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Effects of insecticides and defoliants applied alone and in combination for control of overwintering boll weevil (Anthonomus grandis; Coleoptera: Curculionidae)—laboratory and field studies

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Abstract
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Keywords
insecticide, defoliant, boll weevil, cotton, Anthonomus grandis, tribufos, thidiazuron, thibensulfuron-methyl, lambda-cyhalothrin, azinphos-methyl

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Effects of insecticides and defoliants applied alone and in combination for control of overwintering boll weevil (*Anthonomus grandis*; Coleoptera: Curculionidae) — laboratory and field studies†‡

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Abstract: In laboratory, greenhouse and field tests, we determined the effects of combining full rates of the defoliants tribufos and thidiazuron and the herbicide thifensulfuron-methyl with half rates of the insecticides lambda-cyhalothrin or azinphos-methyl, and the combination of tribufos and thidiazuron, both in half rates, on mortality of the boll weevil, *Anthonomus grandis grandis* Boheman and on the quality of defoliation. Tribufos, 0.47 kg ha^{-1} and tribufos, 0.235 kg ha^{-1} + thidiazuron, 0.125 kg ha^{-1} exhibited a slightly toxic effect to boll weevil, while tribufos, 0.47 kg ha^{-1} + lambda − cyhalothrin, 0.019 kg ha^{-1}, tribufos, 0.47 kg ha^{-1} + azinphos − methyl, 0.14 kg ha^{-1}, and tribufos, 0.235 kg ha^{-1} + thidiazuron, 0.125 kg ha^{-1} + azinphos − methyl, 0.14 kg ha^{-1}, provided control of boll weevil as good as or better than full-rate azinphos-methyl or lambda-cyhalothrin alone owing to synergistic effects. Thidiazuron or thifensulfuron-methyl alone or in combination with insecticides did not affect boll weevil mortality. Treatment with tribufos + thidiazuron, both at half rate, significantly increased defoliation compared to full rates of tribufos or thidiazuron alone, and provided adequate defoliation for approximately the same cost per hectare.
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1 INTRODUCTION

The boll weevil, *Anthonomus grandis grandis* Boheman, remains a key pest of cotton in non-eradicated areas of the US cotton belt. Total costs for boll weevil (damage and control) exceeded $381 million in 2000.[1] Chemical control programs against this pest that rely on broad-spectrum insecticides have associated environmental problems, and may lead to insect resistance. Alternatively, integration of pesticides with cultural practices, such as defoliation, may provide opportunities for reducing insecticide input.

Chemical defoliants of cotton are commonly used as a harvest aid, causing leaf abscission, earlier boll opening and shedding of young fruiting forms, so reducing boll rot and preventing deterioration in quality of seed and fibre.[2–6] Application of defoliants is an important component of short-season cotton production practices, and shortens the time that cotton is vulnerable to insect attack. The change from conventional long-season cotton to a short-season system alters the strategies of control of pink bollworm, boll weevil, bollworm and tobacco budworm complex, whiteflies, aphids and spider mites by eliminating leaves, squares and small bolls which provide feeding and oviposition sites for insect pests.[7–14] Defoliation is thought to facilitate dispersal of boll weevils out of

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the fields, and some of these weevils may overwinter to infest young cotton the following year.[8,15] However, the possibility that defoliants may have lethal or sublethal effects on boll weevil has not been examined. Thus, in many regions, insecticides are applied to fields just prior to defoliation ('diapause treatment') to reduce the potential overwintering population. Some authors indicate that defoliant combined with insecticide resulted in additive and synergistic effects on insect mortality.[16–19] Most experiments with cotton defoliants are concerned with their effects on plant physiology, yield and crop quality. Effects of defoliants on insects have been suggested in many cases only by observations or assumptions, but are poorly known, and detailed studies are necessary. The objective of the current study was to examine combinations of insecticides with defoliants for their ability both to control boll weevils and to achieve adequate and cost-effective defoliation. The study was conducted in laboratory, greenhouse and small field plots. Knowledge of how various defoliants alone and in combination with insecticides affect boll weevil and other insect pests in cotton may reveal opportunities for reducing the number of late-season insects and the dispersal of some of them to other host plants.

2 MATERIALS AND METHODS

2.1 Plants and boll weevil culture

Cotton (*Gossypium hirsutum* L), DPL-5415 RR (Delta Pine Land, Scott, MS), was used in all tests. Boll weevil adults were obtained from (1) an established colony at the USDA APHIS-PPQ, Mission Plant Protection Center, Mission, Texas and were reared on an artificial diet,[20,21] and from (2) infested squares collected in a cotton field in the Lower Rio Grande Valley of Texas and held in an environmental chamber for adult emergence.

2.2 Defoliants and insecticides

Two formulated defoliants, tribufos (*S,S,S*-tributylphosphorotrithioate) 720 g liter^{-1} EC (Def 6; Bayer, Kansas City, MO) and thidiazuron 490 g kg^{-1} WP (Dropp 50WP; AgrEvo, Wilmington, DE), and a herbicide against volunteer cotton on fallow ground, thifensulfuron-methyl 750 g kg^{-1} WP (Harmony Extra; EI Du Pont de Nemours and Company, Wilmington, DE) were tested alone and in combination with selected standard insecticides (an organophosphate and a pyrethroid) that are commonly used in cotton for boll weevil control in the Lower Rio Grande Valley (Norman JW, pers comm). The pyrethroid was lambda-cyhalothrin as a 118 g liter^{-1}EC (Karate Z; Zeneca, DE); the organophosphate was azinphos-methyl as a 118 g liter^{-1} EC (Guthion 2 L; Bayer; Kansas City, MO). The defoliants and insecticides were applied at the following rates: tribufos 0.47 kg AI ha^{-1}, thidiazuron 0.23 kg AI ha^{-1}, thifensulfuron-methyl 0.043 kg AI ha^{-1}, lambda-cyhalothrin 0.038 kg AI ha^{-1} and azinphos-methyl 0.28 kg AI ha^{-1}. Other application rates used are expressed as rates relative to the full rates (1×) listed above.

2.3 Experimental design

2.3.1 Laboratory tests

Boll weevil adults of two age categories (3- and 14-day-old) from a colony reared on artificial diet (Series 1) and from infested squares (Series 2) were exposed directly and indirectly to chemical treatments (year 2000). Emerged weevils were provided with artificial pellets or squares until they reached the required age.

Series 1. Treatments were as follows: tribufos (1×), thidiazuron (1×), thifensulfuron-methyl (1×), azinphos-methyl (1× and 0.5×), lambda-cyhalothrin (1× and 2×), tribufos (1×) + azinphos-methyl (0.5×), thidiazuron (1×) + azinphos-methyl (0.5×), thifensulfuron-methyl (1×) + azinphos-methyl (0.5×), tribufos (1×) + lambda-cyhalothrin (1×), thidiazuron (1×) + lambda-cyhalothrin (1×), thifensulfuron-methyl + lambda-cyhalothrin (1×), and control [sprayed with reverse osmosis (RO) water].

Series 2. The choice of treatments was based on the result of the more extensive suite of treatments in Series 1 as follows: tribufos (1×), thidiazuron (1×), azinphos-methyl (0.25×), lambda-cyhalothrin (1×), tribufos (1×) + azinphos-methyl (0.25×), tribufos (1×) + lambda-cyhalothrin (1×), and control (sprayed with RO water).

Series 3. In these experiments, conducted in 2001, boll weevil adults of two age categories (3- and 14-day-old) and reared on artificial diet were exposed to leaves which had been sprayed with the following combinations of defoliants and insecticides: tribufos (1×), lambda-cyhalothrin (1× and 0.5×), azinphos-methyl (0.5×), tribufos (1×) + lambda-cyhalothrin (1×), tribufos (1×) + lambda-cyhalothrin (0.5×), tribufos (1×) + azinphos-methyl (0.5×), tribufos (0.5×) + azinphos-methyl (0.5×), tribufos (0.5×) + thidiazuron (0.5×) + azinphos-methyl (0.5×), and control (sprayed with RO water). There were three replicates per treatment. Each replicate consisted of a vented Petri dish (15-cm diameter with a 5-cm diameter circular nylon screen window) containing 10 boll weevils. In direct treatments, all 10 weevils were sprayed and then placed in a new non-treated vented Petri dish. For indirect treatments, a cotton leaf placed in a vented Petri dish was treated and then 10 non-sprayed weevils were placed on the treated leaf.

To apply defoliants, insecticides and combinations, we used a laboratory spray chamber (De Vries Mfg, Hollandale, MN), calibrated to deliver 56 liter ha^{-1} using one TXVS-4 nozzle at 1.7 kg cm^{-2} pressure and 4.8 km h^{-1}.

2.3.2 Greenhouse tests

Cotton was planted in 30-cm diameter pots. Three to four plants per pot were grown until bolls

started to open, and then were used for treatments. Treatments were: tribufos (1.0×) sprayed once (9 plants) and twice at an interval of 4 days (9 plants); thidiazuron (1×) sprayed once (9 plants); tribufos (0.5×) + thidiazuron (0.5×) sprayed once (12 plants), and control, sprayed once with RO water (10 plants). Pots were aligned in a row and treated similar to a row of cotton. Treatments were applied with a carbon dioxide pressurized (40 psi = 2.76 MPa) backpack sprayer with 3 TX10 hollow cone nozzles using a total volume of 10 gall per acre (93.5 liter ha^{-1}).

2.3.3 Small field plot tests

Year 2000. There were five treatments: tribufos (1×), lambda-cyhalothrin (1×), tribufos, (1×) + lambda-cyhalothrin (1×), azinphos-methyl (0.5×), tribufos (1×) + azinphos-methyl (0.5×). The experimental field was located in Weslaco in the Lower Rio Grande Valley of Texas. It consisted of 100 rows, 1.02 m wide and 45 m long. The five treatments were replicated three times, in a randomized block design. There were 15 plots (laid out in blocks of 5 plots). Each plot consisted of six rows. All six rows of a plot received the same chemical treatment, but the outside two rows were considered buffer rows and were not sampled. Rows were numbered 1–6 from west to east. One treatment was applied at a time across each of the three blocks. The field was sprayed July 25 with a calibrated Spider Track sprayer. Chemicals were applied six rows at a time, with two drops and one nozzle (8001 EVS) over the top for each row.

Year 2001. There were eight treatments: tribufos (0.5×) + thidiazuron (0.5×), azinphos-methyl (1×), lambda-cyhalothrin (1×), thidiazuron (1×) + azinphos-methyl (1×), tribufos (1×) + azinphos-methyl (0.5×), tribufos (0.5×) + thidiazuron (0.5×) + azinphos-methyl (0.5×), tribufos (1×) + lambda-cyhalothrin (0.5×) and control sprayed with water. There were two experimental fields located in Weslaco in the Lower Rio Grande Valley of Texas. Field 1 was planted in the beginning of March (144 rows, 1.02 m wide and 110 m long), and Field 2 in the end of March (144 rows, 1.02 m wide and 80 m long). The eight treatments were replicated three times, in a randomized block design. There were 24 plots (laid out in blocks of 8 plots). Each plot consisted of six rows. The treatments were applied to Field 1 on July 24 and to Field 2 on August 14 with a John Deere sprayer. Chemicals were applied six rows at a time, with two drops and one nozzle (8003 E) over the top for each row.

2.4 Experimental indices and their assessment

2.4.1 Laboratory tests

For all three set of treatments (young and old boll weevils, sprayed directly and indirectly), boll weevil mortality was determined 24, 48 and 72 h post-treatment. A weevil was considered dead if it did not move when the rostrum was pinched with forceps or when prodded in the abdomen. The weevils were sexed[22] and mortality of females and males was evaluated separately. Weight of living and dead weevils was recorded on an analytical balance. Body fat of living and morbid weevils was evaluated and rated as 3 (fat), 2 (intermediate), 1 (lean) or 0 (extra lean).[23] Eggs oviposited per female per day for the first 5 days after 72-h mortality was also checked. Ten females per treatment were placed individually in Petri dishes with 10 squares renewed daily and the numbers of oviposition (sealed) punctures in the squares were recorded. We used the number of oviposition punctures as a relative measure of egg production, because reports of boll weevil oviposition are based on such counts.[24]

2.4.2 Greenhouse tests

The numbers of non-dry leaves per plant, as a measure of leaf defoliation, were recorded for each pot 7 days post-treatment.

2.4.3 Small field plot tests

Boll weevil mortality 24, 48 and 72 h post-treatment was recorded for all treatments. This was evaluated from screen and vacuum samples.

2.4.3.1 Screen samples

Three 3-m-long screens were placed in the center furrow of each plot. Two of the screens were placed beginning 10 m in from the respective ends of the furrow, and the third in the center of the plot. The screens consisted of nylon screen stapled to 2.5 × 2.5 cm boards along the sides. The wooden frame was secured flush against the base of the cotton plants on each side of the furrow. All weevils and elytra (ants sometimes carried off dead weevils but left their elytra behind) were removed from the screen and returned to the laboratory. Live weevils were placed in Petri dishes designated by treatment and held for 48 h, if they were collected on the first day post-treatment, or for 24 h if collected on the second day post-treatment. The Petri dishes were held in an environmental chamber at 28 (±1) °C and a photoperiod of 14:10 h light:dark. For analysis, the number of weevils that died in Petri dishes was added to those that were already dead when collected on the same day. The number of dead weevils estimated from elytra was computed by pairs of left and right elytra. Boll weevil field mortality was calculated by dividing the average number of dead weevils per row-meter of screen by the average number of live weevils per row-meter in the field. The latter was estimated from beat bucket samples[25] taken from 60 plants, and calculated as the number of boll weevils collected per plant multiplied by the number of plants per row-meter.

2.4.3.2 Vacuum samples

Vacuum samples were taken with a tractor-mounted vacuum sampler[26,27] from the entire length of one row of each plot. The first sample was taken the day

before treatment from row 2, the second at the first day post-treatment from row 5, the third at the second day post-treatment from row 3, and the fourth at the third day post-treatment from row 4. Ten live weevils (unless <10 were available) from each plot were placed in Petri dishes and held in an environmental chamber as described in Section 2.3.4.1 for 48 h if they were collected on the first day post-treatment, or for 24 h if they collected on the second day post-treatment, and checked for mortality.

Three days before treatment, we recorded the number of plants per row-meter, boll weevils per plant and row-meter, plant height, number of leaves per plant, including the number of desiccated leaves, and bolls per plant, including how many were open. Samples were made by crossing the experimental field diagonally from one corner to another. Measurements were taken from 40 plants or 25 row-meters. Seven days post-treatment, we again sampled the number of leaves per plant for each plot, with 30 plants examined per plot.

2.5 Statistical analyses

Data were analyzed using analysis of variance (ANOVA), and means were separated by Tukey Studentized range honestly significant difference (HSD) test ($\alpha = 0.05$).[28] Percentage data were transformed using the arcsine-square root method, but are presented as non-transformed means.[29] Differences in pairs of means were tested for significance with *t* tests.

3 RESULTS AND DISCUSSION

3.1 Laboratory tests

Mortality of boll weevil (after direct spray) was caused only by direct contact with the chemicals. However, those placed with treated leaves (indirect treatments) could have obtained a lethal dose both through contact and ingestion, because, in these experiments, we observed boll weevils feeding on the leaves.

The effects of chemical defoliants alone and in combination with insecticides on boll weevils reared on artificial diet are presented in Table 1. After being sprayed with thidiazuron (1×) or thifensulfuron-methyl (1×), the mortality of young and old boll weevils was similar to that in the controls (2.4–11.1% vs 3.3–9.0%), while those sprayed with tribufos (1×) alone had significantly higher mortality than controls (22.5–30.4% vs 3.3–9.0%). When azinphos-methyl was used at the full rate, boll weevil mortality was 97.2–97.9%, compared with 62.7–80.0% mortality with the half rate. Weevil mortality in treatments with lambda-cyhalothrin at full rate was considerably lower than with azinphos-methyl and not much different from that with tribufos. Boll weevil mortality was not significantly different among any direct and indirect spray treatments, except treatments with lambda-cyhalothrin alone and in combination with defoliants for young weevils, and in thidiazuron + lambda-cyhalothrin for old weevils. Three-day-old weevils in some treatments were more tolerant to insecticides than 14-day-old insects (lambda-cyhalothrin (2×), [$P =$

Table 1. Effects of defoliants alone and in combination with insecticides on boll weevil mortality (%) (total for 72-h post-spray, weevils were reared on artificial diet) in laboratory tests[a,b]

	Young weevils (3-day-old)		Old weevils (14-day-old)	
Treatment	Direct spray	Indirect spray	Direct spray	Indirect spray
Tribufos (1×)	25.5 (±2.1) c	30.4 (±3.4) c	22.5 (±7.1) c	25.1 (±4.1) c
Thidiazuron (1×)	2.4 (±4.1) d	4.6 (±4.0) d	9.8 (±3.2) d	11.1 (±3.8) d
Thifensulfuron-methyl (1×)	4.8 (±4.2) d	6.7 (±6.7) d	4.6 (±4.1) d	8.9 (±7.7) d
Lambda-cyhalothrin (2×)	23.6 (±3.8) c*	68.9 (±3.8) b*	75.2 (±9.7) b	84.6 (±10.4) a
Lambda-cyhalothrin (1×)	13.3 (±6.7) c*	35.6 (±3.8) c*	40.0 (±6.7) c	52.1 (±4.9) b
Tribufos (1×) + lambda-cyhalothrin (1×)	65.5 (±3.4) b*	98.8 (±2.0) a*	97.8 (±3.8) a	96.8 (±3.8) a
Thidiazuron (1×) + lambda-cyhalothrin (1×)	15.6 (±7.7) c*	28.9 (±3.8) c*	32.9 (±10.8) c	57.8 (±3.8) b*
Thifensulfuron-methyl (1×) + lambda-cyhalothrin (1×)	11.1 (±3.8) c*	24.4 (±3.8) c*	34.3 (±8.1) c	44.4 (±3.8) b
Azinphos-methyl (1×)	97.8 (±3.8) a	97.5 (±3.2) a	97.2 (±4.8) a	97.9 (±3.6) a
Azinphos-methyl (0.5×)	62.7 (±5.0) b	71.1 (±7.7) b	72.0 (±4.2) b	80.0 (±6.7) a
Tribufos (1×) + azinphos-methyl (0.5×)	97.6 (±4.1) a	97.8 (±3.8) a	97.9 (±3.6) a	97.6 (±4.1) a
Thidiazuron (1×) + azinphos-methyl (0.5×)	62.2 (±3.8) b	68.9 (±3.8) b	74.9 (±4.5) b	82.2 (±3.8) a
Thifensulfuron-methyl (1×) + azinphos-methyl (0.5×)	71.1 (±3.8) b	82.9 (±7.7) b	67.2 (±5.9) b	86.3 (±13.4) a
Control (water)	3.3 (±3.3) d	7.7 (±1.9) d	9.0 (±3.7) d	8.9 (±7.7) d

[a] Means (±SD) in a column followed by the same letter are not significantly different at the 5% level, as determined by Tukey's Studentized range test.
[b] * Pairs that are significantly different based on *t* test.
Young BW direct spray: $F = 59.3$, $df = 13, 406$, $P = 0.001$; young BW indirect spray: $F = 63.9$, $df = 13, 406$, $P = 0.001$; old BW direct spray: $F = 53.2$, $df = 13, 406$, $P = 0.001$; old BW indirect spray: $F = 30.2$, $df = 13, 406$, $P = 0.001$.

0.001], lambda-cyhalothrin (1×) [$P = 0.008$] and tribufos (1×) + lambda-cyhalothrin (1×) [$P = 0.002$] direct spray). Boll weevil females were more tolerant to insecticides alone and mixed with tribufos. The mean female survival was significantly higher [54.3 (±3.2%)] than that of males [45.7 (±6.8%)] ($T = 2.6$, $df = 98$, $P = 0.025$). Surviving individuals weighed significantly more than dead ones: 14.0 (±2.4) mg vs 8.6 (±1.1) ($T = 4.5$, $df = 98$, $P = 0.001$). Tribufos, thidiazuron, thifensulfuron-methyl, azinphos-methyl and azinphos-methyl + tribufos did not negatively affect the number of eggs oviposited per female per day, compared with the non-treated group [average 5.8 (±0.9) and 6.1 (±1.0) eggs per female per day], but combinations of defoliants with lambda-cyhalothrin reduced these numbers 2.1-fold [2.7 (±0.8) eggs per female per day]. Although mortality from lambda-cyhalothrin was low (Table 1), after spraying with lambda-cyhalothrin (2×) the weevils practically stopped laying eggs (0.12 eggs per female per day) ($F = 73$, $df = 9, 90$, $P = 0.004$).

The effects of chemical defoliants alone and in combination with insecticides on feral boll weevils showed the same trends as weevils reared on artificial diet, and are presented in Table 2. The results indicate that tribufos by itself was toxic to young and old weevils. Total mortality from tribufos for the 72-h post-spray period was significantly higher than that of control and thidiazuron treatments. The mortalities from lambda-cyhalothrin (1×) and azinphos-methyl (0.25×) alone were not significantly different from the treatment with tribufos. The combination of tribufos (1×) + lambda-cyhalothrin (1×) showed synergistic effects in all tests, while tribufos (1×) + azinphos-methyl (0.25×) showed synergism only in old weevils. Mortalities of feral weevils did not differ significantly between direct and indirect treatments, except for lambda-cyhalothrin (1×), azinphos-methyl (0.25×) and lambda-cyhalothrin (1×) + tribufos (1×) against young weevils. Feral weevils surviving after treatment weighed significantly more than dead weevils [11.2 (±1.6) vs 8.2 (±1.3) mg] ($T = 7.2$, $df = 48$, $P = 0.001$). It is possible that dead weevils weighed less than live weevils due to post-mortem water loss. In contrast, young weevils of both sexes surviving treatments of any kind had significantly (non-overlapping 95% CI's) lower-rated levels of body fat [females 1.0 (±0.09); males 1.1 (±0.08)] than morbid weevils dissected shortly before death [females 1.6 (±0.15); males 1.5 (±0.17)]. In the case of old feral weevils, significant differences in body fat were not observed between surviving [females 2.3 (±0.24); males 2.3 (±0.11)] and morbid (females 2.2 (±0.18); males 2.3 (±0.14)] weevils. The feral females were slightly more tolerant to all chemicals used [53.3 (±1.6)% survival 72 h post-spray) than males [46.7 (±1.6)%] ($T = 2.9$, $df = 52$, $P = 0.001$). The number of eggs oviposited per feral female per day in treatments with defoliants alone (tribufos or thidiazuron) [5.1 (±1.3), 5.6 (±1.1), respectively] were not significant different from the control [5.3 (±2.0)], but was significantly higher than in those treated with lambda-cyhalothrin [0.33 (±0.07)] ($F = 2.6$, $df = 3, 36$, $P = 0.048$).

Cumulative mortality of boll weevil through 72 h in Series 3 of laboratory tests is presented in Table 3. Defoliant plus insecticide treatments had a significant effect on boll weevil mortality. Tribufos (1×), lambda-cyhalothrin (1× and 0.5×) exhibited moderate toxic effects, while tribufos (1×) + lambda-cyhalothrin (0.5×) and tribufos (1×) + azinphos-methyl (0.5×) showed synergistic effects.

In conclusion, the results of the laboratory tests indicated that tribufos by itself exhibited a toxic effect on boll weevil. tribufos (1×) + lambda-cyhalothrin (0.5×) and tribufos (1×)+ azinphos-methyl (0.5×) showed synergistic effects on weevil mortality. Tribufos (1×) + lambda-cyhalothrin (1×) and, especially, lambda-cyhalothrin (2.0×) significantly reduced the number of eggs deposited per female per day. These results demonstrate that efficacy equal to that of full insecticide rates can be attained by using half the insecticide rates when combined with tribufos. The effects of chemical defoliants alone and in combination with insecticides on feral boll weevils showed the same trends to those on weevils reared on artificial diet.

Table 2. Effects of defoliants alone and in combination with insecticides on mortality (%) of feral boll weevils (total for 72 h post-spray)[a,b]

	Young (3-day-old)		Old (14-day-old)	
Treatment	Direct spray	Indirect spray	Direct spray	Indirect spray
Tribufos (1×)	30.0 (±6.9) b	32.2 (±8.8) c	42.5 (±2.2) b	38.9 (±9.6) b
Thidiazuron 1×	6.0 (±5.5) c	8.0 (±4.4) d	7.0 (±6.1) c	16.7 (±6.7) c
Lambda-cyhalothrin (1×)	30.0 (±9.2) b*	49.6 (±8.1) bc*	43.9 (±11.3) b	55.0 (±9.6) b
Tribufos (1×) + lambda-cyhalothrin (1×)	69.3 (±9.3) a*	88.0 (±10.9) a*	83.8 (±11.1) a	88.9 (±9.6) a
Azinphos-methyl (0.25×)	33.7 (±7.2) b*	53.8 (±5.8) bc*	43.3 (±8.9) b	44.4 (±9.6) b
Tribufos (1×) + azinphos-methyl (0.25×)	40.0 (±10.0) b	62.5 (±12.5) ab	84.2 (±8.0) a	83.3 (±9.7) a
Control (water)	3.3 (±3.8) c	6.7 (±5.8) d	2.5 (±5.0) c	5.6 (±9.6) c

[a] Means (±SD) in a column followed by the same letter are not significantly different at the 5% level, as determined by Tukey's Studentized range test.
[b] * Pairs that are significantly different based on *t* test.
Young BW, direct spray: $F = 17.7$, $df = 6, 203$, $P = 0.002$; young BW, indirect spray: $F = 28.8$, $df = 6, 203$, $P = 0.001$; old BW, direct spray: $F = 15.9$, $df = 6, 203$, $P = 0.001$; old BW, indirect spray: $F = 20.1$, $df = 6, 203$, $P = 0.003$.

Table 3. Effects of defoliants alone and in combination with insecticides on mortality of boll weevils after placement on leaves treated in the laboratory (total for 72 h post-spray, weevil reared on artificial diet, 2001)

	Mortality (%) (±SD)[a]	
Treatment	3-day-old	14-day-old
Control	3.3 (±3.3) e	3.0 (±1.8) d
Tribufos (1×)	46.7 (±3.3) cd	41.5 (±6.0) c
Lambda-cyhalothrin (1×)	42.5 (±3.8) cd	53.3 (±8.8) bc
Lambda-cyhalothrin (0.5×)	31.8 (±5.5) d	43.3 (±8.8) bc
Azinphos-methyl (0.5×)	66.7 (±3.3) c	76.7 (±6.7) ab
Tribufos (1×) + lambda-cyhalothrin (1×)	93.3 (±6.7) ab	96.7 (±3.3) a
Tribufos (1×) + lambda-cyhalothrin (0.5×)	72.2 (±4.0) b	86.7 (±13.3) a
Tribufos (1×) + azinphos-methyl (0.5×)	100 a	100 a
Tribufos (0.5×) + azinphos-methyl (0.5×)	100 a	100 a
Tribufos (0.5×) + thidiazuron (0.5×) + azinphos-methyl (0.5×)	100 a	100 a

[a] Values in each column followed by the same letter are not significantly different at the 5% level, as determined by Tukey's studentized range test. 3-day-old BW: $F = 41.7$, $df = 9, 290$, $P = 0.017$; 14-day-old BW: $F = 24.8$, $df = 9, 290$, $P = 0.02$.

3.2 Small field plot tests

Mortality values from tribufos (1×) alone and in combination with lambda-cyhalothrin (1×) and azinphos-methyl (0.5×) are presented in Fig 1 (results from 2000, mortality screen data). Tribufos (1×) alone exhibited a toxic effect on boll weevil (cumulative mortality of insect through 72 h was estimated as 0.519 (±0.1) dead boll weevils m^{-2}). In plots treated with lambda-cyhalothrin (1×), we estimated 1.222 (±0.2) dead weevils m^{-2} by 72 h, while in plots treated with azinphos-methyl (0.5×), we estimated 0.741 (±0.1) dead weevils m^{-2}. In tribufos (1×) + lambda-cyhalothrin (1×) plots, the rate of boll weevil field mortality by 72 h was 2.4 times higher than in the lambda-cyhalothrin (1×) plots, and 5.8 times higher than in tribufos (1×) plots. Similarly, in plots treated with tribufos (1×) + azinphos-methyl (0.5×), boll weevil mortality was 2.2-fold higher than in azinphos-methyl (0.5×) plots, and was 3.1-fold higher than in tribufos (1×) plots ($F = 15.8$, $df = 4, 10$, $P = 0.001$). These results suggest a synergistic effect of tribufos (1×) + lambda-cyhalothrin (1×) over tribufos (1×) or lambda-cyhalothrin (1×) alone and also tribufos (1×) + azinphos-methyl (0.5×) over tribufos (1×) or azinphos-methyl (0.5×) alone.

Boll weevil population decrease in the plots evaluated by vacuum samples showed treatment-related trends similar to those observed from the screen data (Table 4). In plots treated with tribufos (1×) + lambda-cyhalothrin (1×) the decrease in population

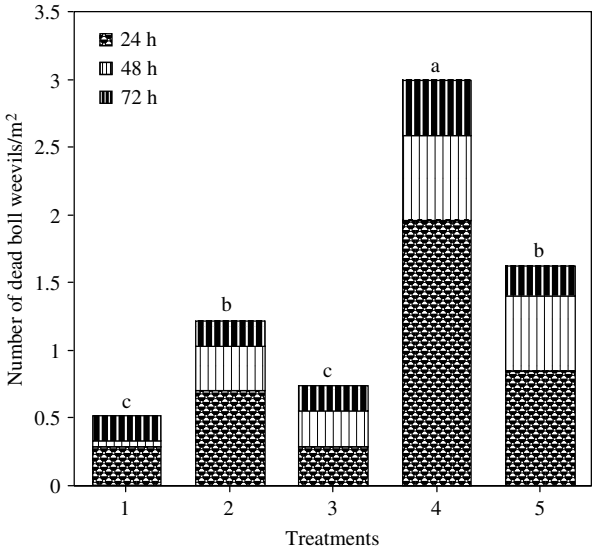

Figure 1. Mortality of boll weevils as determined by screen sampling (2000): **1** tribufos (1×); **2** lambda-cyhalothrin (1×); **3** azinphos-methyl (0.5×); **4** tribufos (1×) + lambda-cyhalothrin (1×); **4** tribufos (1×) + azinphos-methyl (0.5×). Bars with the same letter are not significantly different.

was 2.3-fold greater than in plots treated with tribufos (1×) alone, and 1.6-fold greater than in plots treated with lambda-cyhalothrin (1×) alone. Similar decreases in tribufos (1×) + azinphos-methyl (0.5×) plots were 1.8-fold and 1.5-fold greater than in tribufos (1×) and azinphos-methyl (0.5×) plots, respectively.

Data from the vacuum samples cannot be used directly to estimate mortality in the plots, because a decrease in numbers of live weevils after treatment is the result of not only mortality, but dispersal from the plot as well. Nevertheless, if the numbers of weevils dispersing from the plots is relatively independent of treatment, then differences in percentage population decrease across treatments reflects the relative efficacy of the treatments. Sappington *et al*[30] used a mark-recapture technique on the same experimental field to calculate population size. Dispersal out of the field, number that died after dispersal, and total percentage mortality by treatment were calculated by

Table 4. Effects of tribufos and insecticides alone and in combination on boll weevil mortality and dispersal (Field test, 2000, vacuum samples)

Treatment	Population decrease[a] (%) (± SD)	Mortality via mark-recapture[b] (%)
Tribufos (1×)	36.2 (±21.9) c	26.3
Lambda-cyhalothrin (1×)	52.7 (±10.3) b	52.6
Tribufos (1×) + lambda-cyhalothrin (1×)	84.0 (±3.7) a	93.4
Azinphos-methyl (0.5×)	41.5 (±7.2) bc	51.3
Tribufos (1×) + azinphos-methyl (0.5×)	63.5 (±14.0) b	71.1

[a] Values followed by the same letter are not significantly different at the 5% level, as determined by Tukey's Studentized range test.
[b] From Sappington *et al*[30].

combining data from the mortality screens, vacuum samples, and Petri dishes. Their results are also presented in Table 4, and show a very similar trend to that predicted from the vacuum samples alone. The similarity in trends between the percentage population decrease estimated by vacuum sample and the mortality estimates from Sappington *et al*[30] mark-recapture data suggest that dispersal out of the field was relatively independent of treatment. This conclusion is supported by the lack of differential effects of the treatments on the flight behavior of surviving weevils tested on flight mills.[31]

Interpretation of our field plot results for 2001 assumes that movement of boll weevils between plots during the experiment was low, as evidence from mark-recapture data suggests.[30,32] In field 1 (2001), the number of dead weevils recovered on screens in plots treated with lambda-cyhalothrin (1×) or tribufos (1×) + lambda-cyhalothrin (0.5×) did not differ significantly, but both were significantly higher than tribufos (0.5×) + thidiazuron (0.5×) and the untreated control (Fig 2). The number of dead weevils in plots treated with azinphos-methyl (1×) alone or combined with thidiazuron (1×) was not significantly different from treatments that included azinphos-methyl (0.5×) + tribufos (1×), and were significantly higher than in plots treated with lambda-cyhalothrin (1×) or lambda-cyhalothrin (0.5×) + tribufos (1×) ($F = 4.8$, $df = 7, 16$, $P = 0.005$).

In field 2 (2001), we observed a pronounced synergistic effect of lambda-cyhalothrin (0.5×) + tribufos (1×) over lambda-cyhalothrin (1×) and tribufos (0.5×) + thidiazuron, (0.5×) (Fig 3). The number of dead weevils did not differ significantly

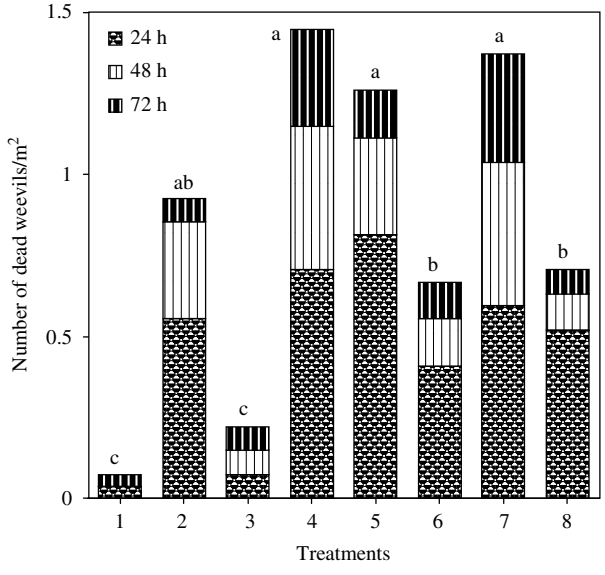

Figure 2. Mortality of boll weevils as determined by screen sampling (Field 1, 2001): **1** control; **2** tribufos (0.5×) + thidiazuron (0.5×) + azinphos-methyl (0.5×); **3** tribufos (0.5×) + thidiazuron (0.5×); **4** thidiazuron (1×) + azinphos-methyl (1×); **5** tribufos (1×) + azinphos-methyl (0.5×); **6** tribufos (1×) + lambda-cyhalothrin (0.5×); **7** Azinphos-methyl (1×); **8** Lambda-cyhalothrin (1×). $F = 4.8$, $df = 7, 16$, $P = 0.005$. Bars with the same letter are not significantly different.

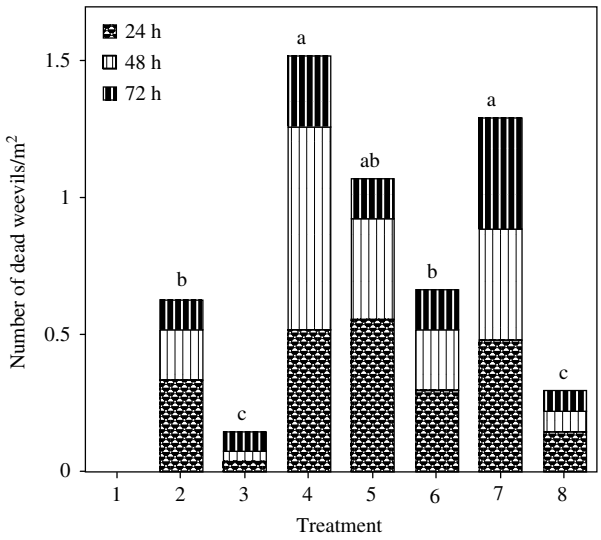

Figure 3. Mortality of boll weevils as determined by screen sampling (Field 2, 2001): **1** control; **2** tribufos (0.5×) + thidiazuron (0.5×) + azinphos-methyl (0.5×); **3** tribufos (0.5×) + thidiazuron (0.5×); **4** thidiazuron (1×) + azinphos-methyl (1×); **5** tribufos (1×) + azinphos-methyl (0.5×); **6** tribufos (1×) + lambda-cyhalothrin (0.5×); **7** azinphos-methyl (1×); **8** lambda-cyhalothrin (1×). $F = 17.9$, $df = 7, 16$, $P = 0.001$. Bars with same letter are not significantly different.

among plots treated with azinphos-methyl (1×) alone or in combination with defoliants compared with those including azinphos-methyl (0.5×), except the treatment tribufos (0.5×) + thidiazuron (0.5×) + azinphos-methyl (0.5×), which was lower. The mortality of boll weevils in Petri dishes after collection from the plots by vacuum sampler showed the same trends as the screen data (Table 5). Combination of tribufos (1×) with lambda-cyhalothrin (0.5×) showed synergistic effects. Mortality with azinphos-methyl (1×) + thidiazuron (1×) was not significantly different from that with tribufos (1×) + azinphos-methyl (0.5×). Population decreases in the plots evaluated by vacuum sampler showed treatment-related trends similar to those observed from the screens and Petri dishes (Fig 4). In plots treated with tribufos (1×) + lambda-cyhalothrin (0.5×), the cumulative reduction in live weevils after 72 h was 4.7-fold greater than in plots treated with lambda-cyhalothrin (1×) alone, and 3.2-fold (24 h post-treatment) and 4.1-fold (48 h post-treatment) greater than in plots treated with tribufos (0.5×) + thidiazuron (0.5×) (Fig 4). Similarly, decreases in the tribufos (1×) + azinphos-methyl (0.5×) plots were 7.1-fold, 2.3-fold and 2.7-fold greater than in the azinphos-methyl (1×), or tribufos (0.5×) + thidiazuron (0.5×) plots at 72, 24 and 48 h post-treatment, respectively. The number of weevils remaining in the tribufos (0.5×) + thidiazuron (0.5×) plot was not significantly different from the control at 24 and 48 h post-treatment ($P > 0.05$), but was significantly different by 72 h (Fig 4). This late decrease presumably reflected dispersal of weevils from the plot as leaves began to fall from the plants. The other treatments may also have contributed to the dispersal of weevils

Table 5. Mortality in Petri dishes of vacuum-collected boll weevils from field test evaluations (2001), total for 72 h post-spray

Treatment	Mortality[a] (%) (±SD)	
	Field #1	Field #2
Control	1.6 (±04) c	3.2 (±06) c
Tribufos (0.5×) + thidiazuron (0.5×) + azinphos-methyl (0.5×)	74.7 (±2.5) a	69.2 (±3.6) a
Tribufos (0.5×) + thidiazuron (0.5×)	23.3 (±2.7) b	24.0 (±2.1) b
Tribufos (1×) + azinphos-methyl (1×)	80.0 (±4.8) a	82.2 (±3.5) a
Tribufos (1×) + azinphos-methyl (0.5×)	74.2 (±5.1) a	81.5 (±3.1) a
Tribufos (1×) + lambda-cyhalothrin0.5×	69.2 (±1.6) a	77.5 (±2.6) a
Azinphos-methyl (1×)	70.0 (±1.1) a	89.2 (±19) a
Lambda-cyhalothrin (1×)	44.7 (±4.3) b	43.0 (±3.8) b

[a] Values in each column followed by the same letter are not significantly different at the 5% level, as determined by Tukey's Studentized range test. Field #1: $F = 28.6$, $df = 7, 16$, $P = 0.001$; Field #2: $F = 35.6$, $df = 7, 16$, $P = 0.004$.

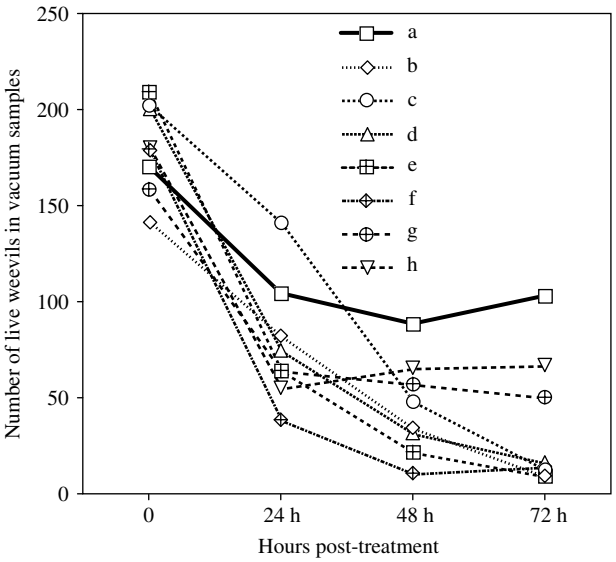

Figure 4. Reduction in number of live weevils after treatment (Field 1, 2001). Captions on figure, in order: (a) control; (b) tribufos (0.5×) + thidiazuron (0.5×) + azinphos-methyl (0.5×); (c) tribufos (0.5×) + thidiazuron (0.5×); (d) thidiazuron (1×) + azinphos-methyl (1.0×) (e) tribufos (1×) + azinphos-methyl (0.5×); (f) tribufos (1×) + lambda-cyhalothrin (0.5×); (g) azinphos-methyl (1×); (h) lambda-cyhalothrin (1×).

from the plots by 72 h, but the screen and Petri dish data suggest that the cumulative reduction in population can be attributed largely to mortality.

In conclusion, the results from the field tests indicated that tribufos (0.5×) + thidiazuron (0.5×) was slightly toxic to boll weevil, and that tribufos (1×) + lambda-cyhalothrin, (0.5×), tribufos (1×) + azinphos-methyl (0.5×), and tribufos (0.5×) + thidiazuron (0.5×) + azinphos-methyl (0.5×) performed as well or better than full rates of lambda-cyhalothrin or azinphos-methyl alone. Application of half rates of azinphos-methyl or lambda-cyhalothrin mixed with defoliant will permit growers to attain the benefits of a diapause control program at reduced cost and insecticide input into the environment.

3.3 Quality of defoliation

The different treatments significantly affected the quality of defoliation in the greenhouse ($F = 193.4$, $df = 4, 44$, $P = 0.001$) (Table 6). The percentage of dropped leaves per plant was significantly higher for tribufos (1×) sprayed twice (97.4%) and a combination of tribufos (0.5×) + thidiazuron (0.5×) sprayed once (97.8%) than that with tribufos (1×) sprayed once (80.2%) or thidiazuron (1×) sprayed once (78.7%).

In small field plot tests treatments including tribufos (0.5×) + thidiazuron (0.5×) showed 93.6–96.4% increase in leaf drop over the control at 7 days post-treatment (Table 7). In the treatments containing only tribufos (1×) or thidiazuron (1×), this was reduced to 59.8–71.8%. The insecticides alone had no significant effect. It is likely that fields with <70% defoliation would be treated a second time, thus increasing costs. Defoliation started by the third day after spraying, as revealed by the weight of vacuum samples which was a function of leaves collected in the bags. The weight of vacuum samples from the plots receiving defoliant treatment was about 3.6-fold greater than

Table 6. Effects of various defoliant treatments on the quality of defoliation in the greenhouse

Treatment	Number of sprays	Average number of initial leaves per plant (±SD) [number of plants]	Dropped leaves per plant 7 days post-spray	
			(Number) (±SD)	(%) (±SD)[a]
Control	—	24.5 (±1.2) [10]	0.8 (±0.2)	3.3 (±1.6) c
Tribufos (1×)	One	22.2 (±1.4) [9]	17.8 (±1.6)	80.2 (±6.1) b
Tribufos (1×)	Two	27.1 (±1.6) [9]	26.4 (±1.6)	97.4 (±1.4) a
Thidiazuron (1×)	One	25.8 (±2.7) [9]	20.3 (±2.0)	78.7 (±4.0) b
Tribufos (0.5×) + thidiazuron (0.5×)	One	27.7 (±2.0) [12]	27.1 (±1.9)	97.8 (±0.9) a

[a] Values followed by the same letter are not significantly different at the 5% level, as determined by Tukey's Studentized range test. $F = 193.4$, $df = 4, 44$, $P = 0.001$.

Table 7. Quality of cotton defoliation in field plots at 7 days post-treatment

Treatment	Increase in leaf drop over control[a] (%) (±SD)	
	Field 1	Field 2
Control	0 c	0 d
Tribufos (0.5×) + thidiazuron (0.5×) + azinphos-methyl (0.5×)	93.6 (±0.6) a	96.4 (±0.3) a
Tribufos (0.5×) + thidiazuron (0.5×)	90.7 (±0.6) a	94.2 (±0.5) a
Thidiazuron (1×) + azinphos-methyl (1×)	66.8 (±1.6) b	67.7 (±0.7) b
Tribufos (1×) + azinphos-methyl (0.5×)	59.8 (±0.8) b	71.8 (±1.1) b
Tribufos (1×) + lambda-cyhalothrin (0.5×)	72.3 (±0.7) b	65.2 (±1.4) b
Azinphos-methyl (1×)	1.3 (±0.5) c	8.5 (±2.9) c
Lambda-cyhalothrin (1×)	4.4 (±1.7) c	13.9 (±2.7) cd

[a] Values in each column followed by the same letter are not significantly different at the 5% level, as determined by Tukey's Studentized range test. Field #1: $F = 126.6$, $df = 7, 232$, $P = 0.001$; Field #2: $F = 137.4$, $df = 7, 232$, $P = 0.001$.

those without them [2.9 (±0.3) kg vs. 0.8 (±0.2) kg] ($F = 37.0$, $df = 7, 16$, $P = 0.001$). By two weeks post-treatment, the plots that were not defoliated or poorly defoliated had more weevils per plant than plots with high defoliation [0.4 (±0.05) boll weevils per plant vs. 0.1 (±0.04) boll weevils per plant] ($F = 4.5$, $df = 7, 247$, $P = 0.001$). Most farmers currently treat their fields only once with tribufos or thidiazuron, and they may not achieve perfect defoliation, which can have negative effects on mechanized harvest. Better defoliation can be accomplished using a combination of tribufos (0.5×) + thidiazuron (0.5×), although the mechanism of this interaction is unknown.

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