EFFECT OF KAOLIN PARTICLE FILM ON BOLL WEEVIL FEEDING AND OVIPOSITION ON COTTON SQUARES

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Abstract

Kaolin, a reflective white mineral, mixed with water and applied as a coating to excised cotton squares, squares on whole cotton plants, or to foliage initially resulted in lower oviposition and feeding injuries to squares. When alternative untreated food and oviposition sources were increasingly used and in short supply, boll weevils made greater use of treated squares and squares on cotton plants with treated foliage until there were no significant differences in boll weevil damage between treated and untreated cotton plants and squares. It is likely that boll weevils make host selections based to some extent on color, but this can be overcome if boll weevils also select using other cues. A field trial indicated that boll weevils might be able to distinguish among cotton fields based on color differences caused by application of kaolin, and the ability to distinguish appears to influence relative levels of infestation.

Introduction

Insect and disease injury to some crops can be prevented by coating plants with kaolin as hydrophobic particle film (Glenn et al. 1999). The film acts to make the host plant visually or tactically unrecognizable. Arthropod movement and feeding might be hindered by the attachment of particles to the body and pathogen infection can be blocked by a hydrophobic film that impedes direct interface between disease propagules and coated plant surfaces. Kaolin is a white, porous, nonswelling, non-abrasive fine grained platy aluminosilicate mineral ($Al_4Si_4O_{10}(OH)_8$) that disperses in water and is chemically inert over a wide pH range. Coating grade kaolin is >90% pure and has a brightness quality of >85% (Harben 1995).

Application of kaolin particle film has resulted in the suppression of injury caused by pear psylla, *Cacopsylla pyricola* Foerster, on pear; spirea aphids, *Aphis spireacola* Patch, on apple; potato leafhopper, *Empoasca fabae* (Harris), on apple; codling moth, *Cydia pomonella* (L.), on apple and pear; obliquebanded leafroller, *Choristoneura rosaceana* (Harris) on apple; root weevil, *Diaprepes abbreviatus* (L.), on citrus; and twospotted spider mite, *Tetranychus urticae* Koch, on apple (Glenn et al. 1999, Puterka et al. 2000, Knight et al. 2000, Lapointe 2000, Unruh et al. 2000). Fireblight, *Erwinia amylovora* L., and apple scab, *Venturia inaequalis* (Cooke), were reduced in pear and apple, respectively (Glenn et al. 1999, Puterka et al. 2000).

During the cotton growing season, especially where eradication programs are not completed or have not been implemented, principle commercial practices for improving yield are reducing cotton square exposure to late-season boll weevil, *Anthonomus grandis* Boheman, populations by planting early and using early maturing varieties (Slosser 1993, Roach & Culp 1984) and insecticide application (Loera-Gallardo et al. 1997, Page et al. 1999). Predators (Sterling 1978, Sturm et al. 1990), parasites (Morales-Ramos & King 1991, Summy et al 1997), trap crops (Moore & Watson 1990), and plant extracts (Miles et al. 1993, 1994) have not been shown to suppress boll weevil damage to cotton on an area-wide basis. This study was undertaken to examine kaolin particle film as an alternative tactic to conventional insecticides for protecting cotton squares against boll weevil oviposition and feeding damage.

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Materials and Methods

The kaolin used in these experiments was Surround™ WP processed to a bright white color of > 85%, ≤ 2 µm particle diameter, and coated with a proprietary synthetic hydrocarbon (Englehard, Iselin, NJ) to impart hydrophobic quality. Sixty g of Surround™ per liter water was used for all experiments in this study. All applications, whether by painting, dipping, or spraying, were done twice to ensure complete coverage.

In order to quantify kaolin particle deposition after application, 20 leaves were excised from different cotton plants 4 h after being twice sprayed with Surround™. The kaolin was washed from the leaves with methanol into pre-weighed plastic dishes (a 6 mm flat oxhair paint brush was used to dislodge particles that adhered to the leaf and square surfaces), the methanol was evaporated off, and the dried particles plus beaker were weighed. Twenty leaves from cotton plants grown in a USDA-ARS Kika de la Garza Subtropical Agricultural Research Center, Hidalgo Co., TX, cotton field that had been twice sprayed with Surround™ from a tractor mounted boom were excised one wk later, and another 20 leaves were excised for kaolin particle density measurement 2 wk later (the plants had been sprayed twice previously at biweekly intervals). The difference between the dish + particle weight and the empty beaker weight yielded the mass of kaolin on each leaf. The upper surface area of each leaf was measured using a Model 3100 Area Meter (Lycor, Lincon, NB). The mass of kaolin collected from each leaf was divided by 2 X the upper surface area of the leaf to give the mass of kaolin deposited per cm^2. Twenty 7 mm diameter (± 0.5 mm) squares were twice dipped in the kaolin spray mix and air dried at ambient room temperature after each dip. Mass of kaolin deposited was determined in the same way as for the leaves, but the diameter of the square was given instead of leaf surface area. Mean particle density on leaves 4 h after application was 360 ± 18.68 µg kaolin per cm^2 leaf surface. After 1 and 2 wk in the field, particle densities were 319.88 ± 20.80 and 200.96 ± 13.16, respectively. On squares, mean particle density was 145.00 ± 20.84 µg per 7 mm diameter square.

In assays that required gravid female boll weevils, field captured boll weevil adults were kept in 0.5 m^3 cages, 60 weevils per cage, at a male:female ratio of 1:1 for six d and fed fresh squares every day. A sample of five females was dissected from each cage population to confirm the presence of eggs; if eggs were present in all five females, the female populations in the cage was assumed to be gravid.

All cotton in this study was USDA cultivar C-208. Greenhouse pots were 7.5-liters in volume, each had three cotton plants. Significant differences between treatment means were detected using the paired *t* test and Tukey's HSD multiple range test (Analytical Software 1998).

Kaolin Application to Squares on Whole Cotton Plants, Choice Assay

At the 7 mm diameter square stage, all of the squares of potted cotton plants were coated with Surround™ using a 6 mm flat oxhair paint brush, and upon drying, 20 pots of these plants were placed in separate 1 X 0.65 X 1 m cages alongside a pot of cotton plants with unpainted squares. Five pairs of boll weevils were released into each cage and permitted to feed and oviposit. Damage to 15 squares from each pot of plants was recorded after 1 and 7 d.

Kaolin Application to Excised Squares, Choice and No-choice Assays

In the choice assay, two 7 mm diameter squares dipped in Surround™ were placed with two untreated squares in a ventilated petri dish. One gravid female boll weevil was released into each petri dish and observed at 10 min intervals up to 90 min, then at hourly intervals for four h, then again at 24 h, and the positions of the boll weevils relative to the squares were recorded: on a kaolin-dipped square, an untreated square, or no square. At

24 h, all squares were examined for feeding and oviposition damages. In the no-choice assay, all conditions were the same, except that there were either two kaolin-dipped or two untreated squares in each petri dish. In both the choice and the no-choice assays, 10 separate petri dishes constituted each of the 100 and 185 replications, respectively.

Kaolin Application to Foliage on Whole Cotton Plants, Choice Assay

Greenhouse cotton in pots was sprayed with a Greenlawn (Gilmour, Somerset, PA) manual pump sprayer. Because they are shielded by bracts, squares received partial or no coverage, which is representative of field conditions when Surround™Ô is applied. One pot of treated cotton plants and one pot of untreated cotton plants were placed together in 1 X 0.65 X 1 m cages kept outdoors. Five pairs of boll weevils were released in each cage and allowed to feed and oviposit for 7 d. Fifteen randomly selected squares from each pot of cotton plants were examined for oviposition punctures after 2, 24, 72 h, and 1 wk.

Small Plot Trial, Application to Foliage Sprays to Protect Squares

Twenty-four plots, each 0.0125-ha and arranged in a completely randomized design, on the Kika de la Garza Subtropical Agricultural Research Center were used to determine the effect of kaolin particle film on suppressing boll weevil injury in field cotton. Deltapine-50 cotton was planted to 101.6 cm rows on March 6, 2000. Pendimethalin at 2.34 liters a.i./ha was applied by tractor immediately after planting, and weed control was thereafter conducted with a rolling cultivator and by hand-roguing. Beginning April 11, when the cotton plants had reached pinhead square stage, Surround™ was applied by tractor mounted boom sprayer weekly to 8 plots, biweekly to 8 plots, and the remaining 8 plots were used as untreated controls. No insecticides were applied during the growing season.

Two adjacent 10-ha Deltapine-50 cotton fields, both planted on the same date as the small plot field, each located < 20 m east and southeast of the small plot field served as external controls. One of the fields received two "pre-emptive" guthion (22.95 ml a.i./ha) applications by tractor boom sprays at pinhead square stage on May 2 and 5 d later. Guthion was sprayed at a rate of 68.84 ml a.i./ha by tractor when square damage exceeded 10% (based on 50 randomly examined squares from each of the field's four quadrants); May 26 and 31, June 14 and 21, and July 7. The second field was not treated with any insecticides over the course of the entire growing season.

In the small plot field, boll weevil damage was determined by examining 50 randomly selected squares per plot each week, May 5 to June 2. Numbers of squares and bolls in 7.6 m row in each plot were counted on May 19 and June 9, respectively. On June 26, heights of 25 randomly selected cotton plants in each plot were recorded. All fields in this study were defoliated on July 7 with Def™ at 1681.3 g ai/ha. Cotton was hand harvested from two 4 m lengths of row in each plot, and from eight 4 m lengths of row in each external control, on July 14, ginned, and weighed.

Results and Discussion

Kaolin Application to Squares on Whole Cotton Plants, Choice Assay

After one d, 1.28 X more unpainted squares had been used for oviposition as compared to the kaolin-painted squares ($P = 0.0003$) (Fig. 1). By the end of a week, all 15 squares that were sampled from every pot of plants were damaged with an oviposition scar. This indicates that boll weevils might find kaolin-coated squares to be tactically or visually less preferable than uncoated squares. Because only the squares of the treated plants were painted with kaolin, it is likely that searching boll weevils could not perceive the presence of kaolin until after they had pushed through the protective bracts and encountered the squares at a distance of several mm or less. Though the difference in the amount of damage was significant at 24 h, it was nevertheless relatively small which suggests that kaolin does not act as a strong repellent. The negation of any difference at the end of one wk suggests that as the availability of undamaged squares declines, kaolin treated squares are more likely to be utilized for oviposition; any barrier effect imposed by kaolin appears to have been overcome by the boll weevils' need to oviposit.

Kaolin Application to Excised Squares, Choice and No-Choice Assays

Gravid boll weevils in the choice assay mostly moved first to the untreated squares at every 10-min interval during the first 90 min ($P \leq 0.01$) (Fig. 2), but by 200 min, the boll weevils were positioning themselves on the kaolin-treated squares as much as on the check squares. After 24 h, the number of egg punctures on the treated and the check squares were equal (Table 1), and the numbers of squares that contained no eggs, and the number with feeding punctures were not significantly affected by a coating of kaolin. However, there were 7 X more undamaged kaolin-coated squares than check squares ($P = 0.0136$), but the average numbers (0.01 – 0.07), taken in the context of practical application, were small.

In the no-choice assay, the gravid boll weevils tended to position themselves on the check squares for the first 90 minutes ($P \leq 0.001$) (Fig. 3), but unlike the results of choice assay, the tendency to position themselves mostly on the check squares continued throughout each of the hourly intervals thereafter up to 330 min ($P \leq 0.05$). After 24 h, check squares had 1.4 X and 1.6 X higher numbers of egg punctures ($P = 0.0282$) and feeding punctures ($P < 0.0001$), respectively. Kaolin-treated squares without any egg punctures and undamaged kaolin-treated squares were 1.1 X ($P < 0.0114$) and 2.01 X ($P < 0.0001$), respectively, more abundant than among the check squares.

This assay demonstrates that gravid boll weevils tend to prefer squares without a kaolin coating. These assays support the results of the assay wherein squares were painted with kaolin while still attached to the cotton plant. Because the boll weevils tended to move immediately to the check squares without first physically contacting the treated squares, it appears that boll weevils prefer the untreated squares based upon differences in color. It might be that once a gravid female begins to oviposit, it will continue to oviposit on any suitable host, even if there is a coating of kaolin. If, however, the gravid female first finds a potential host to be wholly unsuitable, possibly because of a film of kaolin, then some appear to refrain from ovipositing on kaolin-coated squares in future, though others appear to make use of the squares despite the presence of kaolin.

Kaolin Application to Foliage on Whole Cotton Plants, Choice Assay

After two h, 5.17 X more ($P < 0.0001$) squares on the check plants were damaged compared to squares on the kaolin sprayed plants (Fig. 4). The difference declined at 24 h to 1.46 X ($P < 0.0001$), at 72 h, the difference was 1.14 X ($P = 0.0277$), and at one wk, all 15 squares on all plants were damaged. This assay showed that boll weevils utilized untreated squares less if the surrounding foliage was treated with kaolin which suggests that the boll weevil cues to some extent on its familiarity with the normal color of cotton foliage. As in the assays where squares only were treated, kaolin's apparent interference with the boll weevil's host selection processes is largely negated where undamaged square abundance is insufficient to meet the oviposition and feeding requirements of the boll weevil population.

Small Plot Trial, Application to Foliage Sprays to Protect Squares

Application of Surround™, particularly at weekly intervals, appeared to play a role in protecting squares; the check plots had 4.17 X ($P \leq 0.005$), 1.79 X ($P \leq 0.05$), and 2.01 X ($P \leq 0.001$) more square damage than the weekly treated plots on three of the five weekly sampling dates (Fig. 5). On a fourth date (May 5), there were 4.24 X ($P = 0.07$) more boll weevil injured squares in the check plots as compared to the weekly-treated plots. On the remaining date, May 26, there were no significant differences among the three treatments. This might be the result of heavy rain that occurred on May 20 and washed the kaolin from the cotton foliage. Because of muddy field conditions, the next application of Surround™ occurred four days later. It is possible that during the four days when the deterrent effect of kaolin was absent from the plots that were receiving kaolin sprays, boll weevils made nearly equal use of the squares in all of the plots. Re-application of Surround™, once the distribution of square damage was more uniform, was not associated with a decline in square damage except in the plots that received weekly applications.

There was a tendency, albeit statistically nonsignificant, for the weekly kaolin-treated plots to have more squares, bolls, and lint yield per unit length of row than the biweekly kaolin-treated plots and the controls (Table 2). It is conceivable that if rain had not washed away the foliar kaolin coating, the lint yields in the kaolin-treated plots might have been higher. Plant heights were lower in the weekly kaolin-treated plots than in the check plots (0.92 X, $P \leq 0.05$), but this relatively slight difference appears not to have had an influence on yield in comparison to the effects of boll weevil infestations.

The plots treated weekly with Surround™ yielded 2.36 X ($P \leq 0.001$) more cotton lint than the insecticide-free external control and 1.39 X ($P \leq 0.001$) more lint than the external control that received guthion applications (Fig. 6). The lack of significant differences between the control plots and the kaolin-treated plots in the small plot field, and the detection of significantly higher external control yields suggest that kaolin might provide protection from boll weevil injury more effectively than treatments using a conventional insecticide. The higher yield in the guthion sprayed field than in the unprotected external control shows that the applications of guthion increased yield 1.70 X ($P \leq 0.001$). The 1.37 X ($P \leq 0.001$) and 2.34 X ($P \leq 0.001$) greater lint yield in the small plot controls as compared to the guthion-sprayed external control and the unprotected external control, respectively, indicate that visual perception might play a role in orientation toward areas that are either more recognizable as a source of hosts, or toward areas that appear to offer a greater quantity of host plants. In the second scenario, because the Surround™-treated plots may not have been recognized by some boll weevils as an area containing host plants, hence the area in the small plot field appeared to have only ~ 1/3 of its area with a familiar host in contrast to the two adjacent external control fields which would have appeared to be entirely comprised of cotton plants. The similar lint yields among the three small plot treatments shows that some boll weevils arrived in and made use of the small plots in spite of the white and green checkered appearance of the field. Cotton plant volatiles attractive to boll weevils (Chang et al. 1987, Grodowitz et al. 1992) or boll weevil sex pheromone (Hardee et al. 1969), appear to have offset visual confusion caused by kaolin particle film within the small plot field, but this would only occur if the boll weevils were cued in from locations downwind of the field, or boll weevils emerging from the soil in the spring (Cowan et al. 1963, Summy et al. 1988).

Applications of some particle types for suppression of crop injury have been effective on some pests because abrasion of the cuticle or structural disruption of the epicuticle induced water loss and subsequent desiccation (Kalmus 1944, Hunt 1947, Ebeling and Wagner 1959, David and Gardiner 1950). In other insects, such as the spotted cucumber beetle, *Diabrotica undecimpunctata howardi* Barber (Richardson and Glover 1932) and walnut husk fly, *Rhagioletis completa* Cresson (Boyce 1932), ingested particles plugged the hindgut and resulted in mortality. In other instances, particles that cling to the arthropod's body may disrupt feeding and cause it to leave the plant as has been suggested for pear psylla, potato leafhopper, and root weevil (Glenn et al. 1999, Lapointe 2000). However, white reflective surfaces have been shown to repel some insects by affecting their host-finding and settling responses (Kennedy et al. 1961, Kring 1962). This study suggests that *A. grandis* might be less inclined to utilize kaolin-coated host plants for feeding and oviposition prior to making physical contact with the plant. The deterrent value of kaolin, as shown in laboratory, cage, and field studies, can be overcome by the boll weevil if untreated plants become unavailable or by the boll weevil's need to oviposit even when untreated squares are not available.

The field data shows that kaolin particle film can protect cotton squares from boll weevil injury to an appreciable degree, but also that application should occur weekly and that continuous coverage might be important to maintaining deterrence. This study provides evidence that *A. grandis* feeding and oviposition orientation behavior might, at least in part, be affected by color of the plant, and that orientation toward fields might be confused by how the field, or part of the field, appears from a sufficiently broad visual perspective to result in selection between fields or portions of fields.

Because kaolin particle film must be applied repeatedly during the part of the season when cotton lint yield is vulnerable to destruction by the boll weevil, including immediately after rainfall, costs of kaolin application and soil compaction arising from ground application might outweigh the profit from any increases in yield. Research on kaolin particle film as a deterrent to boll weevil injury to cotton should concentrate to a large extent on extending the effectiveness of single applications and reducing the area requiring coverage. For example, early season kaolin particle film coverage of commercial cotton, especially in fields where conventional insecticide application is unlawful or undesirable, could possibly enhance concentration of boll weevil populations in small-area early-squaring cotton trap crops. Also, enhancing particle adhesion to plant surfaces could reduce the number of kaolin applications and protect against loss of coating by rainfall. Further, spraying parts of fields, rather than entire fields, might effect an acceptable level of protection.

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Table 1. Boll weevil damage to excised cotton squares (± SE) in choice and no-choice assays.

Assay[1]	No. egg punctures	No. squares w/o eggs[2]	No. feeding Punctures[2]	No. undamaged Squares
CC	2.9 ± 0.2	0.5 ± 0.1	4.3 ± 0.6	0.01 b
CK	2.9 ± 0.2	0.6 ± 0.1	4.2 ± 0.5	0.07 a
NC	0.7 ± 0.1a	1.5 ± 0.1 b	4.4 ± 0.2 a	0.35 b
NK	0.4 ± 0.1b	1.7 ± 0.1 a	3.1 ± 0.2 b	0.70 a

[1]CC = choice, control; CK = kaolin; n = 100. NC = no-choice, control; NK = no-choice, kaolin; n = 185.
[2]Treatment differences are shown within each assay type. Values followed by different letters are significantly different ($\underline{P} < 0.05$).

Table 2. Cotton plant heights, fruiting structures, and cotton lint yields (± SE) in kaolin treated and check plots[1].

Trtmnt[2]	Plant height (cm)[3]	No. squares[4]	No. bolls[5]	Lint (kg/ha)[6]
C	67.8 ± 2.0 a	148.1 ± 20.6	299.9 ± 12.8	515.3 ± 25.0
K 14 d	68.1 ± 2.9 ab	161.5 ± 21.8	279.5 ± 17.4	507.0 ± 28.2
K 7 d	62.3 ± 1.9 b	165.4 ± 24.0	317.0 ± 9.9	520.5 ± 18.4

[1]Means followed by different letters are significantly different ($\underline{P} \leq 0.05$).
[2]C = control, K = kaolin
[3]n = 400, June 26, 2000.
[4]Per 7.6 m row, n = 8, May 19, 2000.
[5]Per 7.6 m row, n = 8, June 9, 2000.
[6]Per 4 m row, n = 16, hand-harvested July 14, 2000.

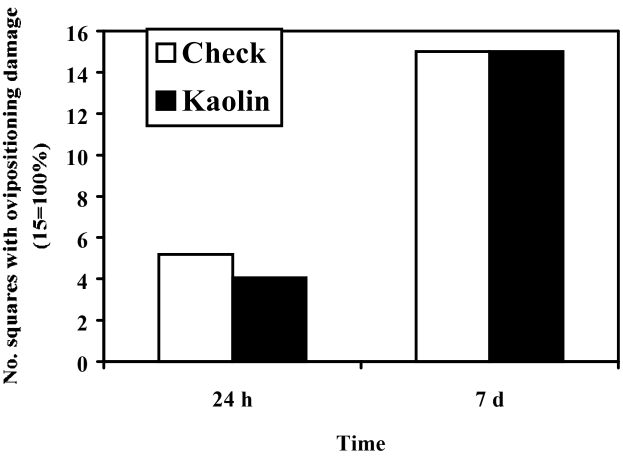

Figure 1. Kaolin-painted and untreated square damage at 24 h and 7 d; different letters over bars indicate P ≤ 0.0005.

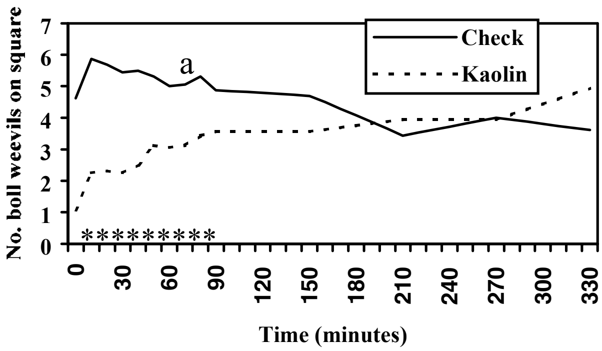

Figure 2. Numbers of gravid female boll weevils positioned on kaolin treated and untreated (check) cotton squares over time, choice assay (*, P ≤ 0.05 at the times shown).

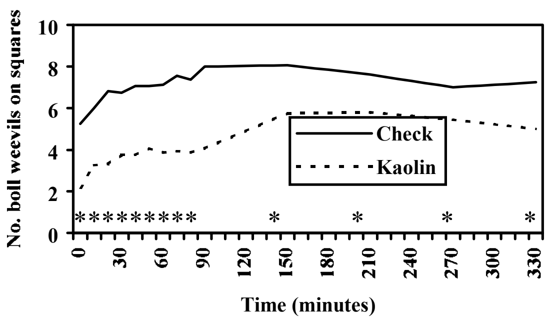

Figure 3. Numbers of gravid female boll weevils positioned on kaolin treated and untreated (check) cotton squares over time, no-choice assay (*, P ≤ 0.05 at the times shown).

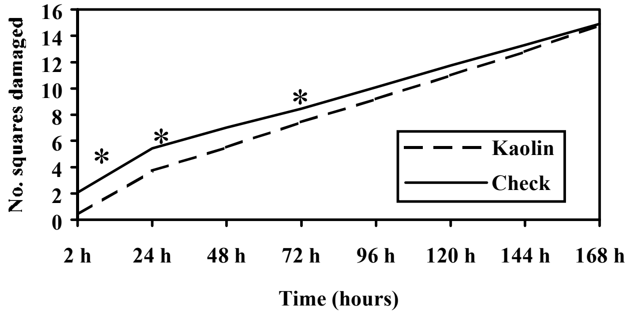

Figure 4. Numbers of squares with boll weevil eggs when cotton foliage was treated with kaolin or left untreated (check) (*, *P* ≤ 0.05 at the times shown).

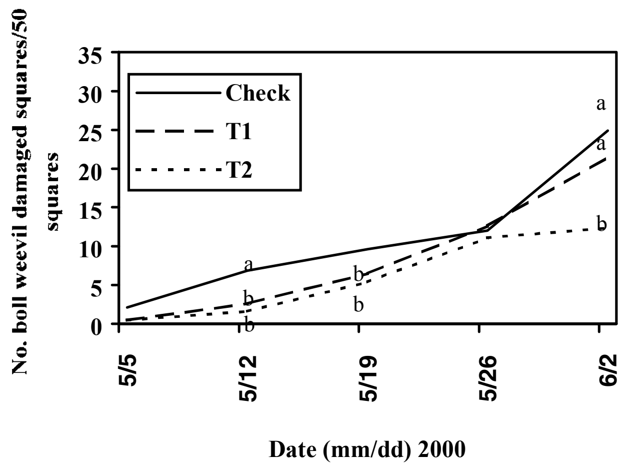

Figure 5. Numbers of boll weevil damaged cotton squares in field plots treated weekly (T2), biweekly (T1), and untreated (check); different letters show significant (P ≤ 0.05) differences that were detected for the days indicated.

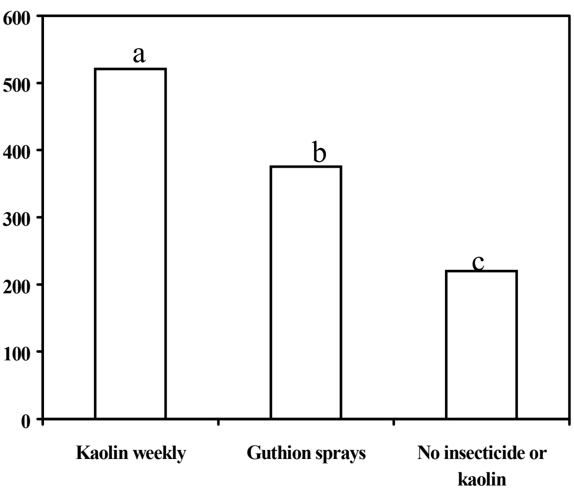

Figure 6. Comparison of cotton lint yield in the weekly kaolin-treated small plots to external control fields; different letters indicate significant (P ≤ 0.001) differences.