Efficacy of an Organophosphate Mixture Against an Organophosphate-resistant Strain of *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae)[1]

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Abstract Efficacy of an organophosphate (OP) mixture acaricide, Ravap® (Bayer, Shawnee, KS) was evaluated as a spray at 0.15 and 0.3% active ingredient (AI) on cattle infested with all parasitic stages of OP-resistant *Rhipicephalus (Boophilus) microplus* (Canestrini). Laboratory bioassays showed ticks were 18.7X more resistant to OP acaricides than a susceptible reference strain. Overall results demonstrated both concentrations produced significantly greater adverse effects on ticks in every measured parameter than were obtained from untreated ticks, except for female engorgement weight. Overall percentage control at 0.15 and 0.3% AI was 85.3 and 87.6%, respectively. Ravap was most effective against ticks treated in the larval stage and least effective against ticks treated in the adult stage. At 0.15 and 0.3% AI, control against adults was 79.8 and 76.2%, respectively, whereas control against ticks in the larval stage was 96.5 and 97.7%, respectively, with no significant differences. Control against ticks treated in the nymphal stage was intermediate (82.5% at 0.15% AI and 93.1% at 0.3% AI) and there was a significant difference between concentrations. Although this OP mixture acaricide provided good control against a highly OP-resistant strain of ticks, the control was still well below the 99% level required for use in the U.S. Cattle Fever Tick Eradication Program. Therefore, a single treatment with this mixture acaricide against OP-resistant ticks would still pose a risk of dispersing cattle harboring viable ticks to uninfested areas. Effect of pesticide application method (spray versus dip) and potential for Ravap use in an emergency tick outbreak situation are discussed.

Key words cattle fever tick, control, acaricide, organophosphate, pesticide-resistance

During the last 106 years the U.S. Cattle Fever Tick Eradication Program (CFTEP), managed by the U.S. Department of Agriculture (USDA), Animal Plant Health Inspection Service (APHIS), Veterinary Services (VS) branch, has been enormously successful in eliminating cattle fever ticks (*Rhipicephalus (Boophilus)* spp.) from an area covering more than 1.8 million km^2 within the U.S. borders (Graham and Hourrigan

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1977, Lohmeyer et al. 2011). The reestablishment of these serious disease vectors has been prevented through the institution, implementation, and enforcement of regulations strictly limiting the entrance and/or movement of potential host livestock into and within the U.S. from endemic areas such as Mexico. The success of the CFTEP has been largely the result of the systematic treatment of livestock in a total immersion dipping vat using a high-dose pesticide treatment strategy. Whereas the use of the high-dose, total immersion treatment strategy has served the CFTEP well throughout the history of the program, the incidence of pesticide resistance in cattle fever tick populations has created great concern. In particular the development of acaricide resistance in cattle fever ticks in Mexico is problematic because not only are the ticks endemic there, but an enormous number of Mexican cattle are imported into the U.S. each year, thereby increasing the risk of reintroduction of both resistant and susceptible ticks. The risk factor is heightened by the fact that since about 1968 the organophosphate (OP) compound, coumaphos, has been the only acaricide approved for use in the CFTEP.

In Mexico pesticide resistance to most classes of pesticides used for control of cattle fever ticks has become widespread and intense. The first report of OP resistance in Mexico occurred in 1982 after intensive use of OP chemicals in the national eradication campaign (Aguirre et al. 1986). Eleven years later, in 1993, the first resistance to pyrethroid acaricides in Mexico was reported (Santamaria and Fragoso 1994). Subsequently, formamidine (amitraz) resistance was reported in 2002 (Soberanes-Cepedes et al. 2002) and more recently, in 2010, macrocyclic lactone (ivermectin) resistance was reported (Perez-Cogollo et al. 2010). Cattle fever tick resistance to acaricides was unknown in the U.S., until cases of OP and pyrethroid resistance were reported in 2005 and 2007, respectively (Miller et al. 2005, 2007). Thus far, there has been no confirmed resistance to formamidines or macrocyclic lactone acaricides in the U.S.; however, the only geographic barrier to the movement of resistant ticks into the U.S. is the Rio Grande River that oftentimes provides little or no impediment to movement of stray animals harboring ticks. In addition, the risk of resistant tick incursion into the U.S. is further enhanced by the presence of dense stands of the invasive giant weed, *Arundo donax* L. that occurs throughout the permanent quarantine zone, making detection of tick-infested animals extremely difficult, whereas also potentially providing suitable habitat for ticks that may detach (Racelis et al. 2012).

Among other possibilities, the use of pesticide mixtures has been proposed as one method of dealing with pesticide-resistant arthropods. A pesticide mixture is composed of two or more pesticides combined into a single treatment solution (Cloyd 2001), such that the arthropod pest population is exposed to each of the chemicals in the mixture simultaneously (Tabashnik 1989). Mixing pesticides with different modes of action may delay the development of resistance because the pest is unable to cope with exposure to multiple pesticides simultaneously (Curtice 1985). In New Caledonia on-animal studies resulted in a dramatic reduction in cattle fever tick numbers on cattle when amitraz was used as a synergist for deltamethrin against ticks that were highly resistant to both acaricides (Barre et al. 2008). Conversely, in the beginning of the 1990s, mixtures of pyrethroid with OP chemicals, as well as mixtures of one OP chemical with another OP chemical were used in Brazil as a means of controlling populations of cattle fever ticks that were resistant to both classes of chemicals; these endeavors generally resulted in an even greater reemergence of resistance (Mendes et al. 2011). Whereas no pesticide mixtures have ever been used in the fever tick eradication program, the mixture acaricide, Ravap E.C.® (Bayer, Shawnee, KS) containing both tetrachlorvinphos (23%) and dichlorvos (5.3%), is registered for use in the U.S. Although the registration label for this material does not include treatment of cattle fever ticks, it does include the lone star tick, *Amblyomma americanum* L.

The purpose of this study was to evaluate the efficacy of the OP mixture acaricide, Ravap, applied as a whole-body spray to cattle infested with an OP-resistant strain of *R. (B.) microplus* (Canestrini) at two different concentrations. Results may provide insight into whether synergism or potentiation occurs between the mixed acaricides that increases efficacy beyond the level of a single chemical administered individually. Positive results could provide the CFTEP with an important alternative acaricide against OP-resistant ticks.

Materials and Methods

The study was conducted at the USDA, Agricultural Research Service (ARS), Cattle Fever Tick Research Laboratory (CFTRL), Edinburg, TX. The product used in the evaluation was the mixture acaricide, Ravap E.C. (rights formerly owned by KMG-Bernuth, Inc., Houston TX; rights now owned by Bayer Animal Health, Shawnee, KS). The formulated material was an emulsifiable concentrate containing the two OP acaricides, tetrachlorvinphos (23% active ingredient (AI)) and dichlorvos (5.7% AI). All cattle used in the study were Angus heifers weighing approx. 180 kg each. Twelve calves were randomly assigned to 1 of 3 equal groups, with each group consisting of 4 animals. Throughout the duration of the study, calves were stanchioned individually inside of 3.3 × 3.3 m stalls with concrete floors that were separated from each other by 1.7 m high sealed cinder-block walls. The study was conducted in an open-sided barn under ambient temperature and humidity conditions (no direct rainfall or sunlight reached the animals because of the roof).

The OP-resistant strain of *R. (B.) microplus* used in the study was originally colonized from engorged females collected from cattle located in Champoton, Campeche, Mexico in 1998. The ticks have been in continuous colonization at the CFTRL since the original collection and have been selectively exposed to the OP coumaphos intermittently throughout the colonization process. Immediately prior to conducting this study, the level of resistance to coumaphos was assessed for the generation of ticks used in this study by comparing serial dilution bioassay results with results obtained from the OP-susceptible reference strain maintained at the CFTRL.

Prior to treatment, each calf was infested 3 times with ≈5000 OP-resistant *R. (B.) microplus* larvae that were 3 - 5-wk-old at each infestation. The initial infestation was applied at 20 d prior to treatment, whereas additional infestations were applied at 13 and 6 d prior to treatment. This infestation pattern (7 d intervals) provided a means of not only evaluating the overall effect of Ravap, but also evaluating it against each individual parasitic development stage of the tick (adult, nymph, and larva) at the time of treatment.

Two concentrations of Ravap were evaluated. One group of calves was treated at a concentration of 0.15% AI, which is the manufacturer's recommended dosage for control of lone star ticks. A second group of calves was treated at a concentration of 0.3% AI, to provide insight into the level of control that might be expected at 2X the recommended dosage rate. The third group of calves remained untreated to serve as a control group. The treatment procedure for each animal in each of the treatment groups consisted of removing a group of animals from stanchion, herding them to an adjacent cinder block building with a concrete floor and a restraining chute inside, and placing calves individually in the restraining chute for treatment. Ten L of the appropriate concentration of the Ravap was applied to each animal using a Model 61 Bean® electric powered sprayer (John Bean, Lagrange, GA) set at 827 kPa, calibrated to deliver 7.125 L per min. After treatment each animal was held in the chute for several minutes to allow runoff of excess liquid chemical, after which treated animals were held in a drain pen then returned to the individual stanchion from which they had been removed.

Beginning the day following treatment and continuing for 21 consecutive days, engorged females that detached from each calf in each treatment group were collected and counted. A random sample of up to 10 female ticks per calf per day was collected to obtain ovipositional data on the ticks. Female ticks in each sample were weighed collectively, placed in a Petri dish (9 cm diam), maintained in an incubator at 27 ± 2°C with 92% RH and a photoperiod of 12-h light and 12-h dark, and allowed to oviposit for 20 d. After oviposition the female ticks were discarded and each egg mass was weighed and placed in a coded 25 × 95-mm (36 ml) shell vial, stoppered with a cotton plug, and returned to the incubator. Four weeks after the egg masses were weighed, the percentage of eggs that hatched for each sample was estimated by visually comparing the proportion of larvae to the proportion of unhatched eggs within the vial by use of a stereomicroscope.

The index of fecundity (IF) of the ticks recovered from each calf on each day was calculated using the formula reported by Davey et al. (2001), which was a modification of the index of reproduction (IR) reported by Drummond et al. (1967), as follows:

IF = Total No. of ♀♀ Collected * Weight of Eggs (g) / No. of ♀♀ sampled * Estimated Egg Hatch (%)

The calculated IF value represented an estimate of the fecundity and fertility (reproductive capacity) of all ticks collected on a single day for a single calf.

Overall effect of the treatment. To determine the overall percentage control of the Ravap treatments against all ticks on the animals at the time of treatment, the daily IF values of the ticks recovered from each calf on each day were calculated then summed for each calf within each of the 3 groups for the entire 21-day evaluation period, producing a total IF value for ticks from each calf within a given treatment group. Once all IF calculations were complete, the mean total IF of ticks recovered from the 4 untreated calves was compared with the total IF for ticks from each individual calf in the treated groups, using a modified Abbott's formula (Abbott 1925), to provide a percentage control value, as follows:

% Control = ((Mean Total IF for Untreated Group – Total IF of Each Calf in the Treated Group) / Mean Total IF for Untreated Group) * 100

Effect of treatment on each development stage. The timing of the 3 pretreatment infestations at 7-d intervals, along with the known reported detachment pattern revealing that > 90% of ticks infested at a given time will detach 21 - 27 d after infestation (Hitchcock 1955), provided a means of estimating the level of control achieved against each parasitic stage of the tick (adult, nymph, and larva). All engorged females collected at 1 - 7 d after treatment were considered to have been adults at the time of treatment, because they were detaching at 21 - 27 d after the initial infestation (20 d pretreatment infestation). Likewise, all ticks recovered at 8 - 14 d after treatment were considered to have been nymphs at the time of treatment, because they were detaching at 21 - 27 d after they were infested (13 d pretreatment infestation) at this time interval. Finally, ticks collected on Days 15 - 21 following treatment were considered to have been larvae at the time of treatment, as they were detaching at 21 - 27 d after infestation at this time (6 d pretreatment infestation). Daily IF values for each calf within each treatment group were classified as 1 of the 3 developmental stages (adult, nymph, and larva), summed across the 7-d interval that constituted the developmental stage, and compared with the mean IF value for the untreated control group that had the same developmental designation. The percentage of control achieved was then determined as described previously.

Female ticks recovered from the cattle during the 21-d posttreatment evaluation period were also evaluated to determine whether treatment with various Ravap concentrations had any measurable effect on the surviving ticks. The engorgement weight of female ticks and weight of egg masses produced by the females in each of the 3 groups were compared in both the overall and stage-wise analyses.

All measured variables (number of ticks, IF, percentage control, weight of female ticks, and weight of egg mass) in both the overall and individual developmental stage analyses were subjected to statistical analysis by use of a one-way analysis of variance (ANOVA) using a General Linear Model (GLM) (SAS/STAT 1987). Differences among means within each measured variable were determined using the Ryan-Einot-Gabriel-Welsch multiple-range test ($P < 0.05$). Percentage control values were transformed to arcsin scale, whereas IF values were transformed to log scale prior to data analysis, although for ease of understanding, data within the tables are presented in the form of untransformed values.

Results

The laboratory bioassay conducted with the OP compound, coumaphos, immediately prior to the study using larvae of the same strain and generation of OP-resistant ticks, resulted in an estimated median lethal concentration (LC_{50}) of the treated ticks of 0.707% AI (95% confidence limit [CL]: 0.624 - 0.802% AI). By comparison, results obtained at the same time using an OP-susceptible reference strain produced an LC_{50} value of 0.0378% AI (95% CL: 0.0342 - 0.0416% AI). Thus, at the time of the study, the OP-resistant strain used in the study had a resistance ratio (RR) of 1:18.7 to OP acaricides, meaning they were 18.7X more resistant than susceptible ticks.

Overall effect of the treatment. The mean number (±SD) of ticks per calf recovered from untreated cattle (2833 ± 508) was significantly higher ($F = 47.26$; df = 2,9; $P < 0.0001$) than from either Ravap treatment (Table 1). Whereas there was no significant difference ($P > 0.05$) in the number of females that survived to repletion in either the 0.15% AI treatment (934 ± 191) or the 0.3% AI treatment (801 ± 184), both groups produced 3-to 3.5-times fewer ($P < 0.05$) females per animal than the untreated group.

The IF values showed the same pattern that was observed for the number of ticks per calf, with ticks recovered from the untreated cattle having a significantly higher IF ($F = 104.16$; df = 2,9; $P < 0.0001$) than ticks recovered from either of the Ravap-treatments (Table 1). Untreated females showed a reproductive capacity (IF = 439.8 ± 73.7) that was approximately 7X to 8X higher ($P < 0.05$) than females recovered from the 0.15% AI treatment (IF = 64.8 ± 10.3) or the 0.3% AI treatment (IF = 54.6 ± 2.7), respectively, which were not different from each other ($P > 0.05$).

Table 1. Mean ± SD number of ticks per animal, index of fecundity (IF), female engorgement weight, egg mass weight, and percentage control of an organophosphate (OP)-resistant strain of *B. microplus* recovered from untreated and treated cattle sprayed with an OP mixture of Ravap.

Conc. (%AI)	No. of ticks per animal	Weight of female (mg)	Egg Mass Weight (mg)	Index of Fecundity (IF)	Control of IF (%)
Untreated	2833 ± 508 a	317 ± 59 a	147 ± 47 a	439.8 ± 73.7 a	————
0.15	934 ± 191 b	297 ± 80 a	105 ± 51 b	64.8 ± 10.3 b	85.3 ± 2.4 a
0.30	801 ± 184 b	294 ± 85 a	100 ± 48 b	54.6 ± 2.7 b	87.6 ± 0.6 a

Means within the same column followed by the same letter are not significantly different ($P = 0.05$); tested by Ryan-Einot-Gabriel-Welsch Multiple Range Test.

Results showed the effect of the Ravap treatments on the engorgement weight of female ticks that survived to repletion was minimal (Table 1). There was no significant difference among any of the groups of cattle, treated or untreated ($F = 2.26$; df = 2,244; $P > 0.05$). The mean weight (±SD) of untreated females was 317 ± 59 mg, whereas the mean weight of females recovered from treated animals was only slightly lower at 297 ± 80 and 294 ± 85 mg for females obtained from the 0.15% AI group and the 0.3% AI group, respectively. Whereas the application of Ravap to ticks on cattle had little effect on the engorgement weight of females, analysis of the weight of egg masses produced by the females showed a significant difference ($F = 23.35$; df = 2,244; $P < 0.0001$). Egg mass weights of untreated females (147 ± 47 mg) were significantly heavier ($P < 0.05$) than those of females obtained from either the 0.15 or 0.3% AI treated groups (105 ± 51 and 100 ± 48 mg, respectively), which were not significantly different ($P > 0.05$) from each other.

The overall level of control (defined as reduction in the IF) obtained against the OP-resistant strain of southern cattle ticks showed no significant difference ($F = 3.79$; df = 1,6; $P > 0.05$) between the two treatment concentrations (Table 1). At the 0.15% AI concentration control was 85.3 ± 2.4%, whereas at the 0.3% AI concentration the level of control only increased to 87.6 ± 0.6%. Thus, doubling of the dosage rate of Ravap had very little impact on the level of control afforded by the treatment.

Effect on each development stage of the tick. Effects of the two Ravap concentrations on the various parasitic development stages of the OP-resistant ticks were, in most respects, reflective of the overall effects (Table 2). Ticks that were in the adult stage of development at the time of treatment showed the lowest levels of control of any stage, regardless of concentration. Analysis showed the ticks obtained from untreated cattle had significantly higher mean values for the number of ticks per animal ($F = 15.92$; df = 2,9; $P < 0.05$), IF value ($F = 118.32$; df = 2,9; $P < 0.05$), and egg mass weight of females ($F = 24.22$; df 2,79; $P < 0.05$) than either of the Ravap treated groups, which were not different ($P > 0.05$) from each other. As in the overall analysis, there was no difference ($F = 1.04$; df = 2,79; $P > 0.05$) in the engorgement weight of the females among the three groups. Similarly, there was no difference ($F = 2.78$; df = 1,6; $P > 0.05$) in the level of control achieved at either Ravap concentration against ticks that were treated whereas in the adult stage of development.

Ravap treatments applied against ticks that were in the nymphal stage of development at the time of treatment showed a somewhat greater degree of variability in

Table 2. Mean ± SD values of various biological parameters used to assess the efficacy of an organophosphorus (OP) mixture acaricide (Ravap) against OP-resistant *B. microplus* in different stages of development at the time spray treatment was applied.

Parasitic stage at treatment	Treatment conc. (%AI)	No. of ticks per animal	Weight of female (mg)	Egg mass weight (mg)	Index of fecundity (IF)	Control of IF (%)
Adult	Untreated	1045 ± 160 a	347 ± 32 a	171 ± 39 a	176.3 ± 31.1 a	———
	0.15	596 ± 97 b	339 ± 60 a	95 ± 49 b	35.6 ± 5.2 b	79.8 ± 3.0 a
	0.30	561 ± 141 b	358 ± 48 a	107 ± 42 b	41.9 ± 5.3 b	76.2 ± 3.1 a
Nymph	Untreated	955 ± 208 a	321 ± 53 a	146 ± 34 a	142.0 ± 31.0 a	———
	0.15	271 ± 106 b	322 ± 36 a	142 ± 21 a	24.8 ± 11.8 b	82.5 ± 7.9 a
	0.30	169 ± 34 b	315 ± 52 a	125 ± 34 b	9.8 ± 4.8 c	93.1 ± 3.4 b
Larva	Untreated	833 ± 196 a	281 ± 69 a	123 ± 55 a	121.5 ± 24.7 a	———
	0.15	68 ± 19 b	225 ± 84 b	76 ± 55 b	4.3 ± 3.4 b	96.5 ± 2.8 a
	0.30	71 ± 18 b	213 ± 75 b	67 ± 47 b	2.9 ± 2.3 b	97.7 ± 1.9 a

Means within the same column and within the same parasitic stage followed by the same letter are not significantly different ($P = 0.05$), tested by Ryan-Einot-Gabriel-Welsch Multiple Range Test.

some of the measured parameters than for other parasitic stages (Table 2). Treatment during the nymphal stage showed the number of ticks per calf obtained from untreated animals was significantly higher ($F = 39.56$; df = 2,9; $P < 0.05$) than either of the treated groups, as was the case with ticks treated in the adult stage. Likewise, the engorgement weight of the females showed the same trend as when ticks were treated in the adult stage, producing no differences (F = 0.17; df = 2,81; $P > 0.05$) among the three groups. However, the egg mass weights of the surviving females treated whereas in the nymphal stage produced slightly different results than those of ticks treated as adults or larvae. Whereas there was no difference between the egg mass weight of untreated ticks and that of females treated at the 0.15% AI Ravap dosage ($F = 3.83$; df = 2,81; $P < 0.0258$), the weight of eggs produced by females treated at 0.3% AI was significantly less ($P < 0.05$) than either of the other two groups. The IF of ticks treated as nymphs showed a significant decrease ($F = 61.34$; df = 2,9; $P < 0.05$) in the reproductive capacity of the surviving females with each increase in concentration, such that the IF of untreated ticks was significantly greater ($P < 0.05$) than ticks treated at 0.15% AI, which was, in turn, significantly greater ($P < 0.05$) than ticks treated at 0.3% AI. Consequently, the level of control achieved against ticks treated as nymphs was the only stage of parasitic development during which there was a significant difference ($F = 7.86$; df = 1,6; $P < 0.05$) between the 2 concentrations of Ravap, with the 0.3% AI concentration reducing the survival rate by > 93%, whereas the 0.15% AI rate only reduced the survival by 82.5%.

The results obtained against ticks that were in the larval stage of development at the time the treatment was applied showed that ticks recovered from cattle treated at both concentrations were significantly adversely impacted in every measured parameter as compared with ticks recovered from untreated cattle (Table 2). The number of ticks recovered from treated cattle at both concentrations was significantly lower ($F = 59.9$; df = 2,9; $P < 0.05$) than that of ticks recovered from untreated cattle. Likewise, the IF value, female engorgement weight, and egg mass weight of females obtained from treated cattle, regardless of concentration, was significantly lower than that of females obtained from untreated cattle (IF: $F = 53.19$; df = 2,9; $P < 0.05$; engorgement weight: $F = 6.32$; df = 2,78; $P < 0.05$; egg mass weight: $F = 3.83$; df = 2,78; $P < 0.05$). Whereas there was no difference ($F = 0.59$; df = 1,6; $P > 0.05$) in the level of control obtained against ticks that were larvae when they were treated at either dosage rate, nevertheless, both treatment concentrations provided a level of control that was > 96% and higher than that of the other two parasitic development stages.

Discussion

The LC_{50} value determined for the tick strain used in this study against OP acaricides (0.707% AI) compares favorably to the LC_{50} values obtained against this same strain in 2 previous studies (0.656 and 0.688% AI), indicating the strain had a longstanding and relatively stable resistance profile to OPs (Davey et al. 2003, 2004). Based on the resistance scale used by other investigators, stating that a RR of ≥ 5 is the minimum value necessary to indicate true OP resistance (Shaw et al. 1968, Beugnet and Chardonnet 1995), the laboratory bioassay results obtained against the tick strain used in this study, showing a RR of 18.7, indicated that at the time of the study the strain was highly resistant to OP acaricides.

Whereas overall results demonstrated that neither concentration of the mixture OP acaricide, Ravap evaluated in the study would provide the necessary level of control

(≥ 99%) required for use in the U.S. CFTEP, nevertheless, the outcome suggested promise for use of the product in emergency tick outbreak situations involving OP-resistant tick populations. Using an OP-resistant strain of ticks that had a RR that was only half as high (RR = 9.5) as the ticks used in this study, Davey and George (1999) reported that the level of control using coumaphos compared favorably when evaluated at essentially the same concentrations of Ravap tested in this study. This indicated that the Ravap mixture acaricide had a considerably higher activity level against OP-resistant ticks than a single nonmixture OP acaricide, such as coumaphos. In addition, Davey et al. (2003), using the same tick strain used in this study, reported only 52.9% control using the single nonmixture acaricide, coumaphos at a concentration of 0.165% AI, and only 75.8% control at concentration of 0.299%, both of which were considerably lower than observed in the present study using the mixture acaricide, Ravap at comparable concentrations. Again, this strongly implied that a mixture of two OP acaricides could provide a potentiation effect that would not occur when a nonmixture acaricide was used alone. In yet another study using the same strain of ticks as was used in this study it was found that following a single dip treatment with coumaphos at a concentration of 0.3% AI, the level of control ranged between 46.8 and 65.6% (Davey et al. 2004), even though the RR of the ticks at the time of that study was approx. 30% lower (RR = 13) than the ticks used in the present study. Another positive factor associated with the use of the Ravap mixture in this study is that it was applied as a spray formulation, rather than a dip formulation. This is noteworthy because generally dip treatments are known to be significantly more effective against ticks infested on cattle than are spray formulations (Davey et al. 1997). Thus, the fact that spray treatments with Ravap provided considerably higher levels of control than were achieved using a significantly more effective treatment method, such as dipping, indicated that this mixture OP acaricide had substantially greater activity than a single nonmixture acaricide, such as coumaphos used alone.

Results obtained against each of the parasitic development stages of the tick indicated that the level of control was related to the stage of development at the time of treatment, regardless of concentration. At both concentrations, the Ravap treatment was most effective against ticks in the larval stage of development at the time of treatment (95.6 - 97.7% control), whereas control was somewhat lower against ticks in the nymphal stage at treatment (82.5 - 93.1%). Thus, reasonably good control was achieved against immature stages using the mixture acaricide, even though they were highly resistant to OP acaricides, which compares favorably with results of other studies using the nonmixture OP acaricide, coumaphos (Davey et al. 2003, 2004). In this study, both Ravap treatment concentrations were least effective against ticks in the adult stage of development at the time of treatment. The trend of being least effective against adult ticks was consistent with findings in another study using the nonmixture acaricide, coumaphos, at comparable concentrations against ticks with a 30% lower RR than ticks in this study (Davey et al. 2003). However, the level of control in that study was much lower (range: 4.3 - 42.1%) than was observed in the present study (range: 76.2 - 79.8%). Thus, the findings obtained in this study appeared to indicate that potentiation was occurring among the two OP acaricides present in the mixture acaricide, Ravap that increased the level of control against adult ticks, and to a lesser extent against nymphal ticks.

The continued evolution and magnification of acaricide resistance in cattle fever tick populations in Mexico only serves to intensify the challenge faced by the U.S. CFTEP in preventing the reestablishment of these ticks back into the country. Thus, the evaluation of mitigation strategies, such as the use of mixture acaricides like Ravap, which may enhance the possibility of preventing or at least curtailing the establishment of acaricide-resistant ticks in the U.S., is critical to the continued success of the program. Results of this study clearly revealed that the level of control that could be expected following the single treatment of a highly OP-resistant cattle fever tick strain would certainly place an eradication program at risk for allowing dispersion of viable ticks into uninfested areas within and outside of the permanent quarantine zone. However, the results of this study showing that Ravap had the ability to control 76 - 79% of the adult ticks, whereas reducing the larval ticks by > 96% against a highly OP-resistant tick strain was encouraging. Considering that multiple treatments of the OP acaricide, coumaphos at 7 - 10 intervals against an OP-resistant tick strain resulted in >99% control following 3 consecutive treatments (Davey et al. 2004), it is highly probable that the use of multiple treatments of the Ravap would provide the same or better results. Therefore, in an emergency situation if highly OP-resistant ticks were detected on cattle at a port-of-entry, the systematic, repeated use of this mixture acaricide would likely provide as good or better control than a single nonmixture acaricide.

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