Deltamethrin Resistance Tests: Alamogordo strain

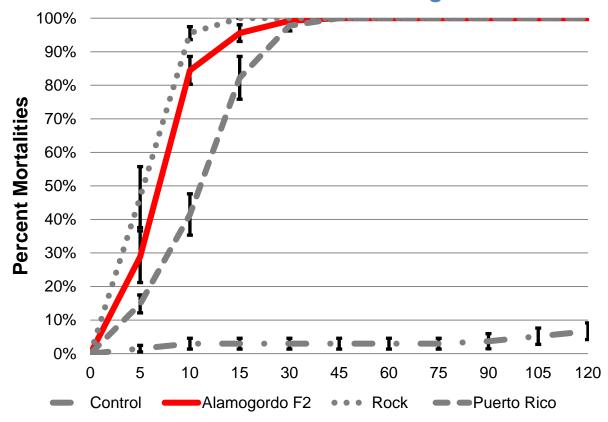


Figure A. Deltamethrin IR Bottle Test Results: 10.5 ug/bottle. Shown are means and standard error bars for different groups of mosquitoes. Rock is considered a pyrethroid-sensitive strain. Puerto Rico is considered a pyrethroid-resistant strain. The control was treated with acetone only.

Results- The control (Acetone) mortality curve was significantly different (P<0.0001) from the curves for all other treatments with little to no mortalities. Kaplan Meier statistical analysis confirmed that there was a significant difference between the the curves plotted for the deltamethrin-exposed Rock Strain and the Alamogordo Strain (P<.0001). The curve plotted for the Puerto Rico Strain (a known resistant strain) is significantly different than that plotted for the Alamogordo Strain (P<.0001). The curves plotted for the Puerto Rico Strain and the Rock strain were also significantly different from each other (P<.0001).

Conclusion- Although we found small but statistical significant differences in the survival curve of the ROCK control and the curve plotted for mosquitoes from Alamogordo, the comparison with the curve plotted for the resistant Puerto Rico strain suggests that the Alamogordo strain is sensitive to deltamethrin.

Deltamethrin Resistance Tests: Las Cruces strain

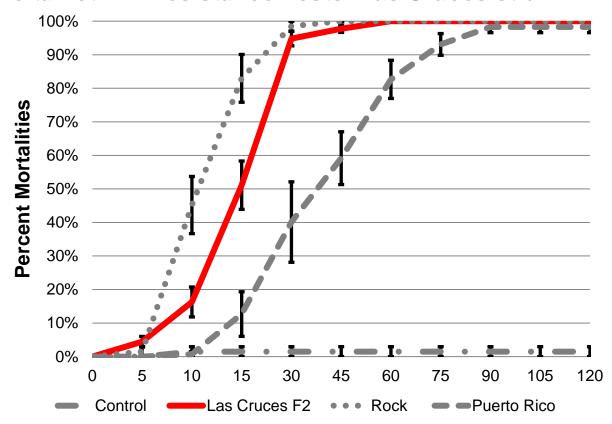


Figure B. Deltamethrin IR Bottle Test Results: 10.5 ug/bottle. Shown are means and standard error bars for different groups of mosquitoes. Rock is considered a pyrethroid-sensitive strain. Puerto Rico is considered a pyrethroid-resistant strain. The control was treated with acetone only.

Results- The control (Acetone) mortality curve was significantly different (P<0.0001) from the curves for all other treatments with little to no mortalities. Kaplan Meier statistical analysis confirmed that there was a significant difference between the curves plotted for the deltamethrin-exposed Rock Strain and the Las Cruces Strain (P<.0001). The curve plotted for the Puerto Rico Strain (a known resistant strain) is significantly different than that plotted for the Las Cruces Strain (P<.0001). The curves plotted for the Puerto Rico strain and the Rock strain were also significantly different from each other (P<.0001).

Conclusion- the Las Cruces *Aedes aegypti* strain showed resistance toward deltamethrin.

Deltamethrin Resistance Tests: Carlsbad strain

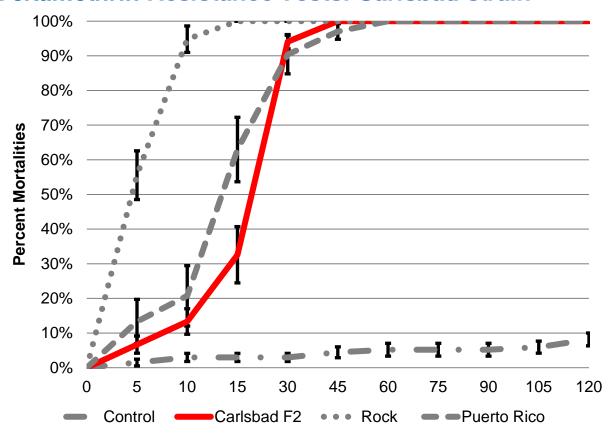


Figure C. Deltamethrin IR Bottle Test Results: 10.5 ug/bottle. Shown are means and standard error bars for different groups of mosquitoes. Rock is considered a pyrethroid-sensitive strain. Puerto Rico is considered a pyrethroid-resistant strain. The control was treated with acetone only.

Results- The control (Acetone) mortality curve was significantly different (P<0.0001) from the curves for all other treatments with little to no mortalities. Kaplan Meier statistical analysis confirmed that there was a significant difference between the the curves plotted for the deltamethrin-exposed Rock Strain and the Carlsbad Strain (P<.0001). The curve plotted for the Puerto Rico Strain (a known resistant strain) is significantly different than that plotted for the Carlsbad Strain (P<.003). The curves plotted for the Puerto Rico strain and the Rock strain were also significantly different from each other (P<.0001).

Conclusion- The Carlsbad *Aedes aegypti* strain shows resistance to deltamethrin.

Deltamethrin Resistance Tests: Roswell strain

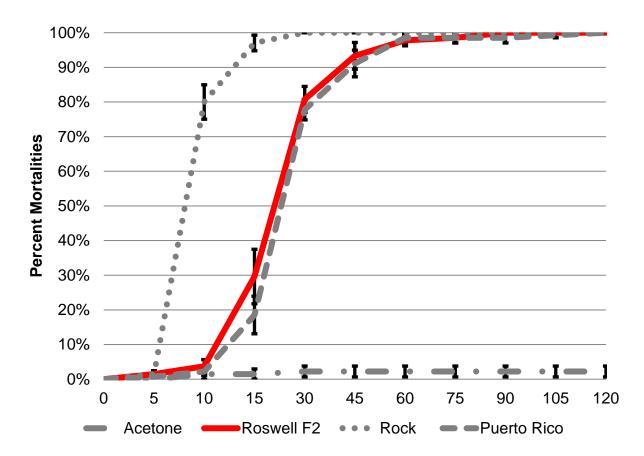


Figure D. Deltamethrin IR Bottle Test Results: 10.5 ug/bottle. Shown are means and standard error bars for different groups of mosquitoes. Rock is considered a pyrethroid-sensitive strain. Puerto Rico is considered a pyrethroid-resistant strain. The control was treated with acetone only.

Results- The control (Acetone) mortality curve was significantly different (P<0.0001) from the curves for all other treatments with little to no mortalities. Kaplan Meier statistical analysis confirmed that there was a significant difference between the curves plotted for the deltamethrin-exposed Rock Strain and the Roswell Strain (P<.0001). The curve plotted for the Puerto Rico Strain (a known resistant strain) is not significantly different than that plotted for the Roswell Strain (P=.091). The curves plotted for the Puerto Rico strain and the Rock strain were also significantly different from each other (P<.0001).

Conclusion- The Roswell *Aedes aegypti s*train shows resistance towards deltamethrin.

Etofenprox Resistance Tests: Roswell strain

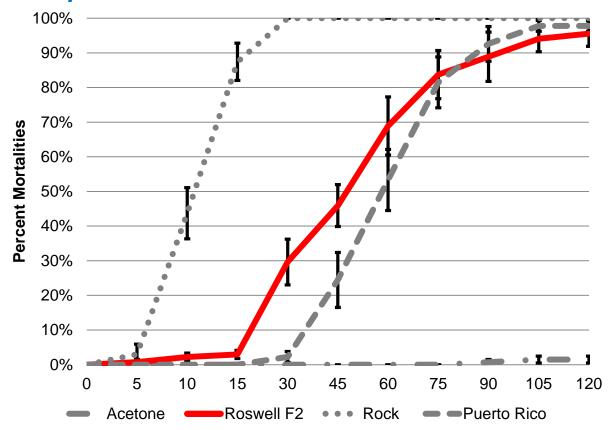


Figure E. Etofenprox IR Bottle Test Results: 37.5 ug/bottle. Shown are means and standard error bars for different groups of mosquitoes. Rock is considered a pyrethroid-sensitive strain. Puerto Rico is considered a pyrethroid-resistant strain. The control was treated with acetone only.

Results- The control (Acetone) mortality curve was significantly different (P<0.0001) from the curves for all other treatments with little to no mortalities. Kaplan Meier statistical analysis confirmed that there was a significant difference between the curves plotted for the etofenprox-exposed Rock Strain and the Roswell Strain (P<.0001). The curve plotted for the Puerto Rico Strain (a known resistant strain) is significantly different than that plotted for the Roswell Strain (P=.034). The curves plotted for the Puerto Rico strain and the Rock strain were also significantly different from each other (P<.0001).

Conclusion- The Roswell *Aedes aegypti* strain shows resistance towards etofenprox.

Etofenprox Resistance Tests: Las Cruces strain

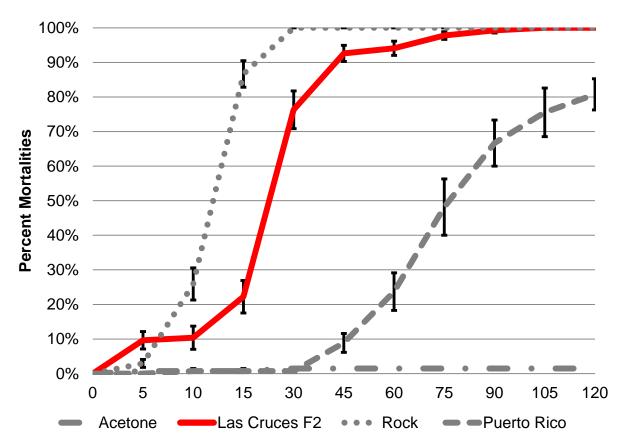


Figure F. Etofenprox IR Bottle Test Results: 37.5 ug/bottle. Shown are means and standard error bars for different groups of mosquitoes. Rock is considered a pyrethroid-sensitive strain. Puerto Rico is considered a pyrethroid-resistant strain. The control was treated with acetone only.

Results- The control (Acetone) mortality curve was significantly different (P<0.0001) from the curves for all other treatments with little to no mortalities. Kaplan Meier statistical analysis confirmed that there was a significant difference between the curves plotted for the etofenprox-exposed Rock Strain and the Las Cruces Strain (P<.0001). The curve plotted for the Puerto Rico Strain (a known resistant strain) is significantly different than that plotted for the Las Cruces Strain (P<.0001). The curves plotted for the Puerto Rico strain and the Rock strain were also significantly different from each other (P<.0001).

Conclusion- The Las Cruces *Aedes aegypti* strain shows resistance towards etofenprox.

Chlorpyrifos Resistance Tests: Las Cruces strain

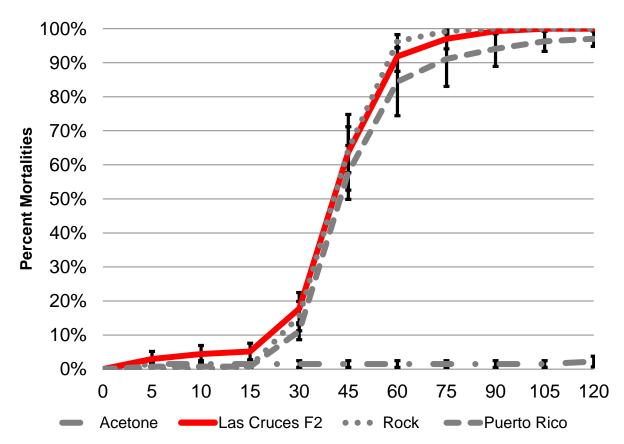


Figure G. Chlorpyrifos IR Bottle Test Results: 40 ug/bottle. Shown are means and standard error bars for different groups of mosquitoes. Rock is considered a pyrethroid-sensitive strain. Puerto Rico is considered a pyrethroid-resistant strain. The control was treated with acetone only.

Results- The control (Acetone) mortality curve was significantly different (P<0.0001) from the curves for all other treatments with little to no mortalities. Kaplan Meier statistical analysis confirmed that there was no significant difference between the curves plotted for the chlorpyrifos-exposed Rock Strain and the Las Cruces Strain (P=.589). The curve plotted for the Puerto Rico Strain (a known resistant strain) is significantly different than that plotted for the Las Cruces Strain (P=.014). The curves plotted for the Puerto Rico strain and the Rock strain were also significantly different from each other (P=.005).

Conclusion- The Las Cruces *Aedes aegypti* strain is sensitive towards chlorpyrifos.

Chlorpyrifos Resistance Tests: Alamogordo strain

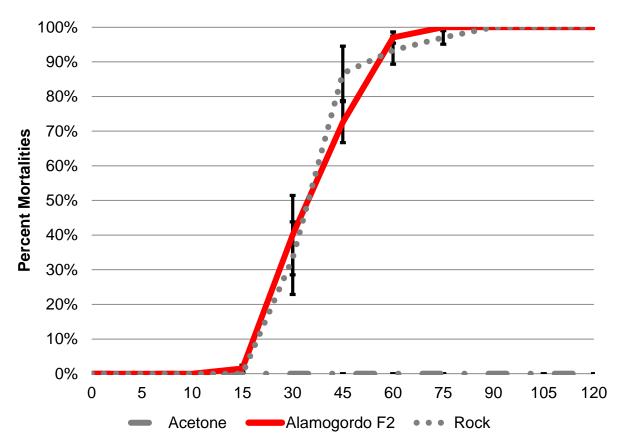


Figure H. Chlorpyrifos IR Bottle Test Results: 40 ug/bottle. Shown are means and standard error bars for different groups of mosquitoes. Rock is considered a pyrethroid-sensitive strain. The control was treated with acetone only.

Results- The control (Acetone) mortality curve was significantly different (P<0.0001) from the curves for all other treatments with little to no mortalities. Kaplan Meier statistical analysis confirmed that there was not a significant difference between the curves plotted for the chlorpyrifos-exposed Rock Strain and the Alamogordo Strain (P=.984).

Conclusion- The Alamogordo Aedes aegypti strain is sensitive to chlorpyrifos.

Chlorpyrifos Resistance Tests: Carlsbad strain

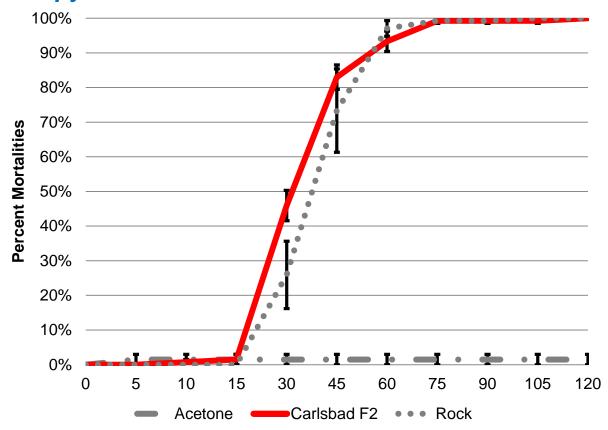


Figure I. Chlorpyrifos IR Bottle Test Results: 40 ug/bottle. Shown are means and standard error bars for different groups of mosquitoes. Rock is considered a pyrethroid-sensitive strain. The control was treated with acetone only.

Results- The control (Acetone) mortality curve was significantly different (P<0.0001) from the curves for all other treatments with little to no mortalities. Kaplan Meier statistical analysis confirmed that there was a significant difference between the curves plotted for the chlorpyrifos-exposed Rock Strain and the Carlsbad Strain (P=.028).

Conclusion- The Carlsbad Aedes aegypti strain shows sensitivity towards chlorpyrifos.

Chlorpyrifos Resistance Tests: Roswell strain

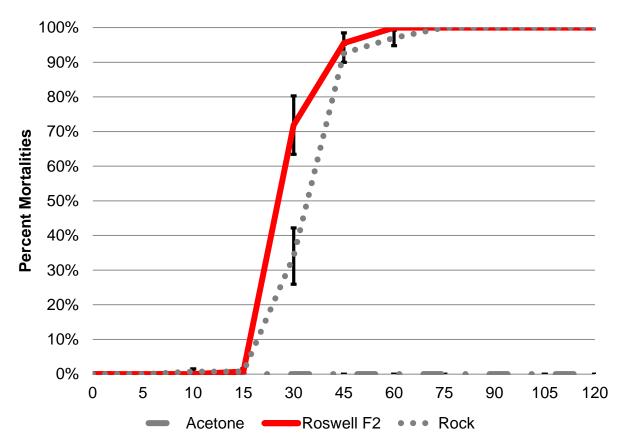


Figure J. Chlorpyrifos IR Bottle Test Results: 40 ug/bottle. Shown are means and standard error bars for different groups of mosquitoes. Rock is considered a pyrethroid-sensitive strain. The control was treated with acetone only.

Results- The control (Acetone) mortality curve was significantly different (P<0.0001) from the curves for all other treatments with little to no mortalities. Kaplan Meier statistical analysis confirmed that there was a significant difference between the curves plotted for the chlorpyrifos-exposed Rock Strain and the Roswell Strain (P<.0001).

Conclusion- The Roswell *Aedes aegypti* strain is sensitive to chlorpyrifos.

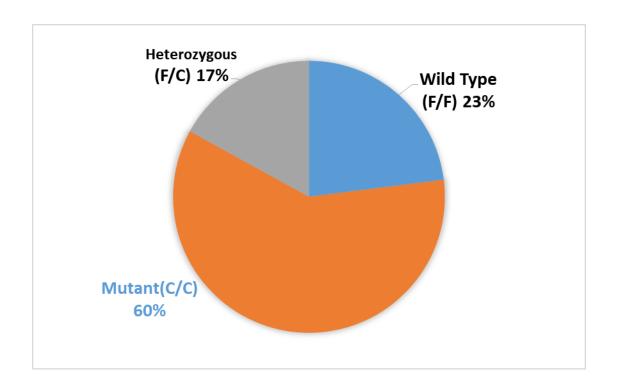


Figure K: kdr position 1534 Genotypes in 50 mosquitoes from New Mexico. The figure shows the percentage of heterozygous and homozygous mosquitoes. The resistant mutants are shown in orange, wild type in blue, and heterozygous in grey.

09_aegypt 410 65908_Musca	MTEDSDSISEEERSLFRPFTRESLAAIERRIADAEAKQRELEKKRAEGETGFGRKKKKKE MTEDSDSISEEERSLFRPFTRESLLQIEQRIAEHEKQ-KELERKRAAEGEQ ***********************************	60 50
NC_035109_aegypt NW_004765908_Musca	IRYDDEDEGPQPDSTLEQGVPIPVRMQGSFPPELASTPLEDIDSYYANQRTFVVVSKG IRYDDEDEGPQPDPTLEQGVPIPVRMQGSFPPELASTPLEDIDPFYSNVLTFVVISKG ************************************	120 110
NC_035109_aegypt NW_004765908_Musca	KDIFRFSATNALYVLDPFNPIRRVAIYILVHPLFSFFIITTILTNCILMIMPSTPTVEST KDIFRFSASKAMWLLDPFNPIRRVAIYILVHPLFSLFIITTILTNCILMIMPTTPTVEST ******:::::**************************	180 170
NC_035109_aegypt NW_004765908_Musca	EVIFTGIYTFESAVKVMARGFILQPFTYLRDAWNWLDFVVIALAYVTMGIDLGNLAALRT EVIFTGIYTFESAVKVMARGFILCPFTYLRDAWNWLDFVVIALAYVTMGIDLGNLAALRT ************************************	240 230
NC_035109_aegypt NW_004765908_Musca	FRVLRALKTVAIVPGLKTIVGAVIESVKNLRDVIILTMFSLSVFALMGLQIYMGVLTQKC FRVLRALKTVAIVPGLKTIVGAVIESVKNLRDVIILTMFSLSVFALMGLQIYMGVLTQKC ************************************	300 290
NC_035109_aegypt NW_004765908_Musca	IREFPMDGSWGNLSDENWERFNNNDSNWYFSETGD-TPLCGNSSGAGQCEEGYICLQGYG IKRFPLDGSWGNLTDENWFLHNSNSSNWFTENDGESYPVCGNVSGAGQCGEDYVCLOGFG *:.**:******* .*.*.***: .: *: *:*** ***** *.*:** 419	359 350
NC_035109_aegypt NW_004765908_Musca	DNPNYGYTSFDTFGWAFLSAFRLMTQDYWENLYQLVLRSAGPWHMLFFIVIIFLGSFYLV PNPNYDYTSFDSFGWAFLSAFRLMTQDFWEDLYQHVLQAAGPWHMLFFIVIIFLGSFYLV ****.********************************	419 410
NC_035109_aegypt NW_004765908_Musca	NLILAIVAMSYDELQKRAEEEEAAEEEALREAEEAAAAKAAKLEAQAAA NLILAIVAMSYDELQKKAEEEEAAEEEAIREAEEAAAAKAAKLEERANVAAQAAQDAADA ****************************	468 470
NC_035109_aegypt NW_004765908_Musca	AAAAANPEIAKSPSDFSCHSYELFVNQEKGNDDNNKEKMSIRSEGLESVSEITRTTAPTA AAAALHPEMAKSP-TYSCISYELFVGGEKGNDDNNKEKMSIRSVEVESESVSVIQRQPAP ****:**:*** :** ***************** :** *: *:	528 529
NC_035109_aegypt NW_004765908_Musca	TAAGTAKARKVSAGVAAFQKASLSLPGSPFNLRRGSRGSHQFTIRNGRGRFVGVPGSDRK TTAPATKVRKVSTTSLSLPGSPFNLRRGSRSSHKYTIRNGRGRF-GIPGSDRK *:* ::*.*	588 581
NC_035109_aegypt NW_004765908_Musca	PLVLSTYLDAQEHLPYADDSNAVTPMSEENGAIIVPVYYANLGSRHSSYTSHQSRISYTS PLVLQTYQDAQQHLPYADDSNAVTPMSEENGAIIVPAYYCNLGSRHSSYTSHQSRISYTS ****.** ***:***************************	648 641
NC_035109_aegypt NW_004765908_Musca	HGDLLGGMTKESRLRNRSARNTNHSIVPPPNMSGPNMSYVDSNHKGQRDFDM HGDLLGGMAAMGASTMTKESKLRSRNTRNQSIGAATNGGSSTAGGGYPDANHKEQRDYEM *******: **:****	700 701
NC_035109_aegypt NW_004765908_Musca	SQDCTDEAGKIKHNDNPFIEPSQTQTVVDMKDVMVLNDIIEQAAGRHSRASDHGVSVYYF GQDYTDEAGKIKHHDNPFIEPVQTQTVVDMKDVMVLNDIIEQAAGRHSRASERG .** ********:**************************	760 755
NC_035109_aegypt NW_004765908_Musca	PTEDDDEDGPTFKDKALEFTMRMIDVFCVWDCCWVWLKFQEWVAFIVFDPFVELFITLCIEDDDEDGPTFKDIALEYILKGIEIFCVWDCCWVWLKFQEWVSFIVFDPFVELFITLCI ********** ***: :: *::****************	820 813
NC_035109_aegypt NW_004765908_Musca	VVNTLFMALDHHDMDPDMERALKSGNYFFTATFAIEATMKLIAMSPKYYFQEGWNIFDFI VVNTMFMAMDHHDMNPELEKVLKSGNYFFTATFAIEASMKLMAMSPKYYFQEGWNIFDFI ***:***:*****************************	880 873
NC_035109_aegypt NW_004765908_Musca	IVALSLLELGLEGVQGLSVLRSFRLLRVFKLAKSWPTLNLLISIMGRTMGALGNLTFVLC IVALSLLELGLEGVQGLSVLRSFRLLRVFKLAKSWPTLNLLISIMGRTMGALGNLTFVLC ************************************	940 933
NC_035109_aegypt NW_004765908_Musca	989 996 IIIFIFAVMGMQLFGKNYIDNVDRFPDKDLPRWNFTDFMHSFMIVFRVLCGEWIESMWDC IIIFIFAVMGMQLFGKNYIDHKDRFKDHELPRWNFTDFMHSFMIVFRVLCGEWIESMWDC ************************************	1000 993

1018 1023

NC_035109_aegypt NW_004765908_Musca	MLVGDVSCIPFFLATVV <mark>I</mark> GNLV <mark>GL</mark> NLFLALLLSNFGSSSL SAPTADNETNKIAEAFNRIS MYVGDVSCIPFFLATVV <u>I</u> GNLV <u>VL</u> NLFLALLLSNFGSSSLSAPTADNDTNKIAEAFNRIA * ***********************************	1060 1053
NC_035109_aegypt NW_004765908_Musca	RFSNWIKSNIANALKFVKNKLTSQIASVQPAGEQHNHLSWIWNEGKGVCPCISAEHGENE RFKNWVKRNIADCFKLIRNKLTNQISDQPSEHGDNE **.**: ***::**::**::**::**::**::**::**::	1120 1089
NC_035109_aegypt NW_004765908_Musca	LELTPDDILADGLLKKGVKEHNQLEVAIGDGMEFTIHGDLKNKGKKNKQLMNNSKVIGNS LELGHDEIMGDGLIKKGMKGETQLEVAIGDGMEFTIHGDMKNNKPKKSKFINNTTMIGNS *** *:::**::**::::**::::***	1180 1149
NC_035109_aegypt NW_004765908_Musca	ISNHQDNKLEHELNHRGMSLQDDDTASIKSYGSHKNRPFKDESHKGSAETMEGEEKRDVS I-NHQDNRLEHELNHRGLSIQDDDTASINSYGSHKNRPFKDESHKGSAETIEGEEKRDVS * ****:*******::**********************	1240 1208
NC_035109_aegypt NW_004765908_Musca	KEDLGIDEELDDECDGEEGPLDGELIIHADE-DEVIEDSPADCCPDNCYKKFPVLAGDDD KEDLGLDEELDEEAEGDEGQLDGDIIIHAQNDDEIIDDYPADCFPDSYYKKFPILAGDED *****:****:* ***::****:: **::* **** **. ****:***:	1299 1268
NC_035109_aegypt NW_004765908_Musca	APFWQGWANLRLKTFQLIENKYFETAVITMILLSSLALALEDVHLPHRPILQDVLYYMDR SPFWQGWGNLRLKTFQLIENKYFETAVITMILMSSLALALEDVHLPDRPVMQDILYYMDR :*****.*******************************	1359 1328
NC_035109_aegypt NW_004765908_Musca	IFTVIFFLEMLIKWLALGFRVYFTNAWCWLDFIIVMVSLINFVASLCGAGGIQAFKTMRT IFTVIFFLEMLIKWLALGFKVYFTNAWCWLDFVIVMLSLINLVAVWSGLNDIAVFRSMRT ************************************	1419 1388
NC_035109_aegypt NW_004765908_Musca	LRALRPLRAMSRMQGMRVVVNALVQAIPSIFNVLLVCLIFWLIFAIMGVQLFAGKYFKCV LRALRPLRAVSRWEGMKVVVNALVQAIPSIFNVLLVCLIFWLIFAIMGVQLFAGKYFKCK ********:** :**:**********************	1479 1448
NC_035109_aegypt NW_004765908_Musca	DKNKTTLSHEIIPDVNACVAENYTWENSPMNFDHVGKAYLCLFQVATFKGWIQIMNDAID DGNDTVLSHEIIPNRNACKSENYTWENSAMNFDHVGNAYLCLFQVATFKGWIQIMNDAID * * * * * * * 1551	1539 1508
NC_035109_aegypt NW_004765908_Musca	SREVGKQPIRETNIYMYLYFVFFIIFGSFFTLNLFIGVIIDNFNEQKKKAGGSLEMFMTE SREVDKQPIRETNIYMYLYFVFFIIFGSFFTLNLFIGVIIDNFNEQKKKAGGSLEMFMTE **** 1520 * 1534 ************************************	1599 1568
NC_035109_aegypt NW_004765908_Musca	DQKKYYNAMKKMGSKKPLKAIPRPRWRPQAIVFEIVTNKKFDMIIMLFIGFNMLTMTLDH DQKKYYNAMKKMGSKKPLKAIPRPRWRPQAIVFEIVTDKKFDIIIMLFIGLNMFTMTLDR ************************************	1659 1628
NC_035109_aegypt NW_004765908_Musca	YKQTDTFSAVLDYLNMIFICIFSSECLMKIFALRYHYFIEPWNLFDFVVVILSILGLVLS YDASEAYNNVLDKLNGIFVVIFSGECLLKIFALRYHYFKEPWNLFDVVVVILSILGLVLS *.:::. *** ** **: ***.*****************	1719 1688
NC_035109_aegypt NW_004765908_Musca	DLIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKGIRTLLFALAMSLPALFNICLLLFLVMF DIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKGIRTLLFALAMSLPALFNICLLLFLVMF *:***********************************	1779 1748
NC_035109_aegypt NW_004765908_Musca	IFAIFGMSFFMHVKYKSGLDDVYNFKTFGQSMILLFQMSTSAGWDGVLDGIINEDECLPP IFAIFGMSFFMHVKEKSGINAVYNFKTFGQSMILLFQMSTSAGWDGVLDAIINEEDCDPP *********** 1763 : ************************************	1839 1808
NC_035109_aegypt NW_004765908_Musca	DNDKGYPGNCGSATIGITYLLAYLVISFLIVINMYIAVILENYSQATEDVQEGLTDDDYD DNDKGYPGNCGSATVGITFLLSYLVISFLIVINMYIAVILENYSQATEDVQEGLTDDDYD *********************************	1899 1868
NC_035109_aegypt NW_004765908_Musca	MYYEIWQQFDPDGTQYIRYDQLSDFLDVLEPPLQIHKPNKYKIISMDIPICRGDMMFCVD MYYEIWQQFDPEGTQYIRYDQLSEFLDVLEPPLQIHKPNKYKIISMDMPICRGDMMYCVD ********:****************************	1959 1928
NC_035109_aegypt NW_004765908_Musca	ILDALTKDFFARKGNPIEETAELGEVQARPDEVGYEPVSSTLWRQREEYCARVIQHAWRK ILDALTKDFFARKGNPIEETGEIGEIAARPDTEGYDPVSSTLWRQREEYCAKLIQNAWRR ***********************************	2019 1988
NC_035109_aegypt NW_004765908_Musca	HKERQAGGGGDDTDADACDNDDGDDGGGGAGDGGSAGGGGVT-SPGVGSGSIVGGGTTP YKNGPPQEGDEGEAAGGEDGAEGGEGEGGGGGGGGDDGGSATGATAAAAGATSP :*: *:**:**:**:**:**:**:**:**:**:**:**:**:	2078 2044

NC_035109_aegypt NW_004765908_Musca	SDPDAGEADGA		2138 2099
NC_035109_aegypt	SITSRSADV	2147	
NW_004765908_Musca	S	2100	

Figure L. Sequence alignment of the Para protein of *Aedes aegypti* and *Musca domestica*. Kdr mutation sites are red and the respective location is given in boxes above and below the alignment.

City	Pesticides - Product Name	Pesticides - Active Ingredient
Las Cruces	not provided	Etofenprox
	·	Deltamethrin
		Chlorpyrifos
		Bifenthrin
		Lambda Cyhalothrin
T or C	Prentox Aqua Perm-X UL 30-30	Permethrin and Piperonyl Butoxide
	Altosid XR Briquets	(S)-Methoprene
	Aqualuer 20-20	Permethrin and Piperonyl Butoxide Technical
Roswell	no info	no info
Lordsburg	none	n/a
Alamogordo	Vectobac	Bacillus thuringiensis subsp. israelensis
Clovis	Delta Guard	Deltamethrin
	Atrapa	Malathion
Sunland Park	Altosid products (2015, 2016, 2017)	(S)-Methoprene
	Fyfanon (2016, 2017)	Malathion
	Kontrol 4x4 (2015)	Permethrin and Piperonyl Butoxide
	Vectolex CG products (2013, 2014)	Bacillus sphaericus
	Evoluer 30-30 (2013, 2014)	Permethrin and Piperonyl Butoxide
	Sustain MBG Pellets (2013)	Bacillus thuringiensis subsp. israelensis
Portales	no info	no info
Carlsbad	Mosquito Master	Chlorpyrifos and Permethrin
	Delta Guard	Deltamethrin
	Altosid	(S)-Methoprene
	Fourstar BTI	Bacillus thuringiensis subsp. Israelensis
	Natular™ products	Spinosad (soil bacterium)
	Permex	Permethrin
	Pursuit	Permethrin and Piperonyl Butoxide
	PyroFos 1.5 ULV (discontinued by	Chlamamifas
Lovington	the manufacturer)	Chlorovrifos and Darmethria
Lovington	Mosquito Master	Chlorpyrifos and Permethrin
Deming	BTI Briquits not provided	Bacillus thuringiensis subsp. Israelensis Etofenprox
Denning	ποι ριονίαεα	Deltamethrin
		Chlorpyrifos
		Bifenthrin
		Lambda Cyhalothrin
Socorro	not provided	Permethrin
3000110	not provided	remedim

Table A. Pesticide-use in selected cities across New Mexico.

City	# sites contributing to adults	# adults added to cage
Alamogordo	9	28
Carlsbad	8	144
Deming	6	28
Las Cruces	9	60
Lovington	5	20
Roswell	8	22
Sunland Park	12	72

Table B. Number of founding individuals for New Mexico *Aedes aegypti* strains used in this study. Traps were set for 24 hours at the respective sites.