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Transaction Detail
Development of Novel UF Patent
For Month Ending: March 31, 2016

Run Date: 04/07/2016

Acctg Date	Flex Code	UFID	CRIS	Transaction ID	Encumbrance ID	ID	Description Detail	Amount
Cost Center: 00125193 - Development of Novel UF Patent~209~60140000 (Project~Fund~Department)								
Subtotal Other Operating Expenses								($0.32)
00125193 - Development of Novel UF Patent~209~60140000 (Project~Fund~Department)								$6,823.14

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FINAL REPORT

Project Title: Development of novel UF patented semiochemical-based insecticides for biting flies of cattle

Principal Investigator: Phillip E. Kaufman

Project Location: Entomology and Nematology Department, University of Florida, Gainesville, FL 32611-0620

Project Type: Research

Project Summary.

Our project sought to further develop three University of Florida patented novel insecticides to better protect Florida's cattle from the horn fly and stable fly, two of the most economically damaging cattle pests. Current estimates put the cost of the horn fly at $1.5 billion and stable fly at $2.2 billion to the U.S. cattle industry. Development of new insecticides is a cumbersome and expensive process that requires years of research and high development costs. Due to overuse, misuse and lack of choices within which to rotate insecticides, horn flies and in a few cases, stable flies have become resistant to many currently available treatments. Implementation of federal re-registration of older insecticides resulted in the loss of many of these insecticides. Replacements are badly needed. In this study, we have tested three University of Florida patented insecticides individually and in combination on adult and immature flies. The knowledge gained from this research will allow us to better partner with a private company to fully develop these into marketable materials. With continued study and using results obtained from this project we hope to continue working toward development of an economically feasible and environmentally friendly technology to lower the impact of flies on beef cattle production.

Project Background.

As many beef producers know, pasture flies are exceedingly difficult to control. In Florida, the horn fly and the stable fly are the most damaging and economically-important pests of pastured cattle, with each occurring at different times of the year. There is a considerable lack of effective and safe chemicals for use against these flies, and most chemicals fall into just a few types, that kill the flies in much the same way. Development of new insecticides is a cumbersome and expensive process that requires years of research and high development costs. Due to overuse, misuse and lack of choices within which to rotate, horn flies and in a few cases, stable flies have become resistant to many currently available insecticides. Implementation of federal re-registration of older insecticides further reduced the numbers of available materials. Pending re-registration of the pyrethroid-based insecticides will further reduce the last major class of insecticides and this includes many commonly used cattle insecticides, such as permethrin and cyfluthrin. The end result is an even greater selection pressure and more rapid resistance development to the few remaining materials. Producers often notice resistance through ineffective applications, such as ear tags that only "work" for a month, when a few years earlier they worked for four or even six months. Our project sought to evaluate combinations of novel existing chemistries that our laboratory has developed against the horn fly and stable fly. These chemicals were drawn from a list of patented insecticides that provided very good pest

management activity (Mann et al. 2010; Kaufman et al. 2011). Additionally, these materials are regularly used in the food and fragrance industry and appear on the U.S. EPA Generally Regarded As Safe list, making registration much more simple.

Objectives.

1. Screen adult horn fly and stable fly for survival against the combinations of the three patented compounds (beta-damascone, cyclemone A and melafleur) that have been identified as insecticides and develop LD_{50} and LD_{90} values for each.
    - 100% completed
2. Screen larval stage horn flies and stable flies against combinations of the previously introduced patented compounds.
    - 100% completed. Note - tested individual compounds due to results from Objective 1.
3. Conduct longer-term efficacy studies on most promising combinations on realistic substrates, such as cattle fur.
    - 0% completed. Note – due to time committed to properly completing Objective 1 endpoints and the subsequent results from Objective 1 it was not possible to complete this objective.

Experimental Methods.

Insects Evaluated.
Horn flies were obtained as pupae from New Mexico State University (NMSU), and maintained at the UF Veterinary Entomology Laboratory of Dr. Phillip Kaufman. The pupae were placed in screen cages and the eclosing adults were provided bovine blood twice per day as a food source. Adult flies used in assays were 2-5 day old females. To obtain eggs for immature assays the cage was placed on top of damp paper towels. Eggs fell through the screen on the bottom of the cage and were collected on the damp paper towels and were harvested the following day. For each assay, eggs were from collected over the previous 12-18 hours.

Stable flies were reared at the UF Veterinary Entomology Laboratory of Dr. Phillip Kaufman following the standard laboratory protocols (Pitzer et al. 2010). Pupae were placed in screen cages and once adults began to eclose the flies received blood every day. To obtain eggs for immature assays an oviposition cup was placed in the cage consisting of a 120 mL plastic cup containing a piece of black cotton fabric wrapped around cotton ball. The fabric and cotton balls were soaked in water that had been previously exposed to eggs to encourage egg laying. For each assay, eggs were from collected over the previous 12-18 hours.

Test Chemicals
Permethrin (98.8%; 40.1% cis and 58.7% trans) was obtained from Chem Service (West Chester, PA) and used as a positive control for the adult fly assays conducted in Objective 1. Methoprene was obtained from Sigma Aldrich (St Louis, MO) and used as a positive control for the immature fly assays conducted in Objective 2. The semiochemicals, i.e. melafleur (93.6%), cyclemone A (89.0%) and beta-damascone (97.0%) were purchased from The John D. Walsh Company Inc. (Ringwood, NJ). All technical-grade insecticides were diluted in analytical grade acetone and acetone was used as a negative control.

Objective 1. Screen adult horn fly and stable fly for survival against the combinations of the three patented compounds (beta-damascone, cyclemone A and melafleur).

This objective was split into two steps, Objective 1a and 1b below. Both objectives tested the effect of the insecticides on the flies using the glass jar exposure method (Kaufman et al. 2011).

Glass Jar Exposure: An aliquot (0.84 mL) of each solution to be tested was placed in a 30 mL vial and swirled to coat the bottom of the vial. To ensure an even insecticide residue, glass vials were rotated on a hotdog roller (Model HDR-535-1805; Nostalgia Products Group LLC, Green Bay, WI) with the heat unit disconnected in a fume hood until the solvent evaporated. Vials were removed from the rollers at this point but were not used in the assay until 60 minutes after the solutions were applied, allowing complete evaporation of the acetone solvent.

Objective 1a. Develop LD_{50} and LD_{90} values for each of the three patented compounds (beta-damascone, cyclemone A and melafleur) that have been identified as insecticides for adult horn fly and stable fly.

Concentrations: All test insects were exposed to insecticides, i.e. permethrin, melafleur, cyclemone A and beta-damascone, applied in serial dilution (5 to 10 doses, plus acetone-treated control) in vials. Concentrations of permethrin to be tested were based on previous literature for each species (Li et al., 2003; Pitzer et al., 2010). In all cases testing of each compound was performed to generate a range of insect mortality of between 5% and 100%. The natural products explored herein have been previously evaluated as individual compounds on stable flies, thus we used the data of Kaufman et al. (2011) to guide our concentrations. For each concentration 3-5 replicates were completed. An acetone control (3-5 replicates) was completed along with every assay.

Assay: Twenty adult female flies (2-5 days old) were lightly anaesthetized and added to each vial. The vials were capped and held horizontally until assessment. The center portion of the cap was replaced with fine metal mesh to provide air circulation. Knockdown was assessed at 2 hours, at which point flies were counted as knocked-down if they were ataxic and could not right themselves. The insects were then lightly anaesthetized and transferred to a holding container, which comprised of a 120 mL plastic cup lined with a small filter paper circle (4.25 cm; P5; Fisherbrand™, Fisher Scientific, Pittsburgh, PA) and fitted with a lid, the center portion of the lid was removed and replaced with a fine mesh fabric to allow for air circulation and feeding. Flies were fed immediately after the assay. At this time both horn flies and stable flies received bovine blood soaked cotton balls and stable flies also were provided with a cotton ball soaked in a 10% sucrose solution. All experiments were conducted and flies were held at 22 ± 2°C. After 24 hours flies were counted as dead if they were ataxic and could not right themselves. The assays were repeated 3-6 times.

Data Analysis: Data from Objective 1a were analyzed using standard probit analysis (Finney 1971). Dose-mortality curves were generated and LD_{50} and LD_{90} values (amount of chemical required to kill 50 and 90% of the insects) were determined.

Objective 1b. Screen adult horn fly and stable fly for survival against the combinations of the three patented compounds (beta-damascone, cyclemone A and melafleur).

Concentrations: Following the completion of Objective 1a the LC_{50} values obtained were used to inform the concentrations to be tested as mixtures. Two- and three-way mixtures of melafleur, cyclemone A and beta-damascone were tested. The 2-way mixtures were tested in a 50:50 ratio at half of the LC_{50} for each chemical. The 3-way mixture was tested in a 33:33:33 ratio at one third of the LC_{50} for each chemical. In addition to the mixtures, permethrin at its respective LC_{50} and the individual chemicals at their LC_{50} were tested in the assay. An acetone control was completed along with every assay. Within each assay, for each treatment, 3-5 replicates were completed.

Assay: The assay followed the description listed above for Objective 1a.

Data Analysis: Proportion knocked-down and proportion mortality were analyzed for each species individually by fitting a generalized linear model for a binomial distribution with a logit link. The model consisted of the fixed effects of trial (for blocking) and treatment (acetone, permethrin, melafleur, cyclemone A, the three two-way mixtures (i.e. beta-damascone, melafleur

and cyclemone A, melafleur and beta-damascone, cyclemone A and beta-damascone), and the three-way mixture (i.e. melafleur, cyclemone A, and beta-damascone). Least significant difference (LSD) tests with the Bonferroni correction were used to compare knockdown and mortality between the treatments ($\alpha = 0.05$). All analyses were conducted in SAS Version 9.3 (SAS 2008).

Objective 2. Screen larval stage horn flies and stable flies against combinations of the previously introduced patented compounds.

Concentrations: Following the completion of Objective 1a the LC_{90} values obtained were used to inform the concentrations of melafleur, cyclemone A and beta-damascone to be tested on immature insects. For each chemical the LC_{90} value and 10 times the LC_{90} value was tested. Additionally, two doses of methoprene, an insecticide that targets immature flies (larvae), were tested 0.1 and 0.01 mg/ml based on previous literature (Schmidt and Kunz, 1980). The negative controls consisted of an acetone application and one container that was not treated with any solution.

Assay: Eggs were collected as described above for use in the assays. Once removed from their respective collection devices the eggs were kept on moistened germination paper in a glass Petri dish in an incubator until they were used in the assay. The incubator was maintained at 26.7 ±0.3°C with a 12:12 (L:D) light cycle and an elevated humidity of 99.7% was provided by the presence of open water-filled pans in the base of the incubator. Immediately prior to use in the assay fly eggs were counted into groups of 50 under a dissecting microscope and placed onto a small strip of germination paper. These strips were stored until application to the manure/media in a glass Petri-dish with a lid to reduce desiccation. Five additional strips were placed individually into glass vials and held for 48 hours in the incubator to determine egg hatch rate.

For the stable fly assays, a batch of stable fly media was prepared and 50g of media was placed in a 120 mL plastic cup. Stable fly media was made by mixing 400 mL calf manna, 1,400 mL coarse vermiculite, 3,600 mL wheat bran and 3,500 water. For the horn fly assays, very fresh cow manure (recently deposited) was collected from the University of Florida Beef Teaching Unit and frozen upon return to the laboratory to kill any developing insects that may have been in the manure. The beef cows that produced the manure had no exposure to antihelmintics (wormers) for at least 6 months. Batches of manure were removed from the freezer to defrost the day prior to setting up the assay. On the day of the assay 70g of cow manure was placed in to a 120 mL plastic cup.

Insecticide-containing solutions were prepared the day of assay set up. Immediately prior to application, the solutions were vortexed and pipetted into 8 mL perfumery spray bottles (QUOSINA, Edgewood, NY). The spray bottles were used to apply 1 mL of each solution to the surface of the media or manure. Following a 15 minute intraval to allow the acetone to evaporate, fly eggs were placed onto the media/manure. There were 10 insecticide and control treatments as described above with 4 replicates completed of each treatment. The germination strips, each with 50 eggs, were flipped over so that the eggs were in direct contact with the manure/media. The center portion of the lid was replaced with fine mesh to provide air circulation. The cups were then placed in an incubator, conditions as described earlier. Horn fly larvae were incubated for 7

days and stable fly larvae were incubated for 14 days in accordance with their typical development times (Moon 2002). After 7 or 14 days the manure/media from each cup was placed in water and the pupae were floated and removed by hand for enumeration. Pupae from each experimental unit were placed in a 30 mL plastic cup with a cardboard lid and left for 7 days for adult eclosion from the puparia. The experiment was repeated four times for each fly species, but only three replicates were statistically analyzed for each.

Data Analysis: Replicate 3 of the horn fly assay was excluded as the hatch rate was well below average (<40%). Replicate 2 of the stable fly assay was excluded, as there was minimal survival of the larvae to pupation even in the insecticide-negative controls. If late instar larvae were present in any rearing cup, they were added to the total pupae for calculation of pupation as we concluded they would have pupated had they not been disturbed.

Three measures of survival were assessed. Proportion pupated, proportion eclosed, and proportion eclosed of those that pupated were analyzed for each species individually by fitting a generalized linear model for a binomial distribution with a logit link. The model consisted of the fixed effects of trial (for blocking) and treatment (blank control, acetone, two doses of methoprene (i.e. 0.1 and 0.01 mg/ml), two doses of melafleur (i.e. LC_{90} and $10xLC_{90}$), two doses of cyclemone A (i.e. LC_{90} and $10xLC_{90}$), and two doses of beta-damascone (i.e. LC_{90} and $10xLC_{90}$). Least significant difference (LSD) tests with the Bonferroni correction were used to compare pupation and eclosion between the treatments and the controls ($\alpha = 0.05$). All analyses were conducted in SAS Version 9.3 (SAS 2008).

Results

Objective 1. Screen adult horn fly and stable fly for survival against the combinations of the three patented compounds (beta-damascone, cyclemone A and melafleur).

Objective 1a. Develop LD_{50} and LD_{90} values for each of the three patented compounds (beta-damascone, cyclemone A and melafleur) that have been identified as insecticides for adult horn fly and stable fly.

Stable flies and horn flies were less susceptible to the semiochemicals compared to permethrin, as expected (Tables 1 and 2). The concentration to reach 90% mortality (LC_{90}) did not differ between stable flies and horn flies for beta-damascone. However, there was a significant difference in the LC_{50} between the two species (Fig. 1A). Stable flies were less sensitive to the chemical with an increased concentration required for 50% mortality. Stable flies and horn flies were similarly sensitive to cyclemone A (Fig. 2B). Stable flies were less sensitive to melafleur with an increased concentration required for 90% mortality (LC_{90}; Fig. 1C). However, there was no significant difference between the species at the LC_{50}. In contrast to the semiochemicals, horn flies were less sensitive to permethrin than stable flies at both 50% and 90% mortality (Fig. 2D). It took twice as much permethrin to kill 50% and four times as much permethrin to kill 90% of horn flies compared to stable flies (Tables 1 and 2).

Table 1. Lethal concentrations (ug/cm^2) for the three semiochemicals compared to permethrin for the stable fly, *Stomoxys calcitrans* L. Upper (UCL) and lower (LCL) 95% confidence limits are included for each LC value along with the slope and Chi-squared test (x^2) result for the regression.

Chemical	LC_{50}	LCL	UCL	LC_{90}	LCL	UCL	Slope	x2
Beta-damascone	24.245	22.885	25.521	35.751	33.146	2.660	7.5	1395.5
Cyclemone A	9.600	9.277	9.907	13.026	12.482	13.737	9.7	260.3
Melafleur	10.956	10.403	11.481	15.839	14.894	17.159	8.0	139.7
Permethrin*	0.011	0.011	0.012	0.016	0.015	0.018	8.1	395.5

*40.1% cis and 58.7% trans

Table 2. Lethal concentrations (ug/cm^2) for the three semiochemicals compared to permethrin for the horn fly *Haematobia irritans irritans* (Linnaeus). Upper (UCL) and lower (LCL) 95% confidence limits are included for each LC value along with the slope and Chi-squared test (x^2) result for the regression.

Chemical	LC_{50}	LCL	UCL	LC_{90}	LCL	UCL	Slope	x2
Beta-damascone	21.164	20.019	22.184	33.266	31.529	35.545	6.525	952.68
Cyclemone A	9.19	8.819	9.535	13.036	12.409	13.877	8.441	432.75
Melafleur	10.224	9.801	10.602	13.909	13.281	14.773	9.588	557.92
Permethrin*	0.031	0.027	0.034	0.067	0.056	0.086	3.774	410.29

*40.1% cis and 58.7% trans

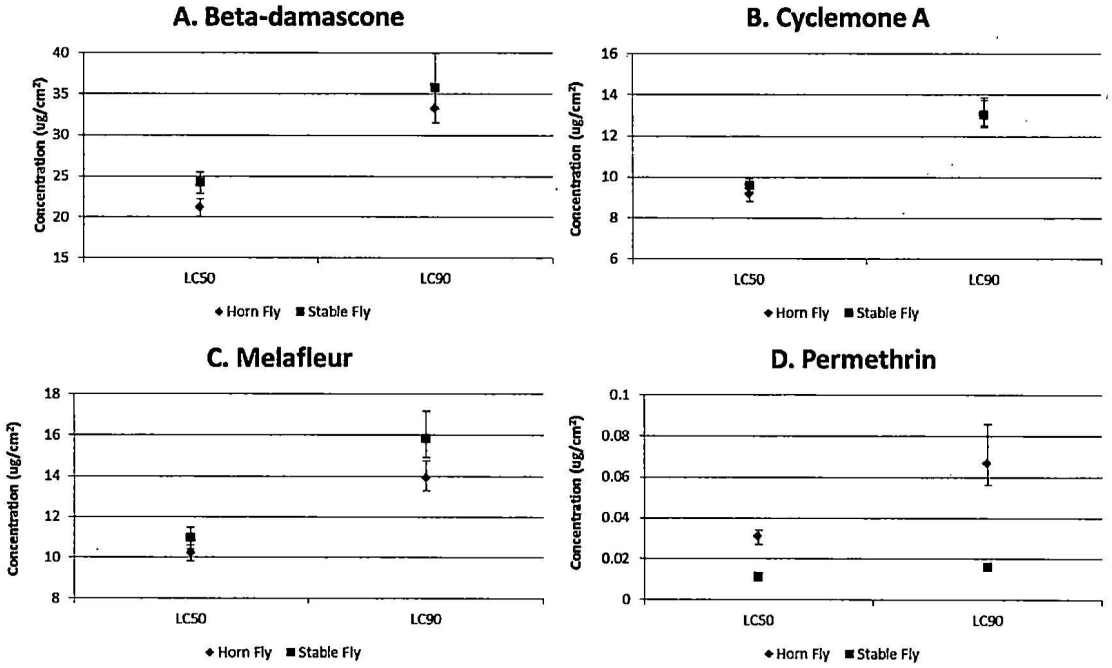


Figure 1. Comparison of adult stable fly, *Stomoxys calcitrans* L. and horn fly, *Haematobia irritans irritans* (Linnaeus), lethal concentration (LC) values (ug/cm^2) for all four insecticides, A – Beta-damascone, B – Cyclemone A, C – Melafleur and D – Permethrin. Error bars represent 95% confidence intervals and when bars do not overlap within a LC level the LC values can be considered to be significantly different.

Objective 1b. Screen adult horn fly and stable fly for survival against the combinations of the three patented compounds (beta-damascone, cyclemone A and melafleur).

The proportion of flies that were knocked down after the two hour exposure was significantly affected by the treatment applied to the glass vial for both stable flies ($F_{8,211} = 9.01$, $P < 0.0001$) and horn flies ($F_{8,211} = 23.33$, $P < 0.0001$). All treatments knocked down significantly more flies than the negative control (acetone). In general beta-damascone was less effective as an individual compound and as a mixture, particularly with horn flies. All treatments resulted in greater than 60% knockdown with stable flies (Fig. 2). Horn flies treated with the two-way mixtures containing beta-damascone and the three-way mixture were significantly less knocked down following two hours of exposure than those treated with the individual chemicals (except beta-damascone) and permethrin (Fig. 3).

The proportion of flies that were dead 24 h after a two hour exposure was significantly affected by the treatment applied to the glass vial for both stable flies ($F_{8,211} = 14.24$, $P < 0.0001$) and horn flies ($F_{8,211} = 11.84$, $P < 0.0001$). All treatments killed significantly more flies than the negative control (acetone), except for the mixture of beta-damascone and cyclemone A on horn flies (Fig. 5). In general, mixtures resulted in the same or significantly less mortality when compared to the individual chemicals (Fig. 4 and 5).

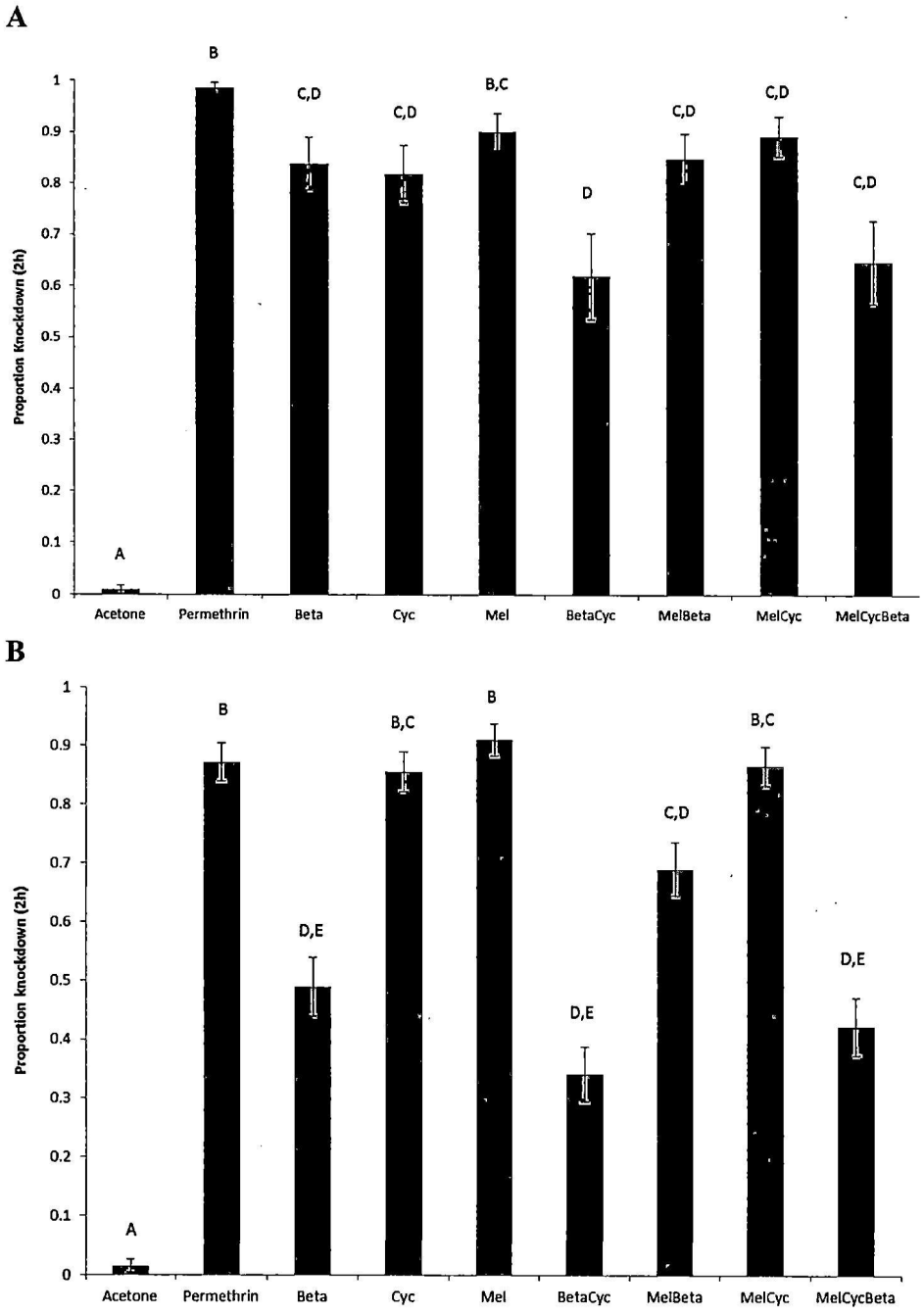


Figure 2. Knockdown of stable flies, *Stomoxys calcitrans* L. (A) and horn flies, *Haematobia irritans irritans* (Linnaeus) (B) exposed to individual and mixtures of insecticides for two hours. Controls also were completed for comparison consisting of permethrin (positive control; LC_{50} from Objective 1a) and acetone (negative control). Columns are predicted means with ± SE; columns capped with different letters were significantly different by means comparison using a LSD test with a Bonferroni correction.

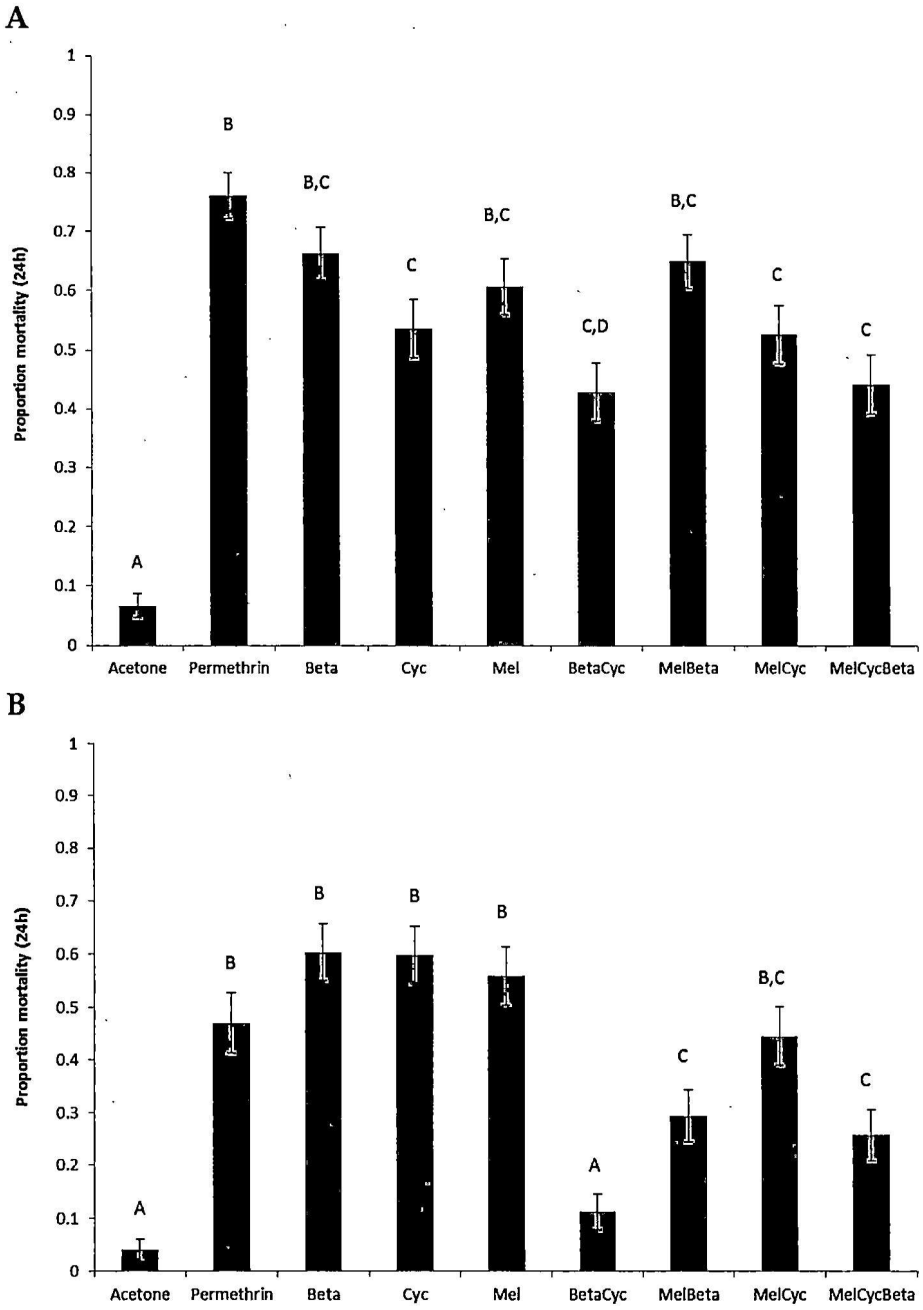


Figure 3. Mortality of stable flies, *Stomoxys calcitrans* L. (A) and horn flies, *Haematobia irritans irritans* (Linnaeus), (B) exposed to individual and mixtures of insecticides for 2 h and held for 24 h. Controls also were completed for comparison consisting of permethrin (positive control; LC_{50} from Objective 1a) and acetone (negative control). Columns are predicted means with ± SE; columns capped with different letters were significantly different by means comparison using a LSD test with a Bonferroni correction.

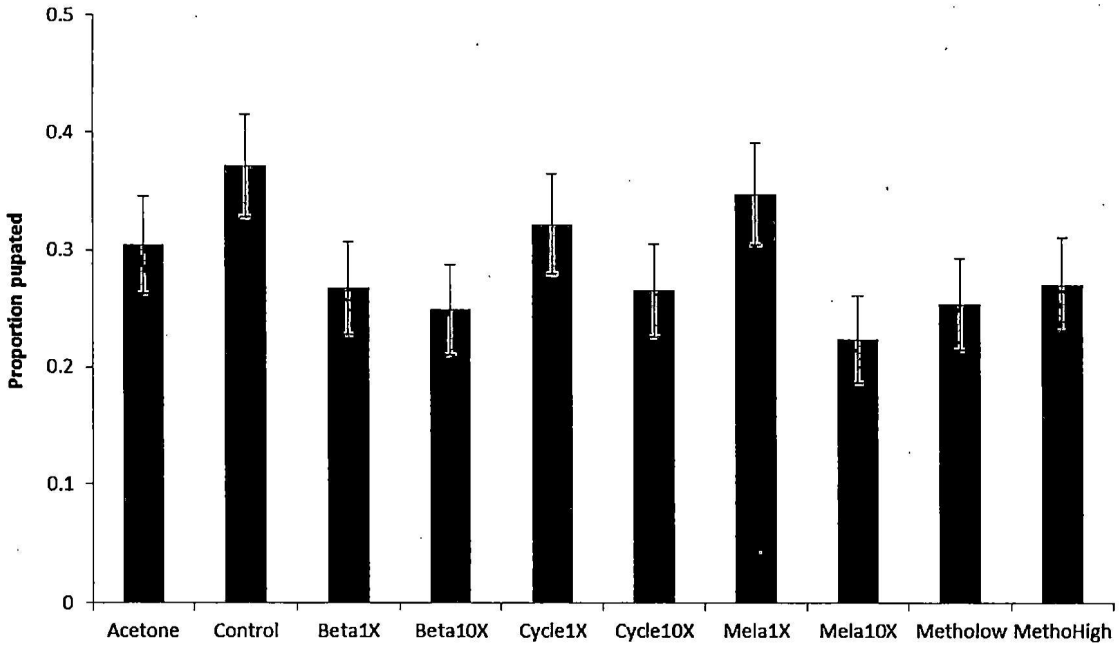
Objective 2. Screen larval stage horn flies and stable flies against combinations of the previously introduced patented compounds.

The average percentage of fly larvae that hatched from eggs was 67.1 ±3.2%; for horn flies the average was 62.1 ±4.8% and for stable flies 73.9 ±3.5%.

There was no significant effect of treatment on the proportion of stable fly larvae that pupated ($F_{9,108}$ =1.33, P = 0.2324; Figure 4A). Eclosion of stable flies exposed to insecticides as larvae was significantly reduced ($F_{9,108}$ = 2.07, P = 0.0382) at the higher dose of methoprene only (Figure 5A). None of the semiochemicals affected the proportion of adults that eclosed. There was a significant effect of treatment on the proportion of adults that emerged from pupae ($F_{9,108}$ = 3.08, P = 0.0469). None of the semiochemicals affected the proportion of adults that emerged from pupae. However, there was a significant reduction in eclosion from pupae after the larvae were exposed to the high dose of methoprene (Figure 6A). Although the percentage hatch of stable flies was >70%, it appears as though superfluous mortality has impacted the results, which is evident by the low pupation and eclosion in the controls.

There was a significant effect of treatment on the proportion of horn fly larvae that pupated ($F_{9,108}$ = 9.19, P <0.0001) and eclosed ($F_{9,108}$ = 7.49, P <0.0001). Pupation and eclosion of horn flies exposed to insecticides as larvae was significantly reduced at the higher dose of 10X LC90 as defined in Table 1. All three semiochemicals resulted in a significant reduction in successful pupation and eclosion at this dose (Figure 4B and 5B). Interestingly despite efforts to use appropriate doses of methoprene there was no significant effect on pupation or eclosion when using either a high or low dose. There was a significant effect of treatment on the proportion of adults that emerged from pupae ($F_{9,108}$ = 3.08, P = 0.0025). None of the semiochemicals affected the proportion of adults that emerged from pupae. However, there was a significant reduction in eclosion from pupae after the larvae were exposed to the high dose of methoprene (Figure 6B).

A.



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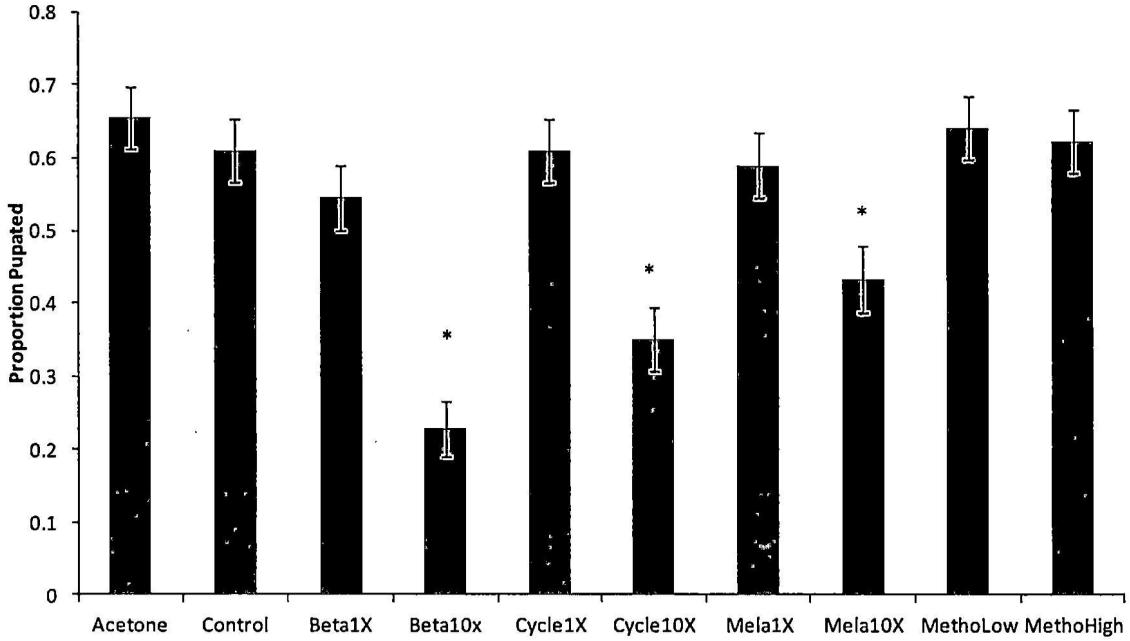


Figure 4. Pupation of larval stable flies, *Stomoxys calcitrans* L. (A) and horn flies, *Haematobia irritans irritans* (Linnaeus), (B) exposed to individual insecticides applied to media or manure, respectively. Controls also were completed for comparisons consisting of methoprene (positive control) and, acetone and control (negative controls; control had no treatment applied to the surface). Columns are predicted means with ± SE; bars and columns with an asterix were significantly different by means comparison using a LSD test with a Bonferroni correction to the negative controls.

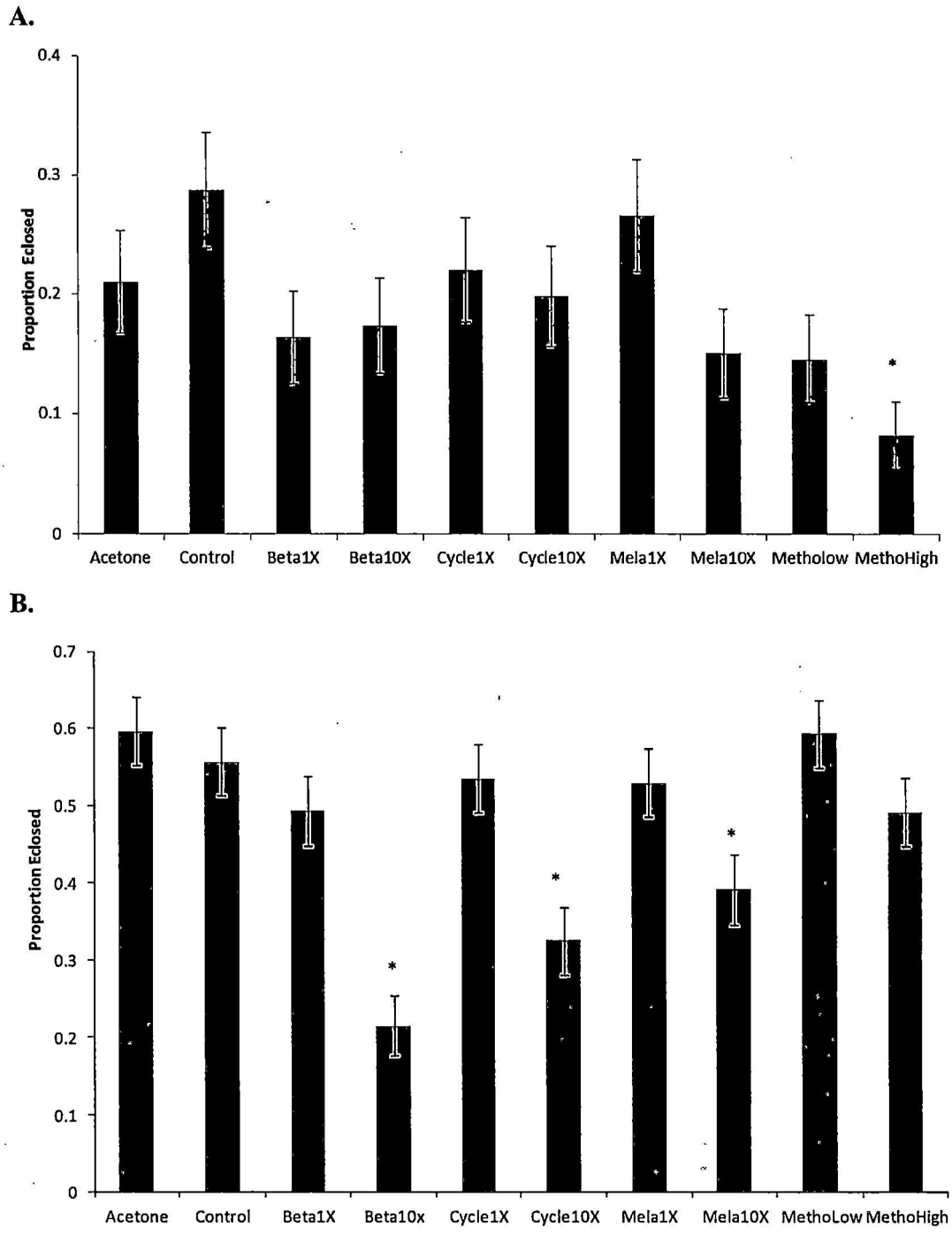
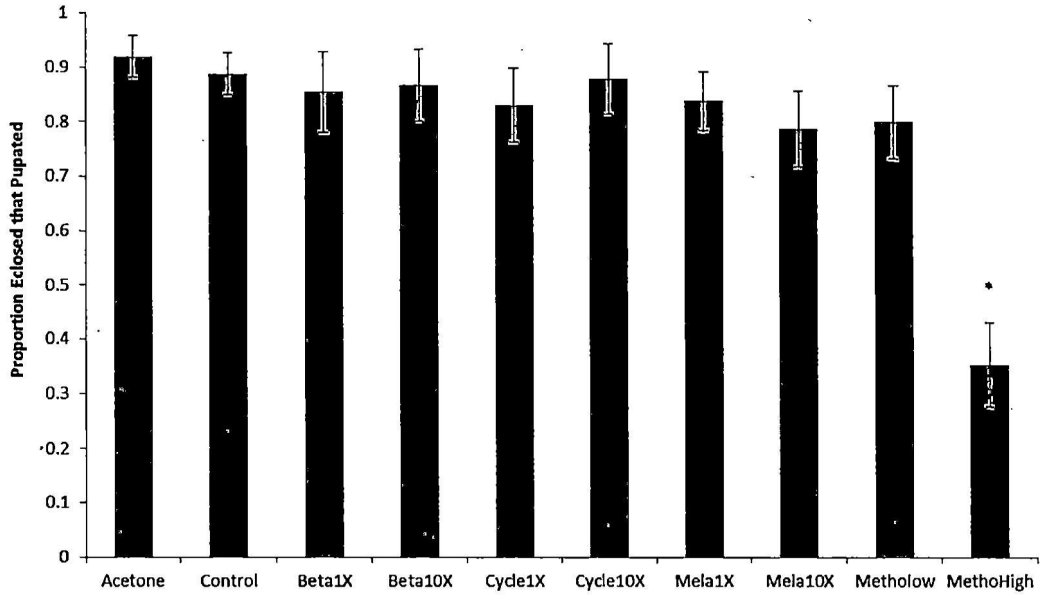


Figure 5. Eclosion of adult stable flies, *Stomoxys calcitrans* L. (A) and horn flies, *Haematobia irritans irritans* (Linnaeus), (B) exposed as larvae to individual insecticides applied to media or manure, respectively. Controls also were completed for comparison consisting of methoprene (positive control) and, acetone and control (negative controls; control had no treatment applied to the surface). Columns are predicted means with ± SE; bars and columns with an asterix were significantly different by means comparison using a LSD test with a Bonferroni correction to the negative controls.

A.



B.

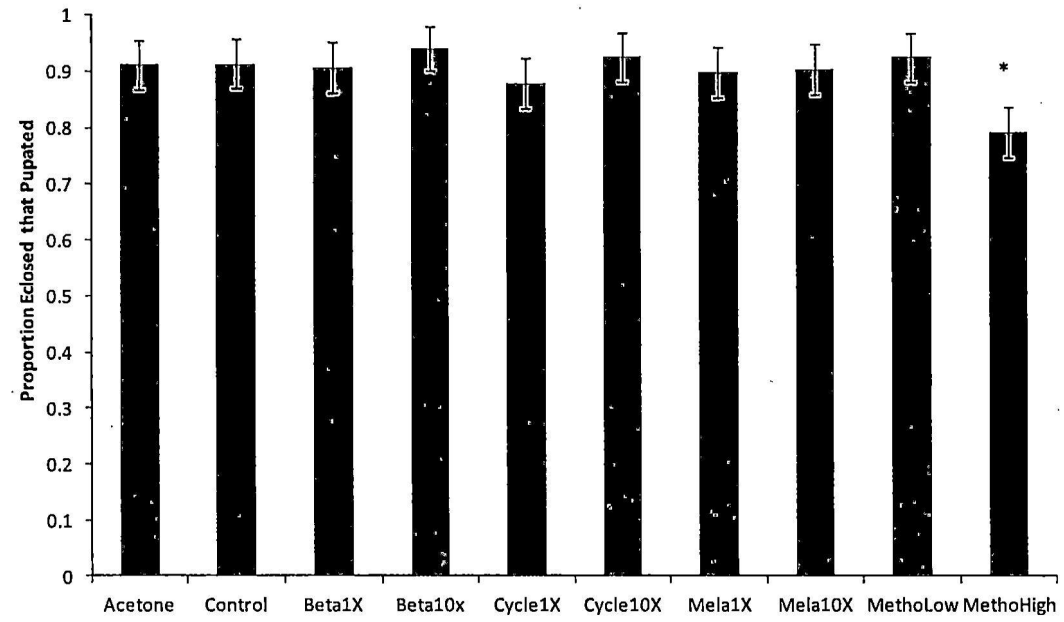


Figure 6. Eclosion of adult stable flies, *Stomoxys calcitrans* L. (A) and horn flies, *Haematobia irritans irritans* (Linnaeus), (B) that successfully pupated after being exposed as larvae to individual insecticides applied to media or manure, respectively. Controls also were completed for comparison consisting of methoprene (positive control) and, acetone and control (negative controls; control had no treatment applied to the surface). Columns are predicted means with ± SE; bars and columns with an asterix were significantly different by means comparison using a LSD test with a Bonferroni correction to the negative controls.

Conclusions

Horn flies and stable flies are economically damaging flies of veterinary importance. Biting and nuisance flies adversely affect animal health and reduce farm profitability, with the horn fly estimated to cause $675 million, extended to be near $1.5 billion in today's dollars (Drummond 1988). As summarized in Taylor et al. (2012), stable fly infestation levels of individual herds, median annual per animal production losses were estimated to be 139 kg of milk for dairy cows, and 6, 26, and 9 kg body weight for pre-weanling calves, pastured stockers, and feeder cattle, respectively. In a study by Sheppard and Noblet (1997), dairy farmers reported using insecticides two to three times per month to manage flies on pastured cattle. Horn and stable fly management on cattle is largely accomplished using broad-spectrum insecticides applied as pour-ons, insecticide-impregnated ear tags, backrubbers and feed throughs, although satisfactory control is not often found.

Due to the heavy use of insecticides against the horn fly, high levels of insecticide resistance have emerged, most dramatically to the pyrethroid materials used in ear tags, such as permethrin (Li et al., 2003; Pitzer et al., 2010). Producers initially observe insecticide resistance in terms of shorter duration of efficacy; but later resistance is demonstrated as immediate product failures – wherein applications simply do not kill the flies. Resistance in stable flies has been examined only a few times, but recent studies on Florida horse farms suggest that it is present (Pitzer et al. 2010). Alternatives to conventional pesticides are needed and this study has provided information that could assist in the development of novel insecticides for adult and immature biting flies.

The semiochemicals that previously had been demonstrated to present insecticidal effects against several medical and veterinary important pests (Mann et al. 2010; Kaufman et al. 2011), were efficacious against adult stable flies and horn flies, greatly increasing mortality when compared with the control. The stable fly was less sensitive to the semiochemical-based insecticides than the horn fly, but the reverse was true for the conventional insecticide permethrin. The adult horn fly was 2-4 times more tolerant of permethrin than the adult stable fly in our assays. Unfortunately combining the semiochemical-based insecticides in two- or three-way mixtures did not prove to have a synergistic effect and the mixtures had an efficacy of equal to or significantly less than the individual chemicals. Therefore, further research should focus on the use of these chemicals individually, particularly in the presence of permethrin-resistant populations.

Our results have demonstrated for the first time that these chemicals can be effective against immature insects developing in breeding sites. There was a significant effect of a surface application with the chemicals on subsequent horn fly development to pupae and adult eclosion at the highest doses evaluated. Further research in this area is needed and should focus on targeting Lethal Concentration values, something beyond the scope of this project.

Our results should provide an economically feasible and environmentally friendly technology to lower the impact of flies on beef cattle production, thereby increasing profitability. Additionally, this project has helped to develop new insecticide-based control technologies for biting flies currently expressing insecticide resistance to these important cattle pests. Development of new pesticides is an extended process and this study provides needed baseline information for follow-up studies.

Selected References

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