Evaluation of Extended-Life Pheromone Formulations Used With and Without Dichlorvos for Boll Weevil (Coleoptera: Curculionidae) Trapping

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ABSTRACT Boll weevil traps baited with a ComboLure (25 of mg grandlure + 30 mg of eugenol + 90 of mg dichlorvos [DDVP]), an extended-release lure (25 mg of grandlure + 30 mg of eugenol + 60 of mg DDVP kill-strip), and extended-release lure with no DDVP were evaluated for boll weevil, *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae), captures in South Texas cotton, *Gossypium hirsutum* L., fields during February–March 2005 and March–April 2006. The traps were serviced once a week for five consecutive weeks by using the same methodology as active boll weevil eradication programs. Mean captured boll weevils from extended-release lures with no DDVP were significantly higher in five of 10 trapping weeks compared with captures of the ComboLure and extended lure. Weekly mortality of boll weevils captured was similar for the ComboLure (72.6 ± 4.7%) and extended lure + DDVP (73.5 ± 4.0%), and both were significantly higher than the extended lure (32.8 ± 5.0%) with no DDVP. The presence or absence of DDVP did not significantly affect the sex ratio of field-captured boll weevils. We found no functional reasoning for using DDVP in large scale trapping of boll weevils regardless of the formulation or presentation in the trap. We conducted two additional trapping evaluations after the 2005 and 2006 studies, but the numbers of boll weevils captured were too low for statistical comparisons, indicating that boll weevil eradication is reducing populations in the Rio Grande Valley of Texas.

KEY WORDS boll weevil, dichlorvos, monitoring, grandlure, trapping

The success of boll weevil, *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae), eradication throughout cotton, *Gossipium hirsutum* L., growing regions of the United States has thus far been due to the ability to monitor the pest with traps baited with grandlure pheromone (Dickerson et al. 2001). A standard boll weevil pheromone dispenser is manufactured with a total of 10 mg of the four components (component I, 30% (+)-*cis*-2-isopropenyl-1-methylcyclobutaneethanol; component II, 40% *cis*-3,3-dimethyl-$\Delta^{1,\beta}$-cyclohexane ethanol; component III, 15% *cis*-3,3-dimethyl-$\Delta^{1,\alpha}$-cyclohexane acetaldehyde; and component IV, 15% *trans*-3,3-dimethyl-$\Delta^{1,\alpha}$-cyclohexane acetaldehyde) of grandlure that attracts both sexes (Tumlinson et al. 1969, Hardee et al. 1974). During active eradication, boll weevil traps are inspected weekly for the presence or absence of captured weevils, whereas the lure-and-kill strips containing 60 mg of dichlorvos ([2,2,-dichlorovinyl dimethyl phosphate]; DDVP) are left to operate for two consecutive weeks before being replaced. Kill strips are used to kill boll weevils after entering the trap; however, 5–8% of the weevils may escape within the first hour of being captured when a kill strip is used (Suh et al. 2003). Hardee et al. (1996) also evaluated the use of kill strips for boll weevil trapping, and they concluded that there was no statistical difference in the capture of weevils; however, servicing the traps was an easier task when the weevils were dead, and the cost was relatively inexpensive. Within the past 6 yr, there has been a significant effort in the manufacture and use of an extended-life or "superlure" dispenser impregnated with 25 mg of grandlure and 30 mg of eugenol (McKibben 2001; McKibben and Dickerson 2002; Johnson et al. 2004, 2005; Armstrong et al. 2006). The higher grandlure content with the addition of eugenol is intended to reduce the cost of trapping by extending the life of the lure from 2 to 3–4 wk to reduce material and labor cost. Eugenol is a clove oil extract added to grandlure to increase the attractiveness and capture of boll weevils (McKibben 2001, McKibben and Dickerson 2002). A more recent amendment to the development of the superlure has been combining grandlure (25 mg) with eugenol (30 mg) and DDVP (90 mg) into a single polyvinyl chloride-impregnated dispenser termed the ComboLure manufactured by Plato Industries (Houston, TX) (Johnson et al. 2005). The ComboLure (ChemTica Internacional, San Jose, Costa Rica) has been tested in

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northern Texas in the spring where mean boll weevil captures were <12 per trap week, and in southern Texas in the fall where >100 boll weevils per trap week were captured while dispersing from mature cotton (Johnson et al. 2005). Our objective was to compare the temporal patterns of boll weevil capture by ComboLures compared with an extended lure used with a standard 60-mg DDVP kill strip, and an extended lure used without DDVP during spring movement of boll weevils into growing cotton. The sex ratio of captured boll weevils was examined to determine whether the combined effects of adding the attractant (grandlure + eugenol) and insecticide (DDVP) would change the ability to attract either sex. We also evaluated the effects of the three different lure types on mortality of field-captured boll weevils. Mortality was recorded every 24 h for 1 wk with freshly captured weevils confined in boll weevil traps. This study was conducted as perhaps the last opportunity for evaluating improvements in boll weevil trapping in the field because active or posteradication programs have been initiated throughout the United States where the boll weevil is known to infest cotton. The Rio Grande Valley of Texas was the last cotton production region to enter active eradication in the fall of 2005, but the number of weevils has decreased to levels that make it difficult to evaluate amendments to lure formulations from field trapping studies.

Materials and Methods

Lure Effectiveness. We evaluated the ComboLure (25 mg of grandlure + 30 mg of eugenol + 90 of mg dichlorovos; Plato Industries) manufactured in a 1.2-g, 2.54- by 2.54-cm polyvinyl chloride dispenser compared with an extended lure (25 mg of grandlure + 30 mg of eugenol) used with a 10% (60 mg., wt:wt) dichlorvos (DDVP [2,2,-dichlorovinyl dimethyl phosphate]) Vaportape (Hercon Environmental, Emigsville, PA) kill strip, and an extended lure used without DDVP for weekly boll weevil captures. Southeast Boll Weevil Eradication Foundation traps (Technical Precision Plastics, Mebane, NC) were supported on top of 1-m conduit poles (2 cm in diameter) and baited with lure–insecticide combinations. Three boll weevil traps baited with the three lure combinations were considered a replicate, and they were spaced 50 m apart. Each of the 16 replicates (48 total traps) were spaced 100 m apart. Traps were stationed on the north sides of brushy irrigation canals oriented in an east–west direction. Boll weevil trapping sites arranged as described have proven to be optimal for capturing boll weevils in South Texas (Sappington and Spurgeon 2000a, Sappington 2002, Armstrong et al. 2006, Spurgeon and Raulston 2006). Boll weevil traps were inspected weekly for five consecutive weeks starting on 15 February 2005, at Russell Plantation, Cameron Co., TX. The second year of study was initiated on 7 March 2006, and it continued for five consecutive weeks at the southern edge of Delta Lake, Hidalgo Co., TX.

Boll Weevil Mortality. At each weekly inspection of traps, all captured weevils were collected in 20-ml glass vials, and they were transported to the laboratory where they were determined to be dead or alive, sexed by the tergal notch method (Sappington and Spurgeon 2000b), and counted. The maximum, minimum, and average daily ambient temperatures were recorded with automated weather stations (Campbell Scientific, Logan, UT) near both trapping sites.

In addition to checking weekly mortality from the field trapping experiment, and to examine mortality to the DDVP over shorter time intervals, freshly captured boll weevils were confined in traps baited with the three lure treatments (ComboLure manufactured with 90 mg of DDVP, extended lure used with a 60-mg DDVP strip, extended lure with no DDVP) and checked for mortality every 24 h, for 1 wk from 14 March to 21 March, 2007. The experiment was a randomized complete block with four blocks of three traps baited with the three lure types, spaced 5 m apart. A group of 20 weevils were enclosed in each trap, and the entrance hole blocked with a cotton ball. Traps with the enclosed weevils were erected outdoors in an open field at the USDA–ARS research station in Weslaco, TX. The source weevils used in the assays were captured with traps baited with extended lures with no DDVP in traps erected at 0900 hours on 13 March and collected at 0900 hours on 14 March, along the Rio Grande River near Santa Anna, Hidalgo Co., TX. Captured weevils were not fed, and care was taken to select healthy weevils with all appendages in tact. A weevil was considered moribund if it was unable to make coordinated movement.

Statistical Analyses. Boll weevil captures for each of the five consecutive weeks of trapping for 2005 and 2006 were analyzed by mixed model analysis of variance (ANOVA, PROC MIXED, SAS Institute 2001). The ANOVA model included the fixed effects of year, week, lure formulation, and lure × week interactions. The denominator degree of freedom was adjusted for the F-tests by using the Kenward–Rogers adjustment (Littell et al. 2002) in the MODEL statement. Differences among least-square means were compared using the Tukey–Kramer adjustment to control the experimental type I error. Weekly and 24-h mortality data were arcsine square-root transformed and subjected to repeated measures analysis (PROC MIXED, SAS Institute 2001), with the degrees of freedom adjusted by Kenward–Rogers as described previously (Littell et al. 2002). Differences among least-square mortality means were separated using the Tukey–Kramer test ($\alpha = 0.05$) to control the experiment-wise type I error rate (adjust = TUKEY option of the LSMEANS statement). Differences among levels of lure type (DDVP versus no DDVP) were further examined using orthogonal contrasts ($\alpha = 0.05$). All data presented in the results are untransformed. The sex ratios of captured male to female boll weevils were statistically compared using PROC MIXED (SAS Institute 2001). Differences in least-square proportions of male to female weevils were compared using the Tukey–Kramer test ($\alpha = 0.05$) for LSMEANS.

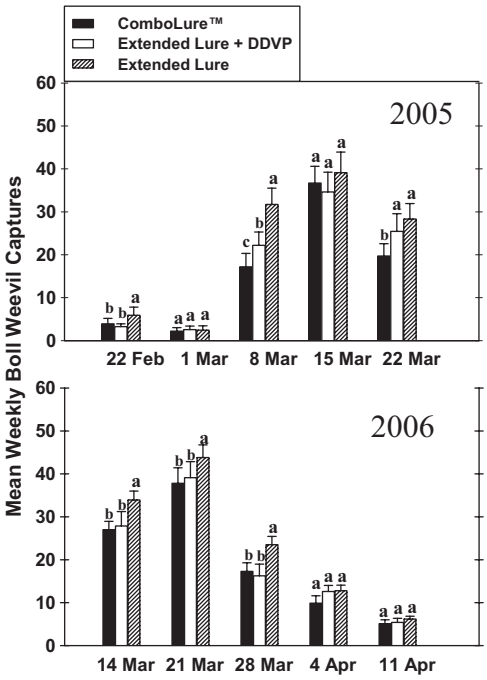

Fig. 1. Mean ± SE boll weevil captures from traps baited with the ComboLure, an extended lure used with a dichlorvos kill strip, or an extended lure without dichlorvos, 2005 and 2006.

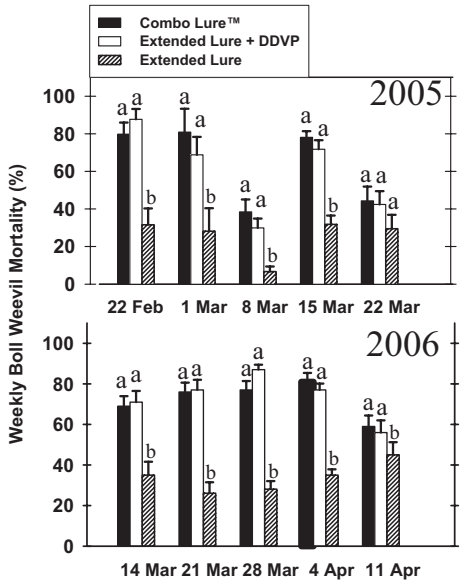

Fig. 2. Weekly percentage ± SE of mortality of boll weevils captured in traps baited with different grandlure and dichlorvos combinations and operated for 1 wk during 2005 and 2006.

Results

Lure Effectiveness. Boll weevil captures for year ($F = 25.75$; df = 1, 419; $P < 0.001$), week ($F = 13.12$; df = 4, 75; $P < 0.001$), and for lure formulation ($F = 3.21$ df = 2, 419; $P = 0.020$) were all significant in this study when the analysis was combined for the two trapping years. The lure formulation by week interaction was not significant ($F = 0.16$; df = 8, 419; $P = 0.591$). Our 5-wk trapping periods during late February and March coincide with an early season spike in boll weevil capture that historically occurs in the Rio Grande Valley (Wolfenbarger et al. 1976, Guerra and Garcia 1982), and the high variability in captures decreases the interaction terms for the weekly trapping effect.

Boll weevil capture for lure formulation ($F = 7.05$; df = 2, 30; $P < 0.001$) and week of capture ($F = 55.12$; df = 4, 180; $P < 0.001$) were significant for 2005, but not for lure formulation by week of capture ($F = 1.15$; df = 8, 180; $P = 0.150$) (Fig. 1) The extended lure resulted in significantly higher boll weevil captures on two of the five trapping dates for 2005, and the orthogonal contrast for the extended lure versus the ComboLure and the extended lure + DDVP kill strip was significant ($t = 3.59$, df = 30, $P = 0.028$).

Boll weevil captures for 2006 were also significant for lure formulation ($F = 11.64$; df = 2, 30; $P < 0.001$), and week of capture ($F = 110.31$; df = 4; 180, $P < 0.001$) but not for the week × lure formulation interaction ($F = 0.56$; df = 8, 180; $P = 0.891$) (Fig. 1). During the first 3 wk of trapping in 2006, the extended lure captured significantly more boll weevils than the ComboLure and the extended lure + DDVP kill strip. The contrast for the extended lure versus the ComboLure and the extended lure + DDVP kill strip was significant ($t = 11.82$, df = 30, $P < 0.001$).

There was no significant difference in the ratio of male to female boll weevils based on the lure formulation in 2005 ($F = 1.09$; df = 2, 189; $P = 0.340$) where a total of 4,416 weevils (51.8% male and 48.2% female) were captured, or 2006 ($F = 1.01$; df = 2, 225; $P = 0.320$) where 4,895 weevils were captured (51.0% male and 49.0% female).

Boll Weevil Mortality. Boll weevil mortality assessed at the end of each weekly trapping interval was significant for lure treatment ($F = 49.62$; df = 2, 30; $P < 0.001$), week of capture ($F = 18.93$; df = 4, 156; $P < 0.001$), and for treatment × week of capture ($F = 69.85$; df = 8, 156; $P = 0.018$) in 2005 (Fig. 2). The mortality of boll weevils was significant when the two lures that contained DDVP were contrast against the extended lure with no DDVP ($t = 9.94$, df = 30, $P < 0.001$). In 2006, boll weevil mortality for lure treatment ($F = 69.22$; df = 2, 45; $P < 0.001$) and treatment by week of capture ($F = 3.96$; df = 4, 180; $P = 0.010$) and the contrast of the two formulations with DDVP were significantly higher ($t = 11.8$, df = 45, $P < 0.001$) that the extended lure alone. The ComboLure and extended lure + DDVP treatments consistently resulted in higher mortality than the extended lure, with the exception of the last trapping week in 2005 in which

Table 1. Ambient temperature for boll weevil trapping where the ComboLure, extended lure + DDVP kill strip, and an extended lure with no DDVP were evaluated

	Temp (°C)		
	Avg	Max	Min.
Week of trapping 2005			
1 (14–21 Feb.)	21.4	29.1	15.3
2 (22 Feb.–1 Mar.)	17.9	29.5	11.1
3 (2–8 Mar.)	19.7	30.2	9.4
4 (9–15 Mar.)	19.5	33.4	10.2
5 (15–22 Mar.)	18.9	29.6	6.4
Week of trapping 2006			
1 (7–14 Mar.)	23.7	35.0	12.8
2 (15–21 Mar.)	23.9	34.3	15.0
3 (22–28 Mar.)	17.2	28.6	3.9
4 (29 Mar.–4 April)	25.1	33.1	20.3
5 (5–11 April)	23.9	35.8	12.2
Week of trapping 2007			
1(14–21 Mar.)	23.2	29.4	18.3

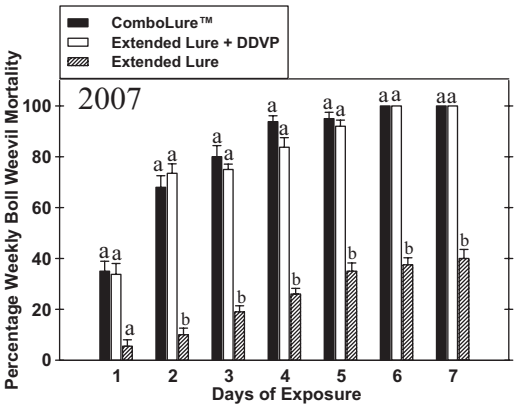

Fig. 3. Mean ± SE cumulative percentage boll weevil ($n = 80$) mortality when confined in a boll weevil trap baited with the ComboLure, an extended lure used with dichlorvos kill strip, or an extended lure without dichlorvos, 14–21 March 2007.

no significant differences in the treatments were detected (Fig. 2). Although mortality declined for all three lure treatments on 8 March 2005, it did not seem to be related to the average, maximum, or minimum temperatures (Table 1.). Mortality on the 22 March, 2005 was not significantly different for any of the lures, but this represented five consecutive weeks of lure operation, which is 1–2 wk longer than the intended 3–4 wk of operation for extended lures.

Mortality observed every 24 h for weevils caught over a 24-h period and exposed to the three lure combinations was significant for lure formulation ($F = 406.54$; df = 2, 6; $P < 0.001$), day of observation ($F = 389.35$; df = 6, 54; $P < 0.001$), and lure formulation × day interaction ($F = 21.01$; df = 12, 54; $P < 0\ 0.001$). An orthogonal contrast of the ComboLure and the extended lure + DDVP versus the extended-lure mortality substantiated the significance ($t = 66.54$, df = 6, $P < 0.001$) of mortality with the use of DDVP. Weevil mortality steadily increased for the ComboLure and extended lure + DDVP formulations for the first 5 d, reaching 100% by day 6 (Fig. 3). Weevils exposed to the extended lure with no DDVP reached 40% after 7 d of exposure (Fig. 3.) This comparison indicates that the ComboLure with 90 mg of DDVP incorporated into the grandlure dispenser, and the extended lure with a 60-mg kill strip show similar mortality trends over 1 wk, whereas the mortality of the weevils confined with an extended lure would represent natural mortality of boll weevils due to starvation and exposure.

Discussion

The results of our 2-yr study of trapping boll weevils with the ComboLure, extended lure + DDVP, and the extended lure with no DDVP indicate that at higher resident populations of boll weevils, there is some repellency in boll weevil captures when DDVP is used in the trap. This is an important point that should not be overlooked because the amount of DDVP has increased from the standard 60-mg kill strip included with a standard 10-mg grandlure dispenser, to 90 mg of DDVP incorporated with 25 mg of grandlure and 30 mg of eugenol combined in the same dispenser.

The threshold for treatment of cotton fields under active boll weevil eradication is determined by capturing a single weevil in a trap over a 1-wk period (APHIS 2006). Therefore, the numbers of boll weevils captured during our field evaluation were far above this threshold, which would have resulted in multiple treatments of ULV malathion provided the cotton was setting flower buds.

The more important result of this study is that the extended lure with no DDVP caught significantly more weevils on 50% of the trapping weeks compared with the other two formulations that used DDVP. This indicates there is no functional reason for using DDVP in large-scale trapping programs. Other studies have compared boll weevil trap captures in which kill strips are included or excluded with the 25 mg of grandlure and 30 mg of eugenol, and where 90 mg of DDVP was incorporated into the 25-mg grandlure dispenser (Johnson et al. 2004, 2005). The results showed no significant differences in boll weevils captured under various densities of trapped boll weevils. Suh et al. (2003) evaluated the use of 60-mg DDVP kill strips, and they found no difference in the number of weevils escaping the trap, regardless of the presence or absence of a kill strip. The study was not designed for the evaluation of capturing weevils, but for the escape and mortality of weevils confined in a boll weevil trap. The study indicates the same as the results presented here, in that DDVP causes significant weekly mortality of captured weevils. An additional related study conducted by Hardee et al. (1996) included the use of different trap designs and lure types used with and without two different kill strips. There were no statistical differences in boll weevil captures regardless of the presence or absence of a kill strip; however, it was concluded that kill strips could still be used because it was easier to count the captured weevils, and the low cost of including the kill strip was minimal. The most

probable reason for using the kill strip other than killing weevils is to deter arthropods from preying on the weevils, or for spiders preying on the weevils or webbing the entrance of the trap. Armstrong and Richman (2007) found that 98% of trap interference (i.e., loss in trapping efficiency) was caused by spiders that blocked the entrance of the boll weevil trap as opposed to predation by spiders or any other arthropod when a kill strip was not included in the trap. The results of current study indicate that higher boll weevil numbers are captured in the absence of using DDVP in the trap. The sum of all the previous mentioned work on the use of DDVP kill strips indicates that there is no advantage for their use, other than servicing the trap is simplified because a greater portion of the weevils are dead. The manufacturing cost of including DDVP, potential handling exposure, and the disposal of used DDVP dispensers does not seem justified for large scale trapping programs for the boll weevil.

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