderated somewhat by an automated misting system. The flight mills used were described by Beerwinkle et al. (1995). The pivot consisted of a stationary pin fitted into a plastic housing attached to the flight arm at its center of gravity. The pin and the housing were fitted with magnets of opposite polarity, so that the arm floated above the pin, resulting in very little frictional drag at the pivot. One rotation of the flight arm represented one meter of flight by an attached boll weevil. An infrared eye counted the revolutions and fed the information into a computer.

A boll weevil was tethered to a flight mill by attaching one end of a 2-cm length of 0.3 mm diameter fishing line to the prothorax with Duro® Super Glue (Loctite Corporation, Rocky Hill, CT). The line was positioned so that it extended forward over the head, taking advantage of its natural curve (arising from its storage on a roll) to sweep upward slightly away from the weevil. The other end of the line was glued into a sleeve (approx. 1 cm long) consisting of insulation stripped from a small copper wire. The weevil was tethered to the flight mill by slipping the distal end of the sleeve over the point of the flight arm. The flight arm was rotated as needed to correct for the curve in the line so that the weevil was suspended parallel to the ground. Suspended weevils were provided with a small piece of tissue paper for tarsal contact to reduce trivial flight activity during placement on the flight mills. Weevils were left with the tissue to drop on their own when initiating flight.

Weevils were attached to the flight mills before 0900 h and were removed no earlier than 1800 h. The software (described in Beerwinkle et al. 1995) recorded revolutions in 10-sec intervals, and the time of day when flight activity occurred. From this information we calculated (for individual weevils over the period 0900-1800 h) total flight distance, total flight duration, number of flights, flight speed, average flight distance, and average flight duration. Individual weevils were tested only once.

Effects of Age and Sex on Flight Behavior

Boll weevils were reared from infested squares collected in the field near Weslaco in the Lower Rio Grande Valley of Texas in April 2000. Pupae were removed from the squares and placed in moist vermiculite and monitored for adult eclosion. The day of eclosion was considered Day 0, and the age of individual weevils tracked accordingly. Adults of mixed sexes were held in groups of 20 or less in cardboard cartons (1-pt.) with screen lids, segregated by date of emergence. They were supplied with small debracted bolls (at a rate of one boll per 10 weevils changed every 3 days), and with distilled water via a cotton wick inserted in a 5-ml glass jar. Usually, eight weevils of each sex (sexed by the tergal notch method, Sappington and Spurgeon 2000) and of a single age were chosen for flight on a given day. The total number of weevils flown per age group over the course of the experiment ranged from 10 (age 1 -- the glue did not adhere well to the cuticle of this age group) to 40, but was usually 32.

Differences in flight parameter means between sexes at a given age were tested for significance ($\alpha = 0.05$) with the t-statistic. Differences among means across ages were tested for significance with the non-parametric

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:973-977 (2001)
National Cotton Council, Memphis TN

Kruskal-Wallis test. All statistical tests were performed with Statistix7 software (Analytical Software 2000).

Chemical Effects on Flight Behavior

Boll weevils were reared from infested squares as described above. Two treatment groups of weevils aged 3 or 14 days were placed in 15-cm Petri dishes (10 weevils per dish, 3 dishes per treatment) each with a cotton leaf treated with water (control), full-rate Def (2.34 L/ha), full-rate Dropp WP (224 g AI/ha) (Drp), full-rate Karate Z (37 g AI/ha) (Kar), ½-rate Guthion (140 g AI/ha) (Gut), full-rate Def + full-rate Karate (D+K), or full-rate Def + ½-rate Guthion (D+G) as described by Greenberg et al. (2001a). The chemicals were applied with a laboratory spray chamber (De Vries Mfg., Hollandale, MN), calibrated to deliver 56 L/ha through a TXVS-4 nozzle at 1.7 kg/cm^2, and 4.8 km/h. Surviving weevils were tethered to flight mills 1, 2, or 3 days after treatment. Thus, treated weevils were tested at 4-6 days old in one trial and at 15-17 days old in the second trial. Weevils in the dishes were chosen at random for flight mill testing without regard to sex. The experiment was repeated later in a third trial using weevils of unknown age captured in pheromone traps in November 2000. By the time of this latter experiment, the flight mills had been moved inside a walk-in environmental chamber. Temperature was maintained at 30^{o}C under a bank of incandescent light bulbs.

Small plot field experiments (described in more detail in Greenberg et al. 2001b, and Sappington et al. 2001) were conducted at the end of July 2000 in which the same treatments (except Drp) were applied in three replications to mature cotton (b open bolls). Weevils collected in these plots with a tractor-mounted vacuum sampler (Beerwinkle et al. 1997, Raulston et al. 1998) on the day before treatment (controls), 2 days after treatment, and 3 days after treatment were tested on the flight mills on the same day. Weevils collected one day after treatment were not tested due to unexpected logistical difficulties encountered on that day.

Results and Discussion

Effects of Age and Sex on Flight Behavior

There were no significant differences between males and females for any of the flight parameters measured at any of the ages tested. For example, in the case of total distance flown, both sexes showed the same pattern: short distances flown at ages 1 and 3, peaks at ages 7 and 11 with a dip at age 9, and a decline in distances flown at age 14 (Fig. 1A). The peak at age 7 in males is quite modest compared to that of females, and the difference may prove to be statistically significant with larger sample sizes. The differences between sexes near the ages tested in the chemical treatment experiments (4-6 and 15-17 day old) were not great, so we concluded that any sex-related differences observed in those experiments would represent differential response to the chemicals rather than age-related differences.

The age-related pattern described above for total distance flown (Fig. 1A-B) was the same for total flight duration (Fig. 2A), suggesting that flight speed did not change with length of flight as it does in some migratory insects (Sappington and Showers 1992b). When mean distance per flight and mean minutes per flight were plotted against age (Fig. 1C and Fig. 2B, respectively), a clearer pattern of age-related flight activity was revealed than when daily totals were plotted. For the first 5 days post-eclosion, flights were short in distance and duration. By age 7 d, flights became longer, reaching a plateau by age 9 d that remained relatively constant through age 28 d. However, there was a great deal of variability in these parameters among weevils 9 days old and older, indicating that factors other than age were generating variability among individuals.

Average flight speed varied little with age, ranging from 1.2 to 2.1 km/h, with the exception of 3 day old weevils, which flew significantly slower (0.5 km/h) (Fig. 3). These results on flight speed are consistent with those found by McKibben et al. (1988) using a different flight mill system, and reiterate the point that boll weevils are not strong fliers. Maximum recorded flight speed was 5.0 km/h, similar to that reported by McKibben et al. (1991) of <4.8 km/h.

Chemical Effects on Flight Behavior

Statistical differences were not detected among treatment groups from any of the trials or combinations of trials for any of the boll weevil flight parameters calculated. However, environmental conditions and weevil developmental history were not equivalent among the different trials, making justification for pooling data from different trials somewhat tenuous. Sample sizes within trials were therefore fairly small especially when multiple treatments per trial were considered. Thus, far more extensive testing is needed and is being planned.

Nevertheless, some potentially interesting trends are evident in the data. Sappington et al. (2001) found that post-treatment dispersal out of the experimental field was substantial. Among the weevils collected from the plots 2-3 days post-treatment, there was a tendency for more individuals to fly long distances (> 2 Km) than among those collected the day before treatment. The same trend is evident when the field data are pooled with that from weevils captured in pheromone traps and exposed to defoliant and insecticide in the laboratory (Fig. 4A). Female weevils collected from the plots at the time of the experiment in late July showed predominantly diapause characteristics upon dissection (Greenberg et al. 2001b), and it is presumed that weevils captured in pheromone traps in November were probably mostly in diapause, but these were not dissected and certainly this is not an entirely safe assumption. Frequency profiles of average flight speeds for weevils in these data sets suggest a trend for slower flight among those that were treated with a chemical compared to untreated controls (Fig. 5B). In contrast, frequency profiles of total flight distance and flight speed among treated and untreated weevils from the initial laboratory trials with weevils of known age (young and old pooled) appear quite similar to one another (Fig. 6).

Although we did not find statistical evidence that the chemical applications used in this study affected flight behavior directly, more experimentation will be necessary to build adequate sample sizes necessary to rigorously assess this question. In addition, we have not yet tested the effects of these chemicals on flight the day of exposure. Sappington et al. (2001) reported evidence that movement out of the experimental field may have been greatest on the day of treatment, and clearly such experiments are called for.

Acknowledgments

We thank Jesus Cabellero, Veronica Cardoza, Peter Carreon, V. Greenberg, Lucas Leal, Elizabeth Razo, and Orlando Zamora for expert technical assistance. We thank Charles Suh and Rosalind James for critical review of the manuscript. Mention of a proprietary product does not constitute an endorsement for its use by USDA.

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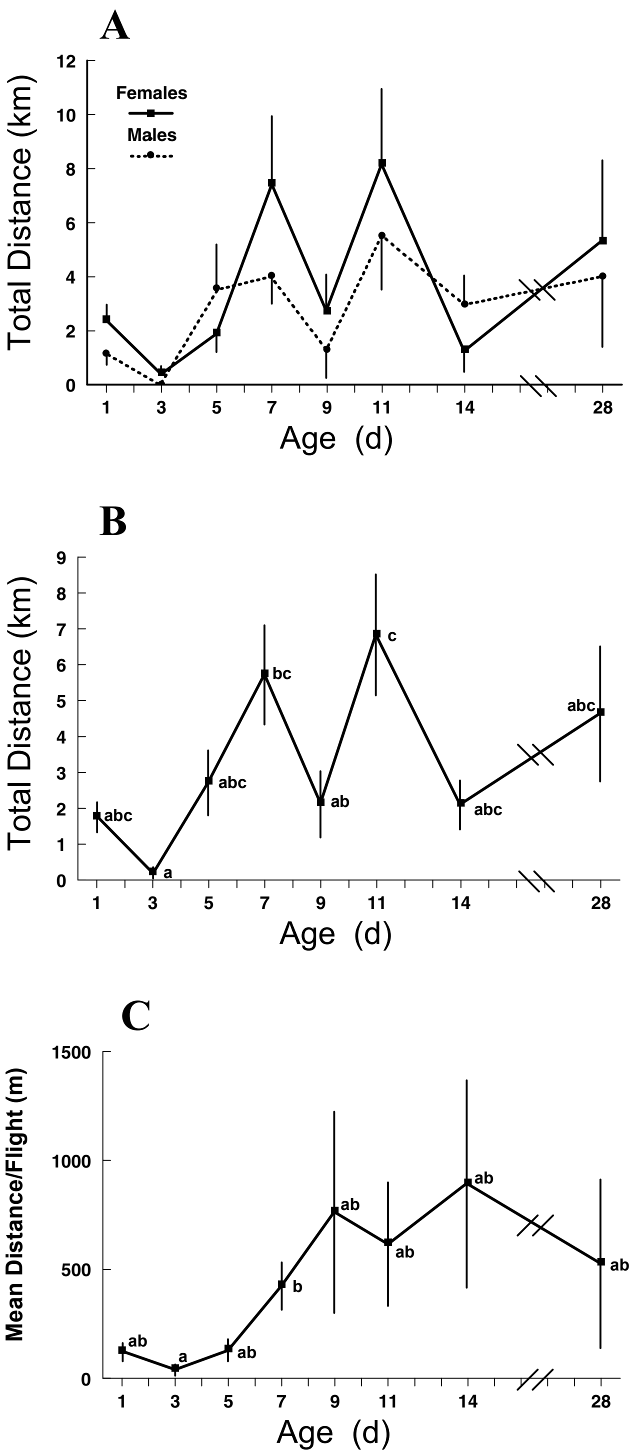

Figure 1. Boll weevil flight distance profiles by age post-eclosion. A) Total flight distance, males and females separately, over a 9-hour period. There are no significant differences between the sexes at any age (t-test, " = 0.05). B) Total flight distance, sexes combined, over a 9-hour period. C) Mean distance per flight by age, sexes combined. Means followed by the same letter are not significantly different (Kruskal-Wallis test; " = 0.05). Bars, S.E.

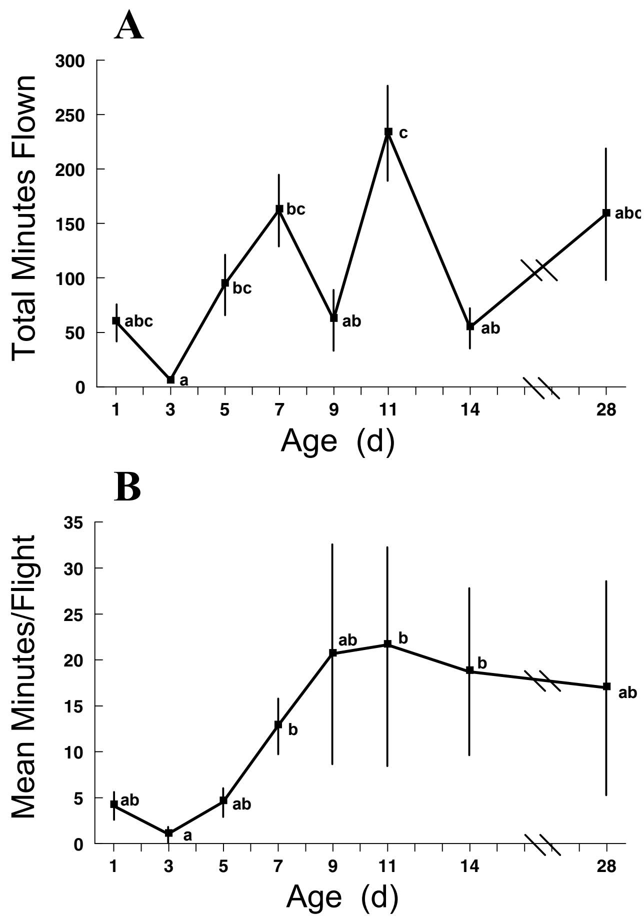

Figure 2. Boll weevil flight duration profiles by age post-eclosion. A) Total time in flight, sexes combined, over a 9-hour period. B) Mean flight duration per flight by age, sexes combined. Means followed by the same letter are not significantly different (Kruskal-Wallis test; " = 0.05). Bars, S.E.

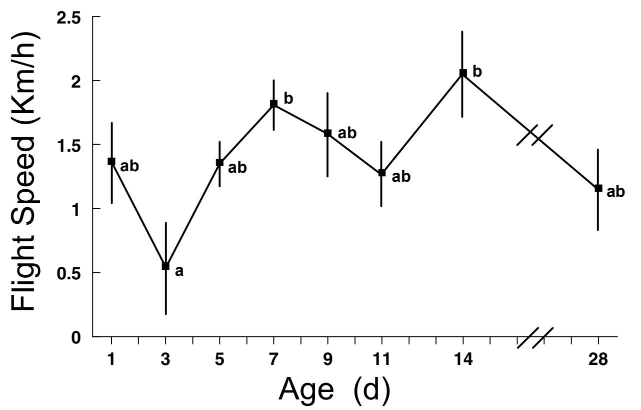

Figure 3. Boll weevil flight speed profiles by age post-eclosion. Means followed by the same letter are not significantly different (Kruskal-Wallis test; " = 0.05). Bars, S.E.

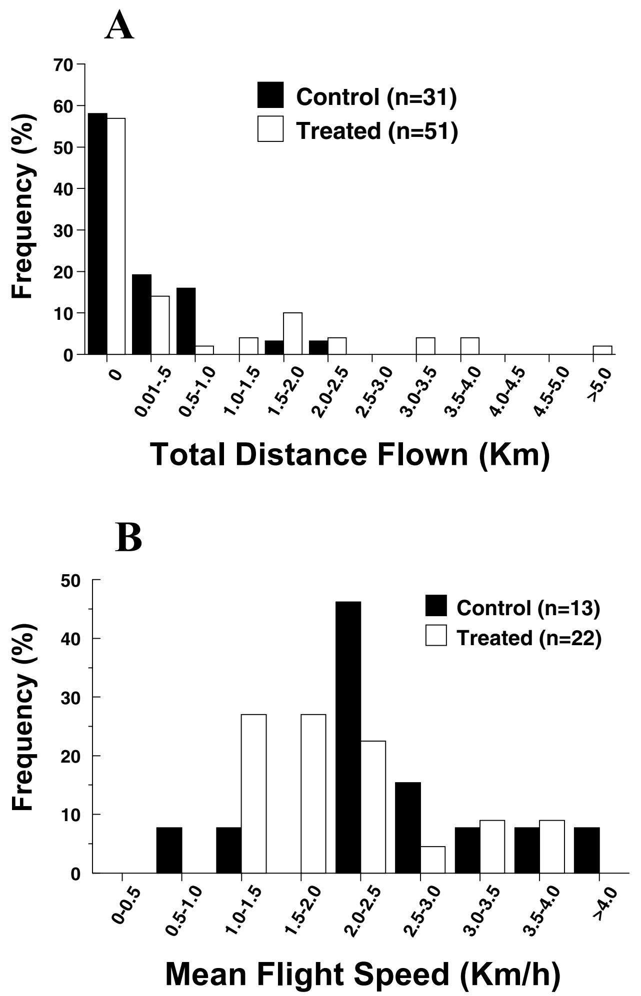

Figure 4. Percent frequency profiles of A) total distance flown and B) mean flight speed on flight mills for untreated control (collected 1 day pre-treatment from the experimental field) and treated (collected 2-3 days post-treatment, all treatments pooled) boll weevils pooled with data from weevils captured in pheromone traps in November.

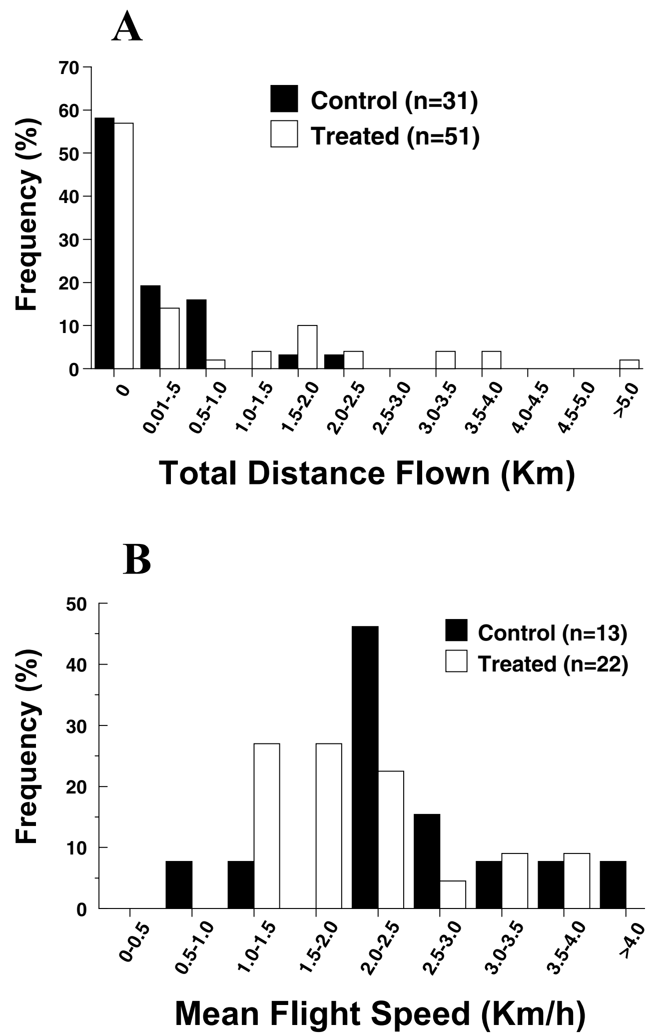

Figure 5. Percent frequency profiles of A) total distance flown and B) mean flight speed on flight mills for untreated control and treated (all treatments and ages pooled) boll weevils reared from infested squares.