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EVALUATION OF CATTLE INSECTICIDE TREATMENTS ON ATTRACTION, MORTALITY, AND FECUNDITY OF MOSQUITOES[1,2]

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ABSTRACT. Attraction, engorgement, mortality, and fecundity were observed on host-seeking *Aedes vexans* and *Psorophora confinnis* given the opportunity to feed on insecticide-treated steers in stable traps. Individual steers were treated with Ectrin® ear tags, Ectrin spray, Terminator™ ear tags, Saber™ ear tags, or Ivomec® injection or were left untreated. *Aedes vexans* and *Ps. confinnis* accounted for over 98% of 20,738 mosquitoes collected. Relative attraction ranged from 0.45 with Saber ear tag to 0.84 with Terminator ear tag. Engorgement rates of mosquitoes from insecticide-treated steers ranged from 55 to 91%. Engorgement was reduced 18–45% by Terminator ear tag, Ectrin spray, and Saber ear tag treatments. Forty-eight percent mortality of unengorged *Ae. vexans* and 61% mortality of unengorged *Ps. confinnis* was observed at collection with the Saber ear tag treatment. Percentage of mortality of engorged mosquitoes of both species was generally much lower. Ivomec injection treatment showed the greatest effect on delayed (48-h) mortality of engorged mosquitoes, with 41 and 82% mortality for *Ae. vexans* and *Ps. confinnis*, respectively. No insecticide treatment had a significant effect on fecundity.

INTRODUCTION

Mosquitoes are estimated to cause annual losses of $38.7 million to the cattle industry in the United States (Drummond et al. 1981). In spite of their importance, control strategies for mosquitoes are limited, usually relying on reduction of breeding sites, application of larvicides and biological control agents, or use of ultra-low-volume adulticides.

The treatment of animals with residual insecticides has been proposed as an additional method to control certain mosquito species (Kuntz et al. 1982, Focks and McLaughlin 1988), but this strategy has received little attention. McLaughlin et al. (1989) found that permethrin oil-based pour-on and emulsifiable concentrate spray formulations applied to cattle were effective against *Anopheles quadrimaculatus* Say the first week post-treatment, but mortality dropped off to less than 50% during the second week. Nasci et al. (1990) found that treating cattle with a permethrin spray reduced the percentage of blood-engorged *Psorophora columbiae* (Dyar and Knab) near the treated herd but did not significantly reduce the proportion that were gravid or parous.

Several insecticides and insecticide delivery techniques have been developed to control arthropod pests of cattle, but not many of the effects of these treatments on mosquitoes have been reported. The objective of this study was to determine the effect of some currently labeled insecticide formulations for cattle on attraction, engorgement, mortality, and fecundity of *Aedes vexans* (Meigan) and *Psorophora confinnis* (Lynch Arrabalzaga).

MATERIALS AND METHODS

Insecticide treatments: Six yearling Hereford steers (200–240 kg) were randomly allocated to receive one of the following treatments: 1) 2 Saber™ (10% λ-cyhalothrin) ear tags, 2) 2 Ectrin® (8% fenvalerate) ear tags, 3) 2 Terminator™ (20% diazinon) ear tags, 4) one Ivomec® (1% ivermectin) subcutaneous injection (200 μg/kg), 5) Ectrin (10% fenvalerate) water dispersible liquid (WDL) spray (0.05% AI in 2 liters whole body spray), and 6) untreated control. To achieve maximal release of insecticide from ear tags and to attain optimal blood levels of ivermectin, ear tags were applied 30 days prior and Ivomec was injected 48 h prior to initiation of the experiment (Miller et al. 1983, Fink and Porras 1989). Ectrin WDL spray treatment was applied 12 h prior to initiation to allow for drying.

Trapping procedure: Four 2.2 × 1.2 × 1.6-m stable traps, constructed of standard aluminum screen (18 × 16 mesh) and steel tubing (1.9 cm) were used for collecting mosquitoes. The

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[2] Animals used in this study were handled with the approval and under the guidelines of the New Mexico State University Institutional Animal Care and Use Committee.

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Table 1. Species composition and abundance of mosquitoes collected from steer-baited traps.

Species	Total	Mean/night/ steer	Proportion
Aedes vexans	11,133	347.9	0.5368
Psorophora confinnis	9,309	290.9	0.4489
Culex tarsalis	238	7.4	0.0115
Aedes dorsalis	26	0.9	0.0013
Culex quinquefasciatus	19	0.6	0.0009
Anopheles franciscanus	13	0.4	0.0006
Total	20,738	648.1	1.0000

basic trap design was similar to that described by Roberts (1965); however, the sides were modified, with openings (1.9 cm) running the length of the traps at heights of 22.8, 77.6, and 121.9 cm from ground level. These openings could be closed easily to prevent mosquitoes from entering or leaving the traps. A further modification was placing nylon netting behind each door to reduce mosquito loss during experimental manipulations. Traps were oriented in a north–south direction 8 m apart in an opening (50 × 200 m) within a large pecan orchard (2,025 ha) in Dona Ana County, NM. Mosquito species composition and relative abundance data collected from CDC traps were used to select this site. Also, no domestic livestock were present within the immediate area.

For each of 8 consecutive nights, the untreated steer and 3 of the insecticide-treated steers were randomly allocated to traps until all treatments had been tested for a minimum of 4 nights, with each treatment in each relative trap location at least one night during the study period. Steers were stanchioned inside the traps at 1900 h, and the side slots were opened, allowing host-seeking mosquitoes to enter. The side slots were closed the following morning at 0700 h, and the steers were removed 30 min later. This delay allowed any unfed mosquitoes in the trap an opportunity to engorge. Following daily collection of mosquitoes, stable traps were washed with tap water and wiped with 75% ethyl alcohol.

Insect collection: Ten bloodfed mosquitoes of each species from each treatment were collected individually in 20-ml glass scintillation vials, then vial tops were covered with nylon netting. Mosquitoes were transported to the laboratory, maintained at 25°C and 80% RH, and fed twice a day by placing moistened raisins on the mesh vial covers. Mortality data were recorded at 48 h. Mosquitoes surviving for 72 h were dissected, and developed eggs were counted to determine treatment effects on fecundity.

The remaining mosquitoes were collected using a battery-powered aspirator (Meek et al. 1985) and frozen. To determine interaction of engorgement on mortality of trapped mosquitoes, live mosquitoes were collected separately from dead mosquitoes.

Relative attraction, percentage of engorgement, and mortality of engorged and unengorged mosquitoes at collection were calculated for each treatment. Relative attraction was determined by dividing the number of mosquitoes collected from a treatment by the number collected from the untreated control. Engorgement was determined by the expansion and coloration of the abdomen of engorged mosquitoes (Edman and Kale 1971). Partially engorged individuals were classified as engorged.

Statistical analysis: Analysis of variance and mean separation procedures were performed using SAS software (SAS Institute 1989) to detect treatment differences in relative attraction, percentage of engorgement, and fecundity of mosquitoes. Significant differences were determined by the least significance difference (LSD) procedure only if a significant F was observed from the analysis of variance procedure. Means were significantly different if $P < 0.05$ using the protected LSD. The Chi-square likelihood ratio statistic was used to analyze percentage of mortality of engorged and unengorged mosquitoes at collection and percentage of mortality of engorged (*in vitro*) mosquitoes at 48 h. The Chi-square test for equal mortality rates was rejected at $P < 0.01$.

RESULTS

A total of 20,738 mosquitoes representing 4 genera and 6 species was collected (Table 1). Averages of 348 *Ae. vexans* and 291 *Ps. confinnis* per animal per night were collected. These 2 species represented 98.6% of the total mosquitoes collected and were used as indicator species. The remaining 1.4% collected, in descending order of prevalence, were *Culex tarsalis* Coq., *Aedes dorsalis* (Meigen), *Culex quinque-*

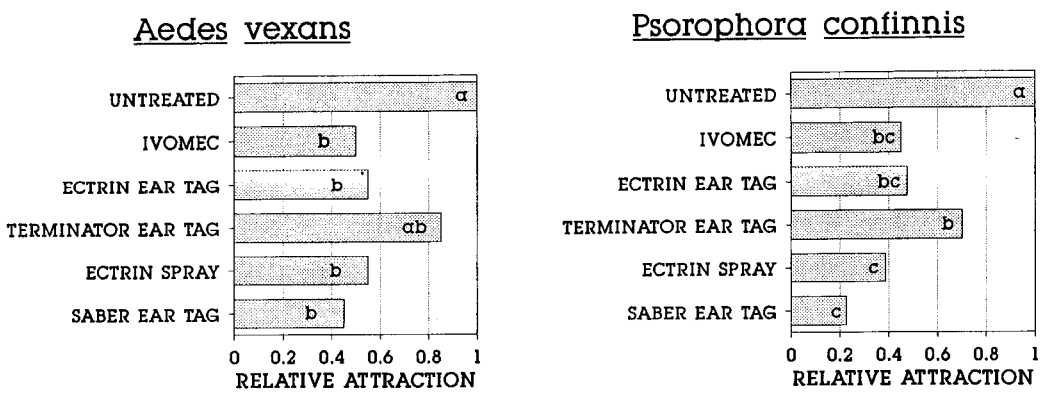

Fig. 1. Relative attraction of mosquitoes to steers treated with selected insecticides. Values followed by the same letter are not significantly different ($P > 0.05$).

fasciatus Say, and *Anopheles franciscanus* McCracken.

Relative attraction of insecticide-treated steers to mosquitoes is shown in Fig. 1. Values for relative attraction to *Ae. vexans* ranged from 0.45 to 0.84. Attraction was significantly reduced by all treatments except the Terminator ear tag treatment. Saber ear tag treatment attracted 55% fewer *Ae. vexans* than did the untreated control. This level of efficacy (% reduction) was followed by Ivomec (50%) > Ectrin ear tag (46%) > Ectrin spray (43%) > Terminator ear tag (17%). Treatment effects on attraction were more pronounced with *Ps. confinnis.* Significantly fewer *Ps. confinnis* were collected from all treatments compared with the control, with relative attraction of treated steers ranging from 0.23 to 0.69. Again, Saber ear tag treatment was the most effective, attracting 78% fewer *Ps. confinnis* than did the untreated control. This level of efficacy was followed by Ectrin spray (62%) > Ivomec (55%) > Ectrin ear tag (53%) > Terminator ear tag (31%).

Percent engorgement of mosquitoes at collection is shown in Fig. 2. Treatment effects were similar between mosquito species. Ninety-eight percent of *Ae. vexans* and 97% of *Ps. confinnis* collected from the untreated control steer were engorged, whereas engorgement rates on insecticide-treated steers ranged from 55 to 91% for both species. Saber ear tag, Ectrin spray, and Terminator ear tag treatments significantly reduced engorgement in *Ae. vexans* by 42, 33, and 18% and in *Ps. confinnis* by 45, 41, and 24%, respectively.

Percentage of mortality of unengorged *vs.* engorged mosquitoes at collection is shown in Fig. 3. Chi-square analyses of equal mortality rates yielded P values of <0.01 for both engorged and unengorged mosquitoes of either species. Mortality of unengorged *Ae. vexans* and *Ps. confinnis* exposed to the various insecticide treatments

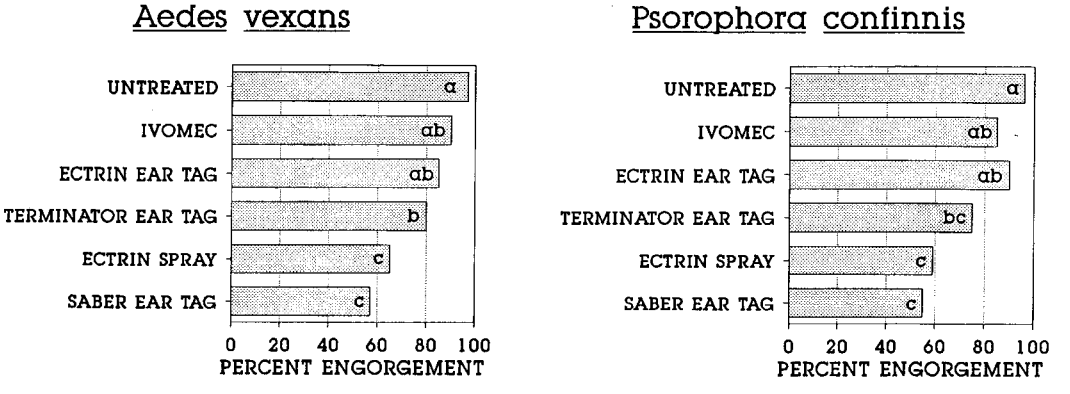

Fig. 2. Percentage of engorgement of mosquitoes allowed to feed on steers treated with selected insecticides. Values followed by the same letter are not significantly different ($P > 0.05$).

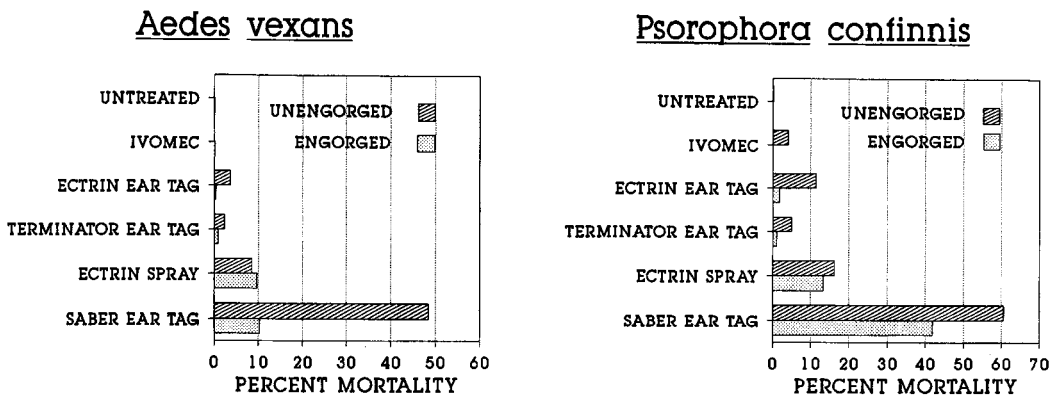

Fig. 3. Percentage of mortality at collection of unengorged and engorged mosquitoes exposed to steers treated with selected insecticides. The Chi-square test for equal mortality rates was rejected at $P < 0.01$.

was similar except a slightly higher mortality was observed for *Ps. confinnis*. Mortality of *Ae. vexans* was <1% for both control and Ivomec treatments, whereas mortality rates of *Ps. confinnis* with control and Ivomec treatments were <1 and 4%, respectively. Mortality with the ear tag treatments varied greatly among different insecticides. Mortality of both *Ae. vexans* and *Ps. confinnis* was much higher with Saber ear tag treatment than with Ectrin or Terminator ear tag treatments. Mortality rates for Saber ear tags were 48 and 61% for *Ae. vexans* and *Ps. confinnis*, respectively, compared with a range of 2–11% for Ectrin and Terminator ear tags. In both species, mortality with Ectrin spray was higher than with Ectrin or Terminator ear tag treatments but much lower than with Saber ear tag treatment.

Mortality of engorged *Ae. vexans* and *Ps. confinnis* was consistently much lower than in unengorged mosquitoes, ranging from <1 to 42%. The same general trends in treatment efficacy were seen in engorged mosquitoes.

Forty-eight-hour mortality of engorged *Ae. vexans* and *Ps. confinnis* maintained *in vitro* is shown in Fig. 4. The Chi-square analyses for equal mortality rates yielded P values of <0.01 for both species. Mortality rates in the control treatment were similar for both species (11 and 13% for *Ps. confinnis* and *Ae. vexans*, respectively), and there was a wide range of mortality among insecticide treatments. The greatest mortality was observed in the Ivomec treatment, with 41 and 82% mortality for *Ae. vexans* and *Ps. confinnis*, respectively.

There were no significant treatment effects on egg production of engorged mosquitoes dissected 72 h post-feeding. Overall, mean egg production ($\bar{x} \pm$ SEM) was 149.7 ± 40.3 eggs per female for *Ae. vexans* (n = 197) and 107.8 ± 35.7 eggs per female for *Ps. confinnis* (n = 112).

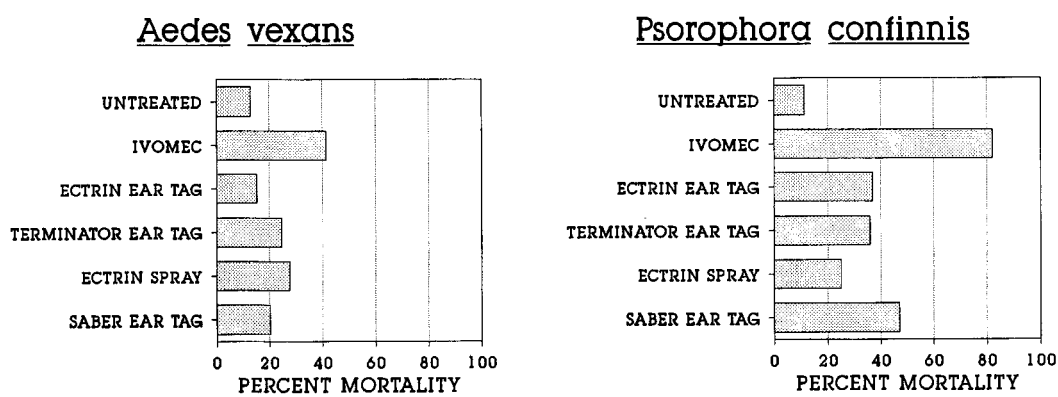

Fig. 4. Forty-eight-hour mortality of mosquitoes engorged on steers treated with selected insecticides. The Chi-square test for equal mortality rates was rejected at $P < 0.01$.

DISCUSSION

Methodologies currently used for controlling arthropod pests of cattle have an effect on attraction, engorgement, and initial mortality but little to no effect on fecundity or delayed (48-h) mortality of *Ae. vexans* and *Ps. confinnis.* The reduced attraction and engorgement rates observed on insecticide-treated steers suggest that some treatments act as repellents.

The greatest repellent response was observed with Saber (cyhalothrin) and Ectrin (fenvalerate) insecticide treatments and to a lesser extent with Ivomec (ivermectin) injection. The repellent activity of pyrethroids is well documented, in fact, pyrethroids have been impregnated into clothing and used as aerosol sprays for personal protection against mosquitoes, black flies, and ticks (Lindsay and McAndless 1978; Schreck et al. 1982, 1986; Frances 1987). Studies by Lockwood et al. (1985) on horn flies and by McLaughlin et al. (1989) on *An. quadrimaculatus* suggest that the repellent activity of pyrethroids is due to the initial contact of an insect with the insecticide. If the reduction in number of mosquitoes trapped with pyrethroid-treated steers observed in our study is a form of repellency, some mechanism other than direct contact may be operating. This assumption is based on the 30-cm distance separating the stanchioned steers from the sides of the screened trap; any repellent effect would have to have been perceived from some distance away. Ivomec treatment also exhibited a small amount of repellency, but the mechanisms of this phenomenon are not understood.

Saber ear tag treatment greatly reduced the survival of feeding mosquitoes, with observed mortality at collection being much greater for unengorged than for engorged mosquitoes. However, this trend occurred with all insecticide treatments except Ivomec. These findings support McLaughlin et al. (1989), who found that engorged *An. quadrimaculatus* were less susceptible to permethrin than were unengorged individuals, and Zyzak et al. (1989), who found similar differences with the horse fly *Tabanus fusciostatus* Hine. A possible explanation for the differential mortality observed in our study is that unengorged mosquitoes may have initially attempted to feed at locations on the steer where the insecticide was in relatively high concentration and either acquired a lethal exposure initially or sensed contact irritancy to the insecticide and moved to another location, thereby acquiring a lethal exposure. Conversely, mosquitoes that survived while engorging may have initially selected feeding sites where insecticide concentration was relatively low. The mechanisms involved in reduced mortality of engorged mosquitoes require further study.

Mortality of both engorged and unengorged mosquitoes was higher with cyhalothrin (Saber) than with the other pyrethroid, fenvalerate (Ectrin). Similar results have been reported with other hematophagous insects. For example, Sparks and Byford (1988) found cyhalothrin more toxic to horn files than were other pyrethroids.

Ivomec showed the most effect on delayed (48-h) mortality in both mosquito species compared with other insecticide treatments, but mortality was greater with *Ps. confinnis.* Pampiglione (1985) found mortality varied among mosquito species that had fed on mice injected with ivermectin. Mortality of *Anopheles stephensi* Liston was 100% within 36 h after feeding on mice treated at a rate of 2.8 mg ivermectin/kg body weight, contrasting with 50% mortality of *Cx. quinquefasciatus* and *Aedos aegypti* (Linn.). Miller et al. (1986) recorded similar results in hematophagous Muscidae, with high mortality in horn flies but only 40% mortality in stable flies *Stomoxys calcitrans* (Linn.) feeding on heifers treated at a rate of 200 μg/kg body weight.

No treatment significantly reduced fecundity in the mosquito species tested, although ivermectin has been shown to reduce both fecundity and hatching rate in other hematophagous pests (Lancaster et al. 1982, Miller et al. 1986). One possible explanation, suggested by the relatively higher rate of 48-h mortality in the Ivomec treatment, is that the amount of ivermectin required to affect egg production is lethal to the mosquito species tested.

The most effective, and therefore most commonly utilized, methodologies for controlling hematophagous pests, including those selected for this study, are designed to be effective for prolonged periods. Prolonged exposure of pests to insecticides leads to the selection of less susceptible individuals and predisposes local populations to insecticide resistance (Taylor and Georghiou 1982). In addition, prolonged exposure of local mosquito populations to repellent chemicals could lead to alterations in their normal feeding behavior, including seeking alternate hosts such as man, wildlife, and companion animals. Therefore, altered host selection could have significant public health and veterinary considerations.

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