Insecticide resistance management in Florida citrus production (CRDF-funded project #17-001C)

Lukasz Stelinski

University of Florida, Entomology and Nematology Department, CREC, Lake Alfred, FL

Objectives

- Objective 1: Conduct statewide survey to identify focal points of resistance using calibrated vial assay and ACP feeding assay.
- Objective 2: Quantify the stability of resistance/reversion to susceptibility in the absence of insecticide selection pressure.
- Objective 3: Define the baseline resistance ratio required for product failure.
- Objective 4: Implement the most effective known insecticide rotation schedules on large-area scale and monitor their effectiveness.

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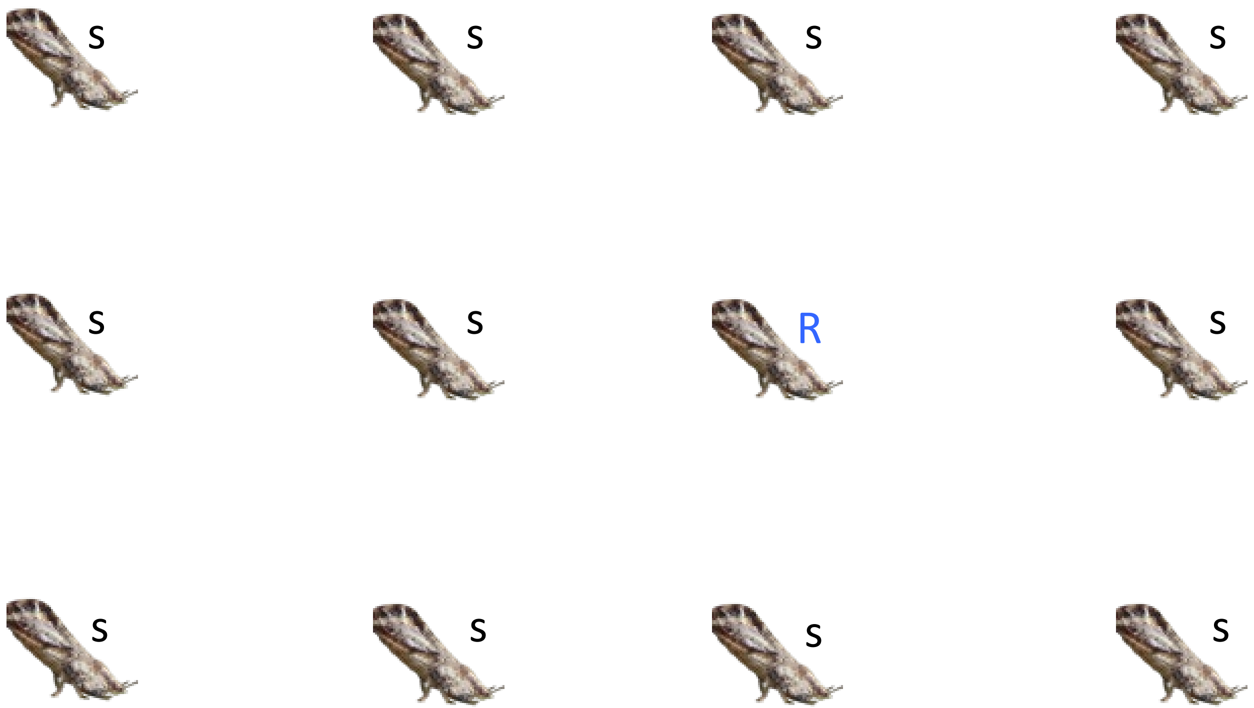
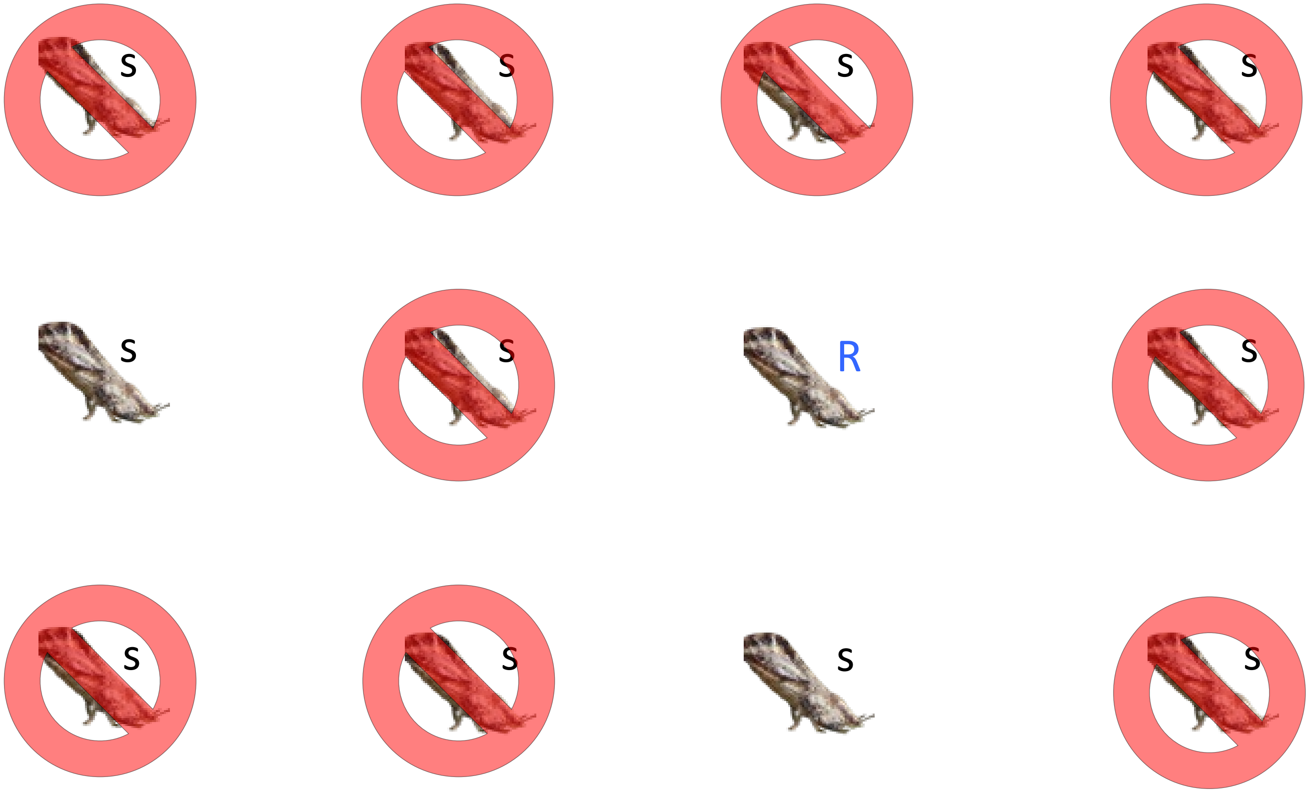
- Objective 1: Fully completed—Resistance occurs sporadically statewide; problem areas associated with overuse.
- Objective 2: Fully completed—Need 5 product rotation or ca. 6 months for full reversion; fitness costs fully defined.
- Objective 3: Fully completed—RR of 80-100 fold associated with product failure.
- Objective 4: Fully completed-Implemented or grove-area scale and proven effective.

Evolution of Insecticide resistance How does it occur?



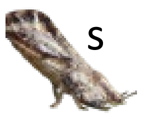
Individuals in a population are never equally susceptible to an Insecticide. Although initially rare, insecticide-resistant R genotype is present.
Frequency is 1/12 = 0.083

Evolution of Insecticide resistance. How does it occur?



An insecticide is used leaving some individuals insecticide-resistant individuals (R genotype) and some susceptible individuals (s genoptype)

Evolution of Insecticide resistance. How does it occur?



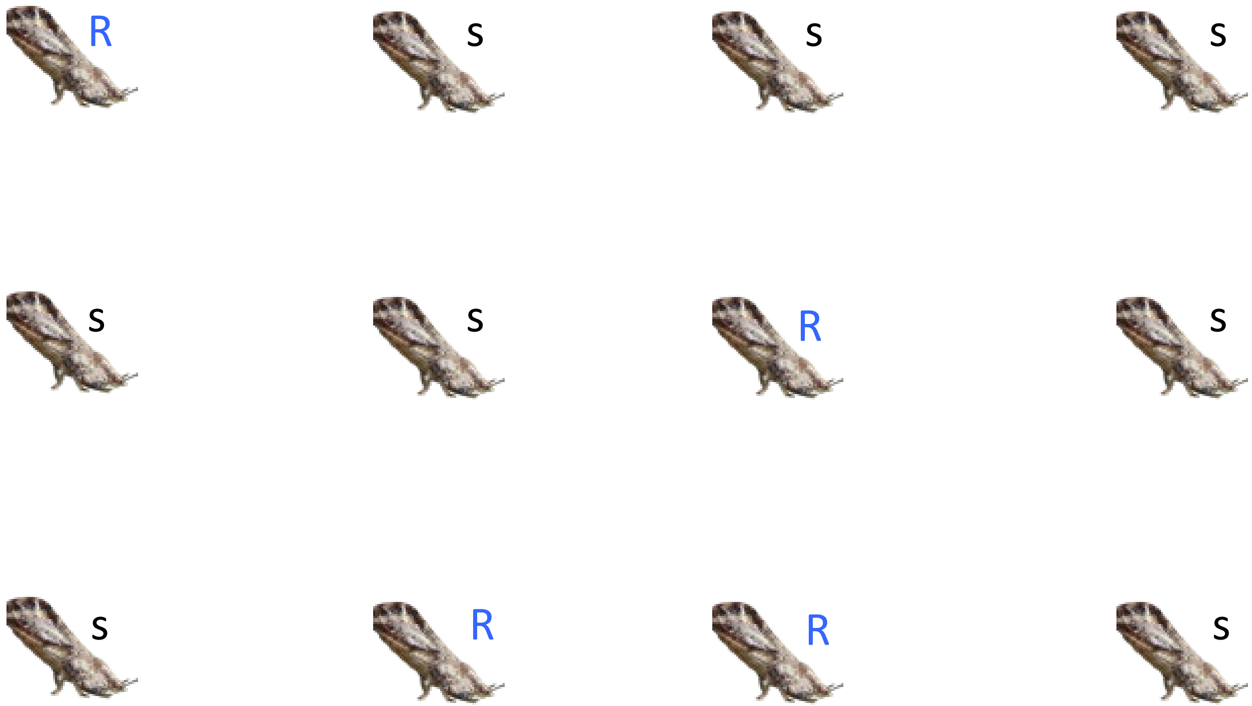
s

R

s

Now, the frequency pf insecticide-resistant R genotype is 1/3 = 0.333

Evolution of Insecticide resistance. How does it occur?



If the R genotype reproduces as well as the s genotype, in the next generation the frequency of the R genotype will be the same as the survivors in the preceding generation, i.e. 0.333

Evolution of insecticide resistance:

- When LD50 ratio ≥ 10, resistance occurs

- Resistance: occur through insecticide selection

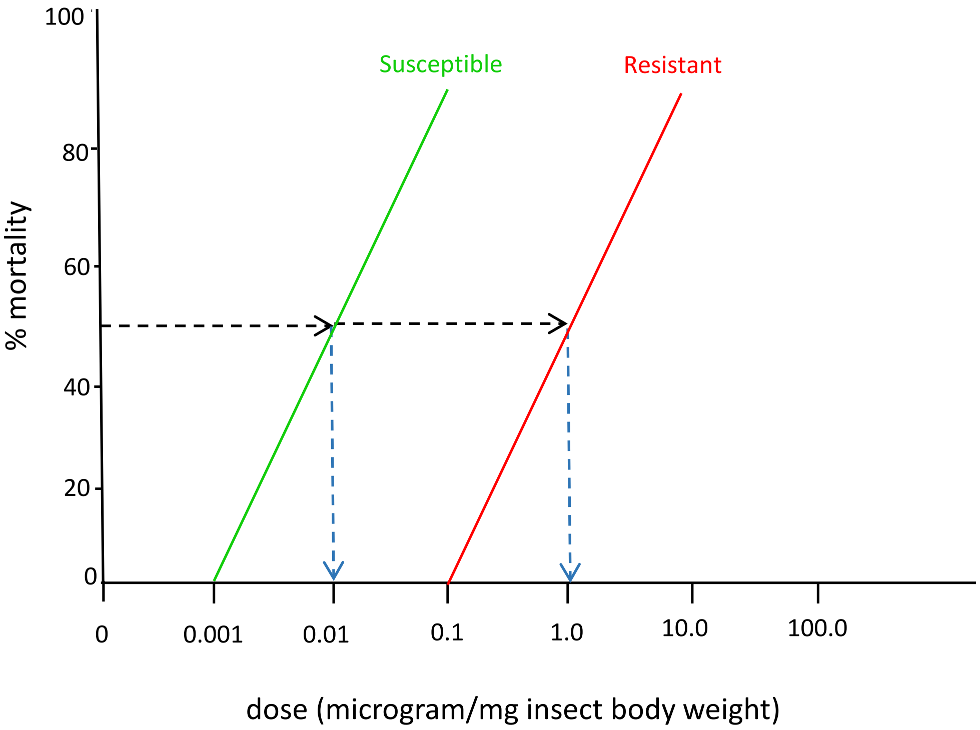
- Selection acts on genetically based variation in susceptibility which arise from:

– Mutation, the source of all new genetic variation

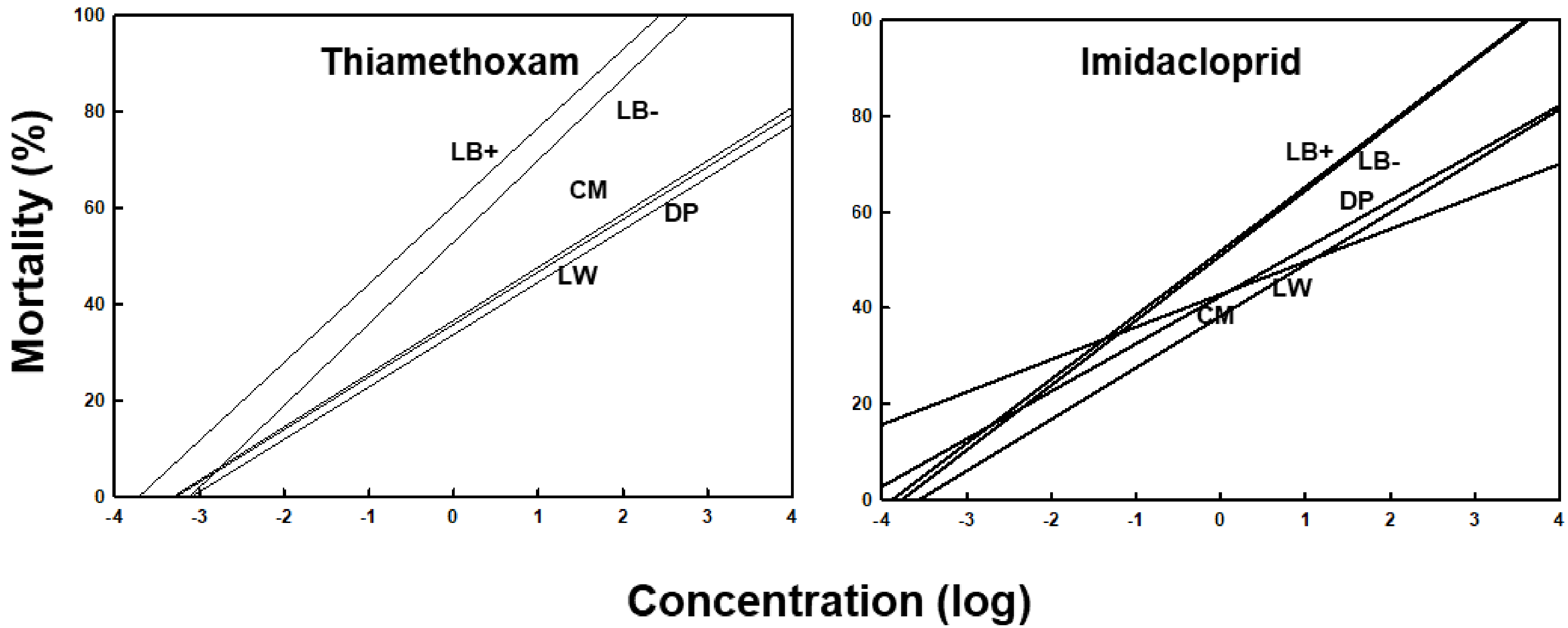
– Genetic recombination that rearranges genetic variation

– Gene flow from populations having different allelic frequencies

LD_{50} = A measure of insecticide toxicity



Dose-response curves for the ACP populations treated with serial dilutions of thiamethoxam and imidacloprid in water. Abbreviations identify geographic origins of each strain. LB^-: Lab *C*Las$^-$; LB^+: Lab *C*Las$^+$; CM: Clermont; DP: Davenport; LW: Lakes Wales (Objective 1).



Susceptibility of laboratory and field collected ACP adults to selected insecticides in Florida, 2018 (Objective 1)

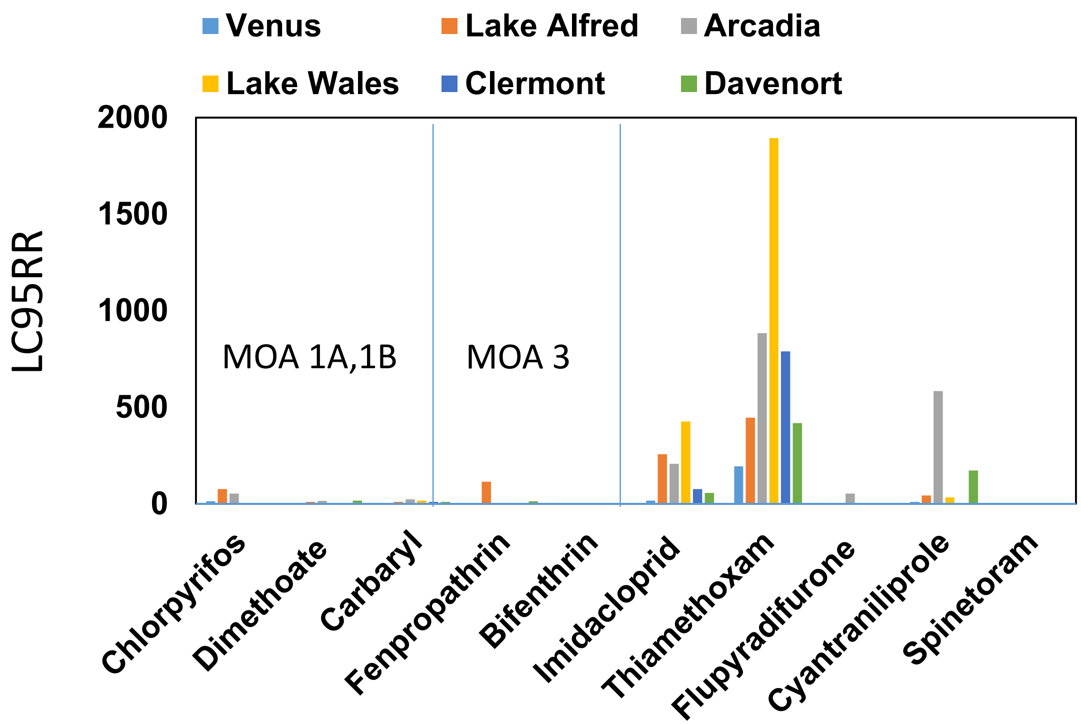
Insecticides	n	χ^2	Slope ± SE	LC_{50}	LC_{95}	RR_{50}	RR_{95}
Dimethoate							
Lab	273	23.68	1.05 ± 0.29	0.07 (0.01-1.56)	2.61(0.29-1188238)	1	1
Venus	274	27.46	0.94 ± 0.26	0.14 (0.06-4.61)	7.83 (0.64-466019)	2.00	3.00
Lake Alfred	274	5.47	0.68 ± 0.07	0.10 (0.05-0.22)	26.71 (7.85-159.40)	1.43	10.23
Arcadia	350	10.77	0.62 ± 0.10	0.09 (0.02-0.53)	38.75 (3.52-12736)	1.29	14.85
Winter Garden	350	38.24	0.72 ± 0.20	0.04 (0.10-6.01)	8.87 (0.37-)	0.57	3.40
Lake Wales	287	6.93	0.76 ± 0.08	0.02 (0.01-0.03)	2.60 (0.91-10.94)	0.29	1.00
Clermont	350	2.37	1.10 ± 0.11	0.06 (0.04-0.10)	2.05 (1.01-5.53)	0.86	0.78
Davenport	300	4.38	0.71 ± 0.08	0.15 (0.08-0.28)	45.21 (14.0-165.3)	2.14	17.32
Chlorpyrifos							
Lab	317	25.16	1.18 ± 0.28	0.03 (0.01-0.20)	0.81 (0.14-337.79)	1	1
Venus	316	22.87	0.80 ± 0.16	0.10 (0.01-0.66)	11.10 (1.30-3701)	3.3	13.70
Lake Alfred	326	6.37	0.75 ± 0.07	0.06 (0.05-0.11)	9.17 (21.7-264.2)	2	11.32
Arcadia	353	7.63	0.56 ± 0.06	0.02 (0.01-0.03)	13.58 (3.90-91.27)	0.67	16.77
Winter Garden	350	40.72	0.62 ± 0.19	0.09 (0.00-1216)	40.72 (0.38-)	3	50.27
Davenport	351	7.70	0.62 ± 0.06	0.18 (0.10-0.37)	85.61 (23-532)	6	105.69
Fenpropathrin							
Lab	270	7.61	0.76 ± 0.08	0.03 (0.02-0.06)	4.27 (1.48-19.67)	1	1
Venus	275	4.33	0.63 ± 0.07	0.08 (0.04-0.17)	34.52 (7.02-230.5)	2.73	5.50
Lake Alfred	407	2.07	0.58 ± 0.05	0.18 (0.10-0.37)	127.86 (40.8-596.9)	6	29.93
Arcadia	350	8.37	0.67 ± 0.09	0.07 (0.02-0.29)	20.73 (2.85-1151)	2.33	4.86
Winter Garden	355	11.28	0.82 ± 0.13	0.18 (0.05-0.74)	18.72 (3.17-914.64)	6	4.38
Davenport	350	6.96	0.60 ± 0.06	0.11 (0.06-0.21)	61.32 (17.1 -377.5)	3.66	14.36
Ft Pierce	405	5.05	0.62 ± 0.05	0.10 (0.06-0.20)	45.83 (16.04-1855)	3.33	34.15
Bifenthrin							
Lab	279	13.48	0.69 ± 0.14	0.03 (0.001-0.20)	6.27 (0.55-7553)	1	1
Venus	289	8.72	0.75 ± 0.11	0.05 (0.01- 0.20)	7.38 (1.89-505.36)	1.67	1.18
Lake Alfred	359	2.88	0.64 ± 0.06	0.02 (0.01-0.20)	4.58 (1.65-18.87)	0.67	0.73
Arcadia	352	9.32	0.76 ± 0.11	0.06 (0.02- 0.25)	9.32 (1.59-340)	2.00	1.49
Winter Garden	350	4.64	0.64 ± 0.06	0.01 (0.01-0.02)	3.67 (1.30-16.71)	0.33	0.59
Lake Wales	282	16.57	0.80 ± 0.17	0.03 (0.003-0.25)	3.02 (0.31-3582)	1	0.48
Davenport	355	3.18	0.93 ± 0.09	0.02 (0.01-0.03)	1.12 (0.52-3.24)	0.67	0.17
Imidacloprid							
Lab	284	6.91	0.75 ± 0.08	0.13 (0.02-0.24)	20.39 (7.34-87.57)	1	1
Venus	282	7.21	0.68 ± 0.07	1.37 (0.70-2.78)	359.01 (107-2085)	10.54	17.61
Lake Alfred	280	3.66	0.46 ± 0.06	1.33 (0.54-3.68)	5236 (710-12874)	10.23	256.79
Arcadia	352	2.75	0.45 ± 0.05	1.07 (0.49-2.59)	4248 (709-64337)	8.23	208.34
Winter Garden	353	3.51	0.63 ± 0.05	1.01 (0.50-2.14)	1242 (289-99407)	7.77	60.91
Lake Wales	352	5.21	0.42 ± 0.05	1.06 (0.18-0.78)	8687 (1162-200162)	8.15	426.04
Clermont	281	0.77	0.53 ± 0.06	1.20 (0.54-2.89)	1556 (304-189733)	9.23	76.31
Davenport	280	6.25	0.53 ± 0.06	0.90 (0.40-2.13)	1150 (228.2-135.8)	6.90	56.40
Ft Pierce	284	7.08	0.60 ± 0.67	1.67 (0.78-3.90)	1353 (288 -14136)	12.65	66.36

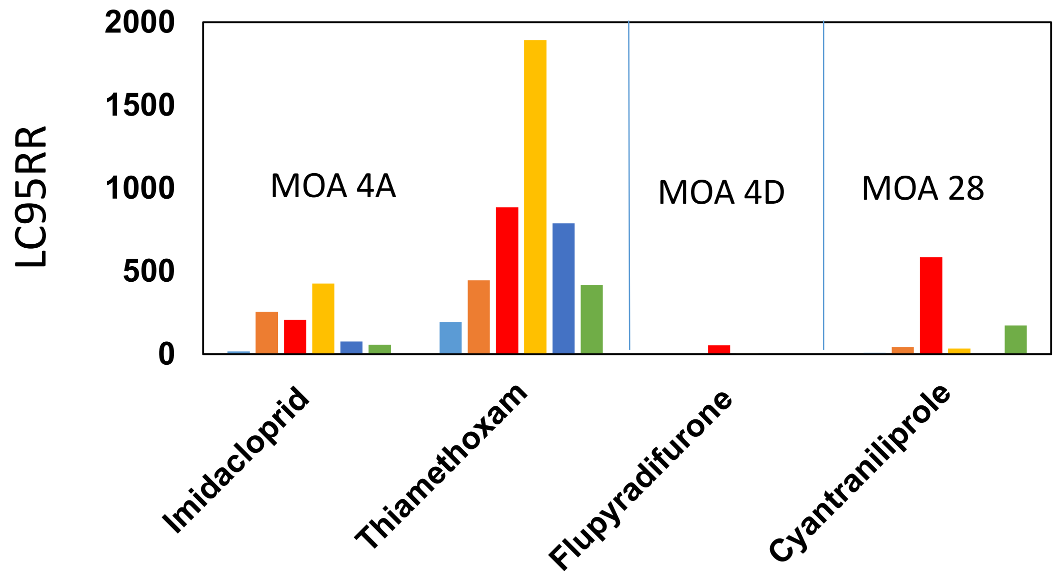
Susceptibility of laboratory and field collected ACP adults to selected insecticides in Florida, 2018, continued (Objective 1)

Thiamethoxam							
Lab	565	2.60	0.91 ± 0.08	0.02 (0.01-0.03)	1.27 (0.66-3.08)	1	1
Venus	232	15.62	0.71 ± 0.19	1.16 (0.01-447)	245.97 (245.70-)	58	193.68
Lake Alfred	288	4.45	0.60 ± 0.06	1.06 (0.52-2.29)	578.51 (144-4522)	53	445.52
Arcadia	351	18.66	0.54 ± 0.12	1.86 (0.20-77.57)	1123 (38.10-$3.2\times^{8}$)	93	884.25
Lake Wales	353	5.69	0.49 ± 0.05	0.96 (0.48-2.44)	2353 (455-27106)	48	1892.75
Clermont	350	1.63	0.60 ± 0.06	1.11 (0.56-2.33)	1002 (248.34-7572)	55.50	788.98
Davenport	350	6.78	0.58 ± 0.09	0.80 (0.41-1.63)	530.15 (146-3320)	40	417.44
Ft Pierce	352	0.95	0.58 ± 0.06	1.18 (0.61-2.43)	861.64 (226.6-5888)	59	678.46
Carbaryl							
Lab	283	1.59	0.90 ± 0.10	0.04 (0.02-0.08)	2.99 (1.22-1.17)	1	1
Venus	285	1.00	0.75 ± 0.08	0.10 (0.05-0.18)	14.21 (5.30-57.55)	3.33	4.75
Lake Alfred	299	3.53	0.17 ± 0.07	0.14 (0.07-0.27)	29.77 (10.8-123.5)	3.50	9.96
Winter Garden	351	5.96	0.76 ± 0.08	0.03 (0.02-0.06)	5.45 (2.19-19.45)	0.75	1.82
Davenport	282	1.87	0.69 ± 0.07	0.12 (0.06-0.24)	29.22 (9.7-143.40)	3	9.77
Flupyradifurone							
Lab	350	22.57	0.77 ± 0.16	0.35 (0.03-3.28)	47.59 (4.6-1.0×10^{5})	1	1
Lake Alfred	348	6.47	0.59 ± 0.06	0.10 (0.05-0.20)	63.16 (19.7-331.3)	1.11	1.32
Arcadia	350	2.30	0.58 ± 0.06	0.39 (0.20-0.76)	255.12 (75.28-1431)	0.86	53.69
Winter Garden	350	3.72	0.59 ± 0.06	0.30 (0.15-0.56)	178.90 (55.1-939.8)	0.31	3.76
Lake Wales	328	7.95	0.56 ± 0.08	0.11 (0.02-0.47)	85.81 (9.45-10120)	0.33	1.80
Clermont	350	2.85	0.64 ± 0.06	0.10 (0.05-0.18)	30.97 (11.2-127.3)	0.34	0.65
Davenport	354	6.51	0.68 ± 0.07	0.12 (0.06-0.21)	39.19 (13.7-170.6)	0.34	0.82
Cyantraniliprole							
Lab	260	6.09	0.66 ± 0.08	0.03 (0.01-0.67)	9.73 (30.4-55.83)	1	1
Venus	268	7.28	0.63 ± 0.07	0.23 (0.11-0.49)	96.44 (26.77-642)	7.67	9.91
Lake Alfred	282	2.84	0.52 ± 0.06	0.30 (0.13-0.67)	416.99 (93.04-4043)	10	42.85
Arcadia	350	9.97	0.37 ± 0.08	0.21 (0.01-2.34)	5682 (106-4.2×10^{9})	7	583.97
Davenport	350	5.85	0.42 ± 0.05	0.02 (0.08-0.48)	1698 (275.93-2855)	0.67	172.73
Spinetoram							
Lab	275	14.49	0.95 ± 0.19	0.27 (0.05-1.79)	14.27 (2.03-4528)	1	1
Venus	287	14.12	0.89 ± 0.17	0.87 (0.17-6.46)	61.05 (7.77-21554)	3.22	4.28
Lake Alfred	288	4.05	0.82 ± 0.09	0.14 (0.07-0.25)	13.50 (5.19-53.00)	0.52	0.94
Arcadia	355	4.68	0.87 ± 0.08	0.14 (0.08-0.23)	10.65 (4.65-33.10)	0.52	0.72
Winter Garden	350	16.55	0.66 ± 0.13	0.19 (0.03-1.55)	57.96 (4.86-83865)	0.70	4.06
Lakes Wales	280	7.32	0.70 ± 0.08	0.10 (0.05-0.20)	23.78 (7.73-113.10)	0.37	1.67
Clermont	351	7.53	0.79 ± 0.07	0.17 (0.10-0.29)	20.19 (8.25-68.77)	0.63	1.42
Davenport	352	5.98	0.70 ± 0.06	0.54 (0.30-1.00)	128.92 (46.3-226.8)	2	9.03

Resistance monitoring in 6 locations in Florida (LD95s) (Objective 1)

MOA	Class	Site of action
1	OPs & carbamate	AChE inhibitors Nerve action
3	pyrethroid	Sodium channel Nerve action
4A	Neonicoti-noid	Nicotinic AChR modulator Nerve action
4D	butenolide	Nicotinic AChR modulator Nerve action
28	diamide	Ryanodine receptor modulator nerves/muscles



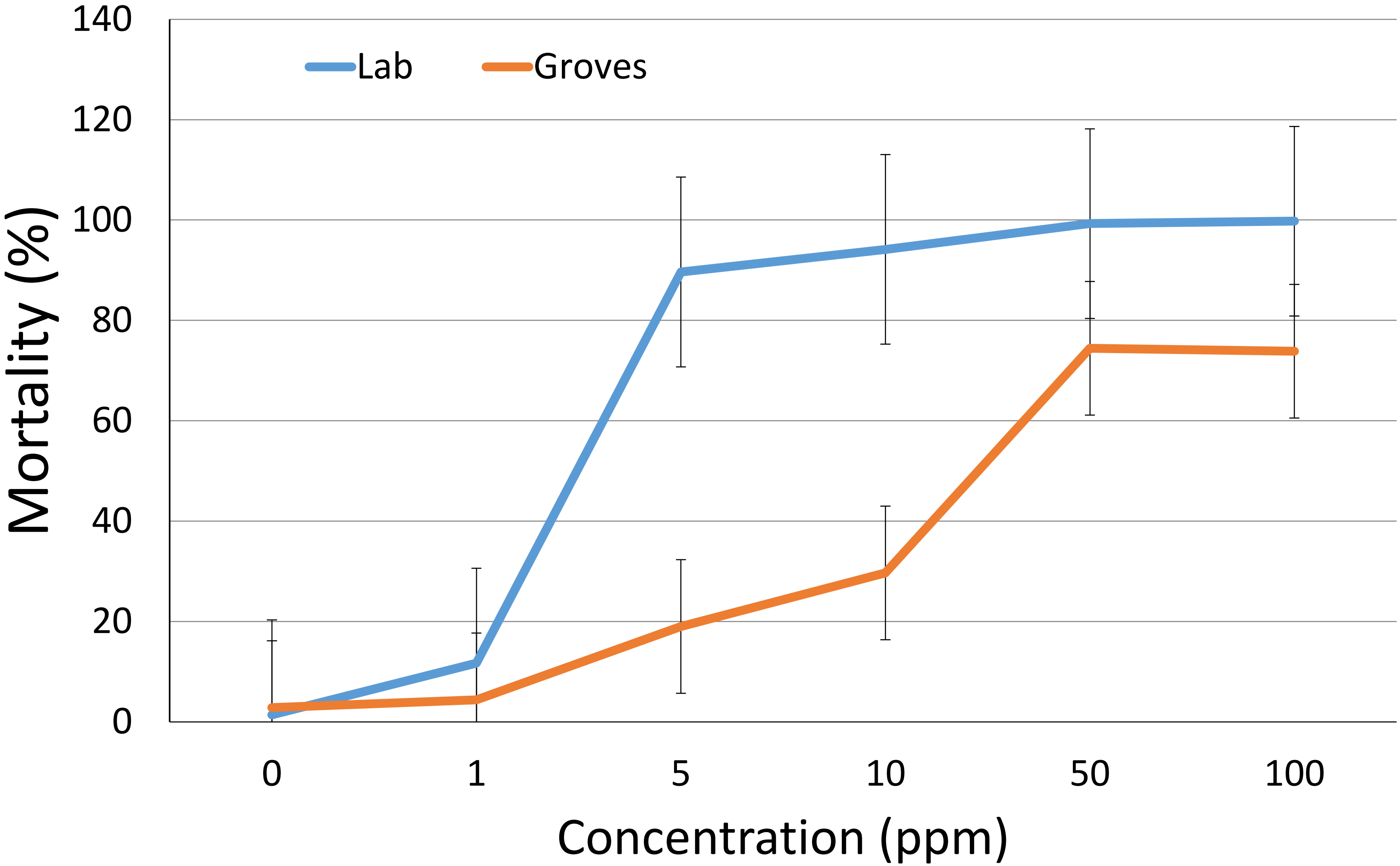


'17-'18 Resistance monitoring in Florida (LD95s) (Objective 1)

Location/Insecticide	n	χ^2	LD95
Imidacloprid			
Laboratory	404	36.53	27.59 (11.34-91.00)
Clermont	282	9.74	**676.05 (36-363445)**
Lake Alfred	405	4.11	**1108 (298-7005)**
Bifenthrin			
Laboratory	233	3.59	6.55 (2.41-29.43)
Clermont	313	4.43	3.55 (1.33-14.04)
Lake Alfred	267	5.94	3.17 (1.40-10.52)
- Dimethoate			
Laboratory	263	9.23	21.08 (3.20-1788)
Clermont	280	9.78	56.75 (8.51-4585)
Lake Alfred	264	4.70	**741 (150-8720)**

ACP Mortality rate at 6 concentrations of imidacloprid (ng/µl).

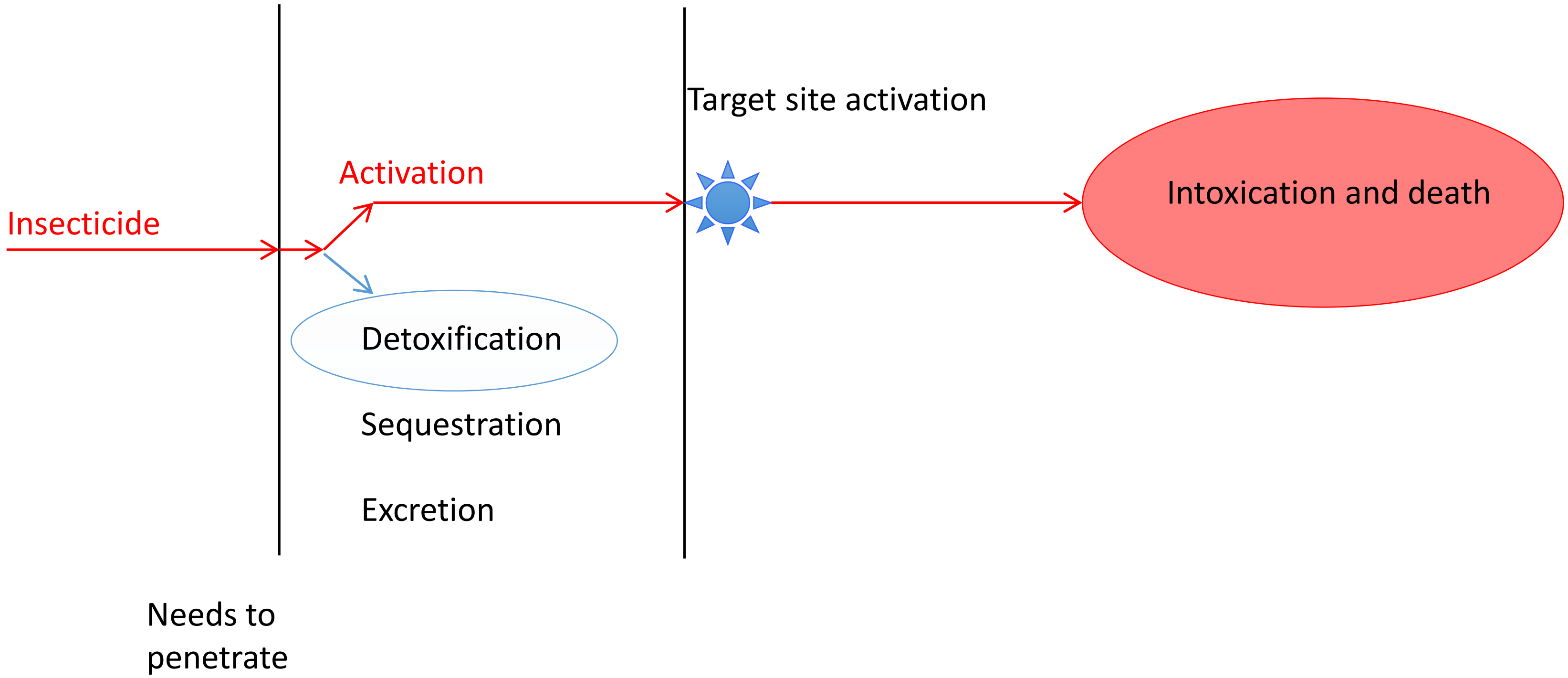
Southwest, FL Gulf Region-6 groves (Objective 1)



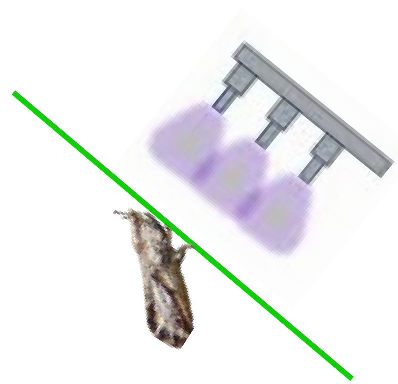
Mortality of *ACP* Adult field populations using Imidacloprid (99.9%) at 10 ng/µl (PPM) (Objective 1)

Susceptible Lab Colony			Locations	Field populations		
ACP total	Dead	Mortality (%)		ACP total	Dead	Mortality (%)
35	35	100	Immokalee North	35	20	57.1
30	30	100	Ave Maria	30	17	56.7
34	34	100	Immokalee South	34	19	55.9
35	35	100	F10- SWFREC	35	18	51.4
35	35	100	Labelle 1	34	17	50.0
25	23	92	F11- SWFREC	25	12	48.0
35	31	89	Felda	35	10	28.6
25	23	92	Labelle 2	25	7	28.0
33	33	100	SWFREC- Main Grove	34	9	26.5
30	30	100	Clewiston	30	7	23.3
25	14	56	Immokalee North	25	5	20.0
35	34	97	Devil's Garden	35	6	17.1
35	35	100	P-Block- SWFREC	105	8	7.6
412	392	95.2		482	155	32.2

Resistance mechanisms: How does an insect become resistant (Objective 2)?



Behavioral resistance-No current evidence



Increased Metabolism (Objective 2):

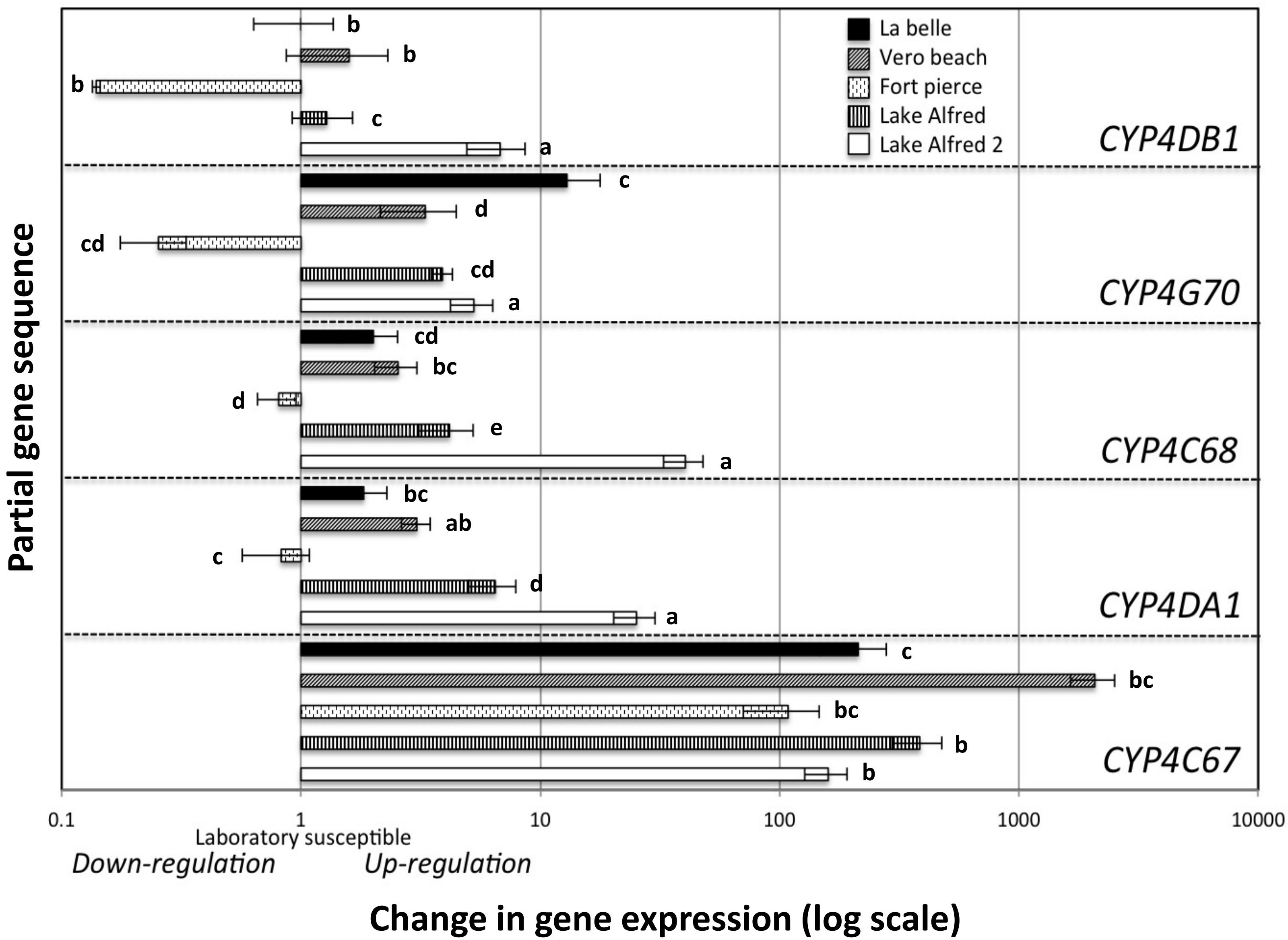
• The most common resistance mechanisms, called metabolic resistance. RR individuals have more enzymes or more efficient enzymes

• P450: Cytochrome P450 monooxygenases, involved in metabolism and resistance to all classes of insecticides

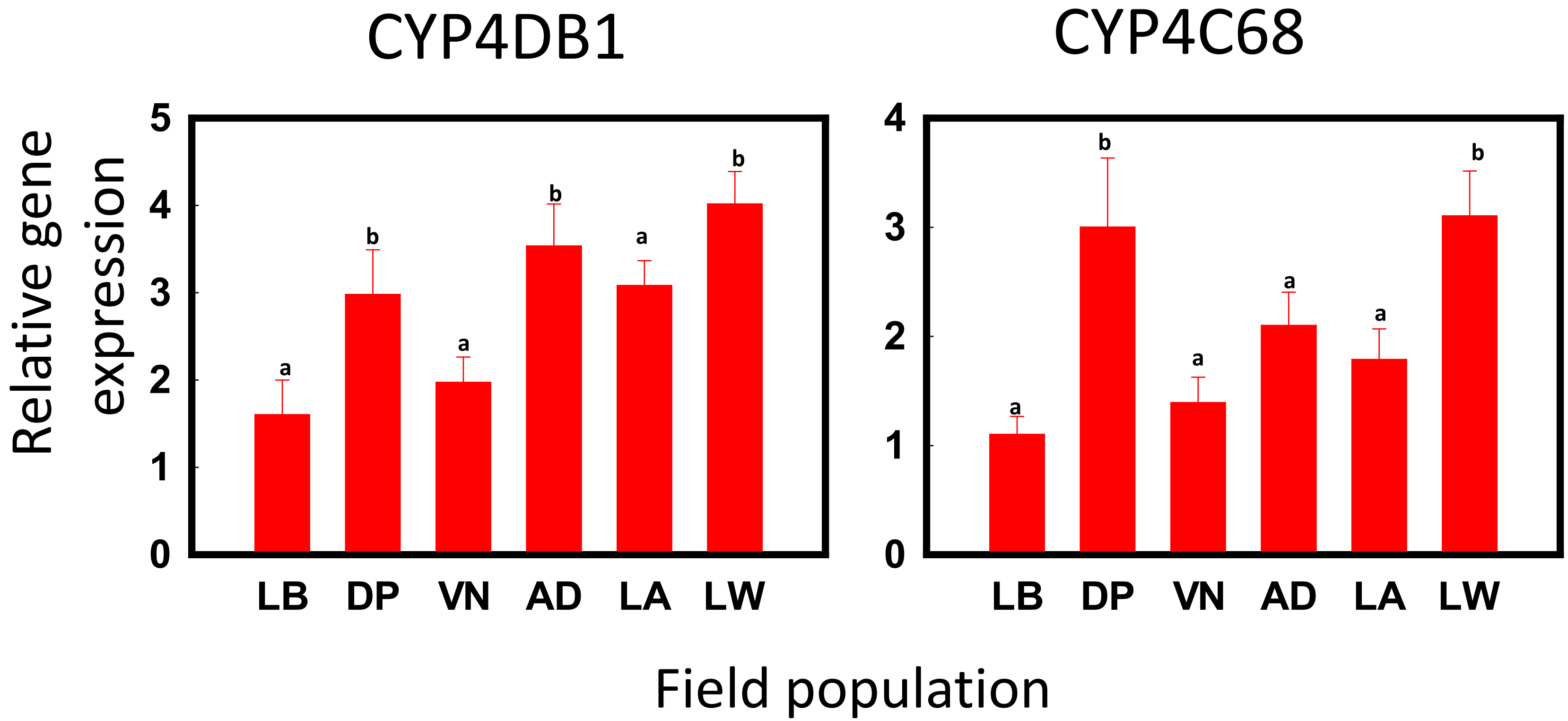
• Esterases: involved in resistance to organophosphates (OP), carbamates (Carb), and Pyrethroids

• GST: involved in resistance to DDT, OP and Py

CYP4 gene expression upregulated in resistant populations throughout Florida



Elevated CYP4 gene expression in resistant ACP (Objective 2)



Persistence of resistance (Objective 2):

- When resistance takes a while to develop, it suggests R alleles are initially rare

- The rarity of R alleles suggests it comes with reduced fitness

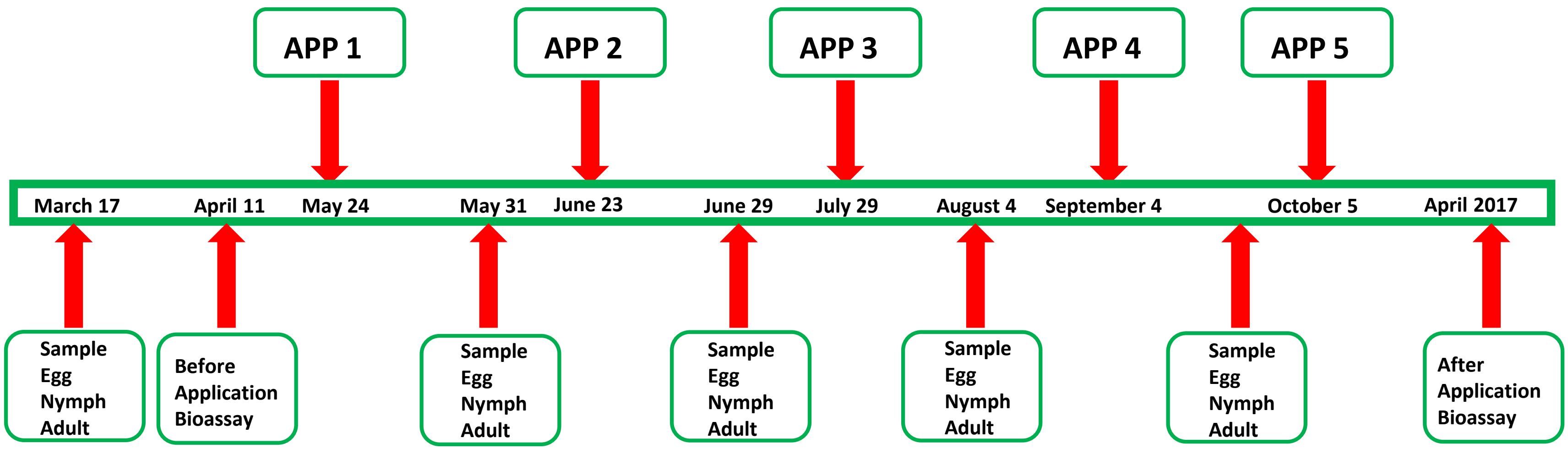
- Thus, R alleles should fall to a very low frequency without insecticide selection

- Is this the case with ACP?: YES

Fitness costs associated with resistance (Objective 2):

- Lay fewer eggs per female (decreased fecundity)
- Egg survival is lower (decreased fertility)
- Populations of resistant individuals are slower to increase due to reduced Net Reproductive Rate and reduced Finite rate of increase
- Resistant individuals are smaller and exhibit reduced maximal dispersal

Procedure for spray applications in rotation schedules (Objective 3, 4)



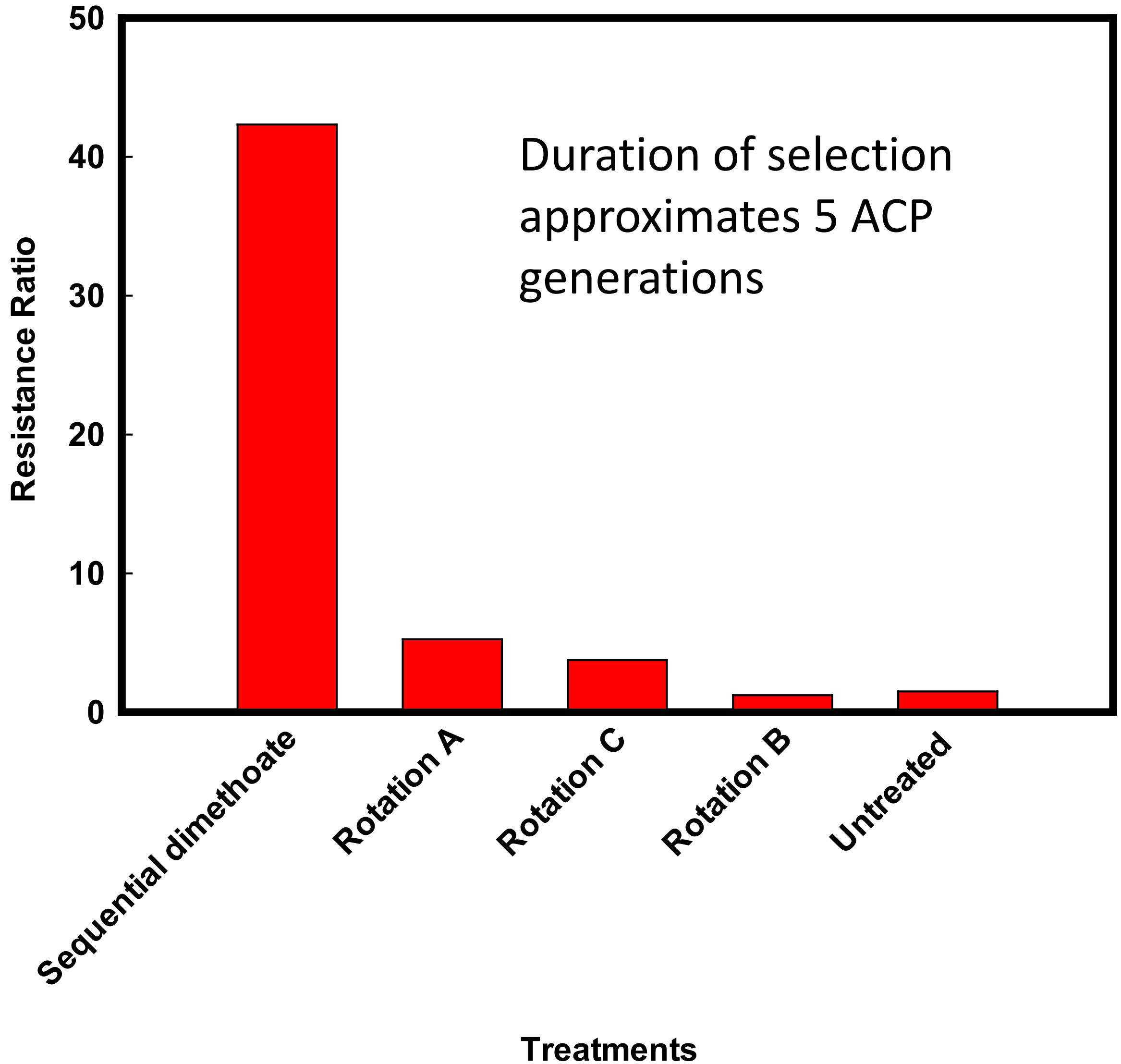
Adult were sampled by tapping method. Eggs and nymphs were ranked on flush collected in the field.

Rotation schedule treatments and dates of application

	Date of Application				
Treatment	**24 May**	**23 June**	**27 July**	**7 September**	**5 October**
Rotation A	**dimethoate**	**abamectin + thiamethoxam**	**fenpropathrin**	**diflubenzuron**	**imidacloprid**
Rotation B	**imidacloprid**	**fenpropathrin**	**abamectin + thiamethoxam**	**dimethoate**	**diflubenzuron**
Rotation C	**abamectin + thiamethoxam**	**diflubenzuron**	**dimethoate**	**imidacloprid**	**fenpropathrin**
Treated Control	**dimethoate**	**dimethoate**	**dimethoate**	**dimethoate**	**dimethoate**
Untreated Control	**-**	**-**	**-**	**-**	**-**

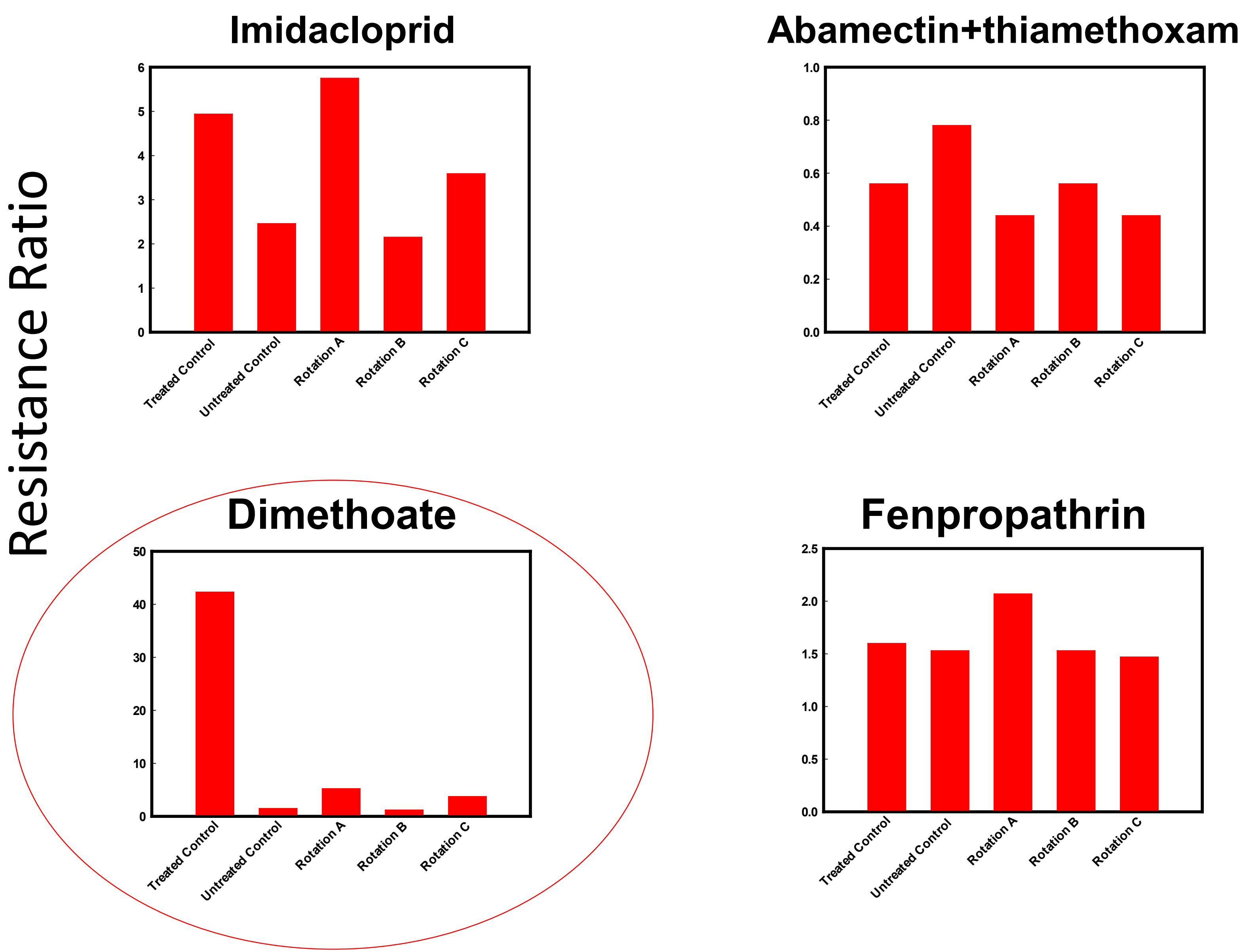
Rotation is effective- experimentally verified (Objective 4)

Susceptibility of ACP following 5 consecutive dimethoate sprays versus various insecticide rotations



Rotation A: organophosphate, microbial, synthetic pyrethroid, insect growth regulator and neonicotinoid;
Rotation B: neonicotinoid, synthetic pyrethroid, microbial, organophosphates and insect growth regulator;
Rotation C: microbial, insect growth regulator, organophosphate, neonicotinoid and synthetic pyrethroid;
Sequential dimethoate: 5 sprays with dimethoate (Resistance Ratio=LC50 of field Pop/LC50 of Laboratory Pop).

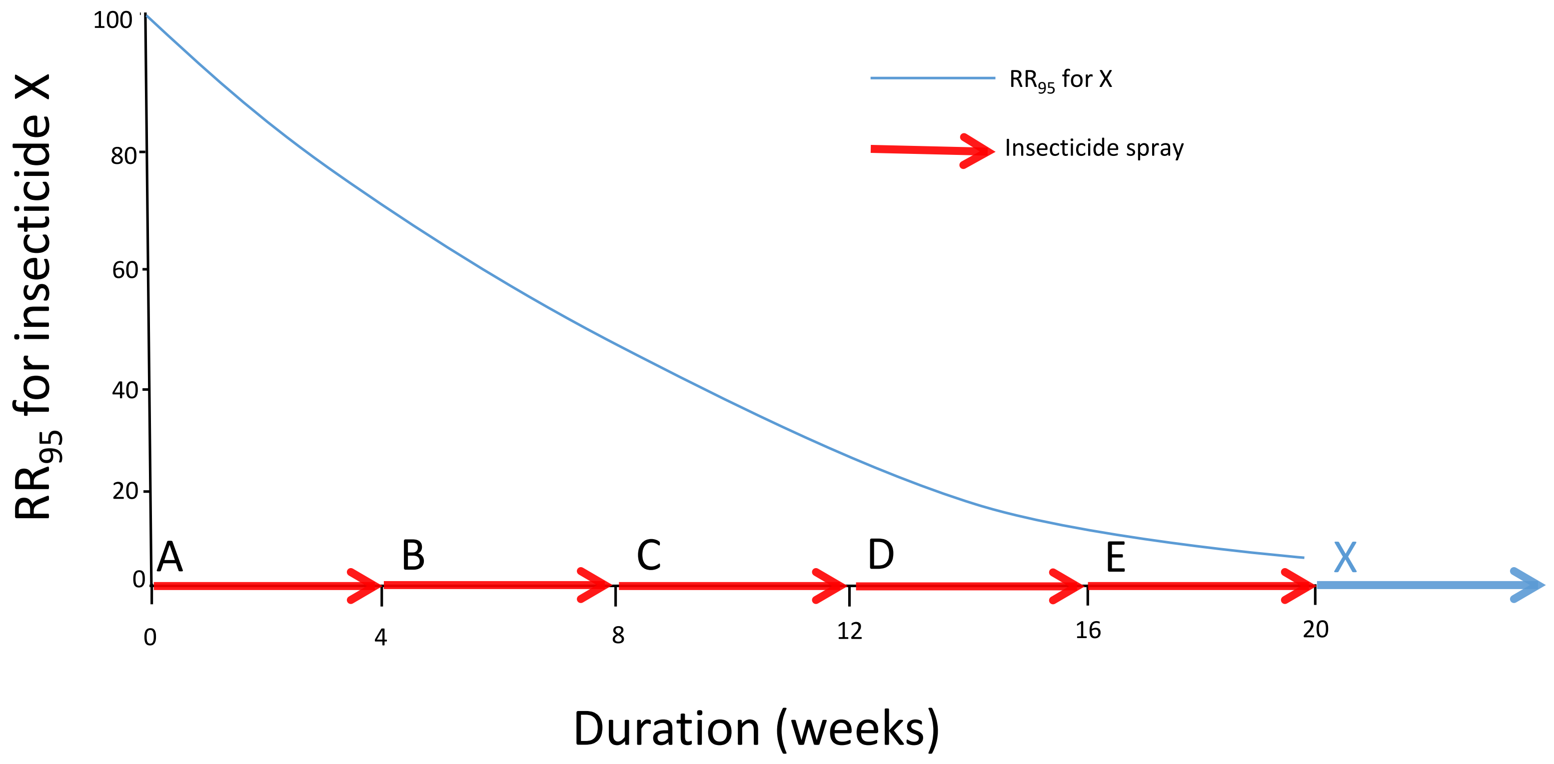
No resistance observed in cases of rotated MOAs where no selection pressure was applied



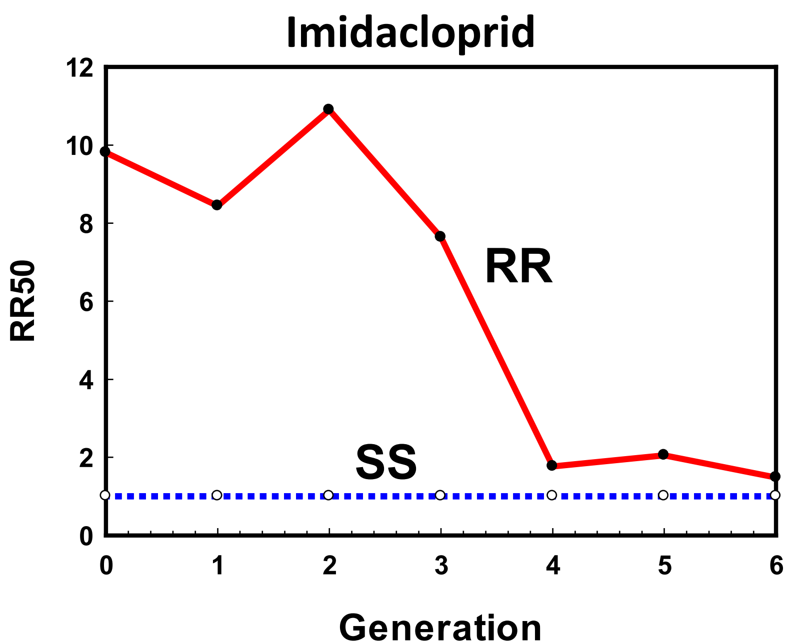
Rotating 5 modes of action in sequence cause reversal to susceptibility for over-selected MOAs (Objective 4)

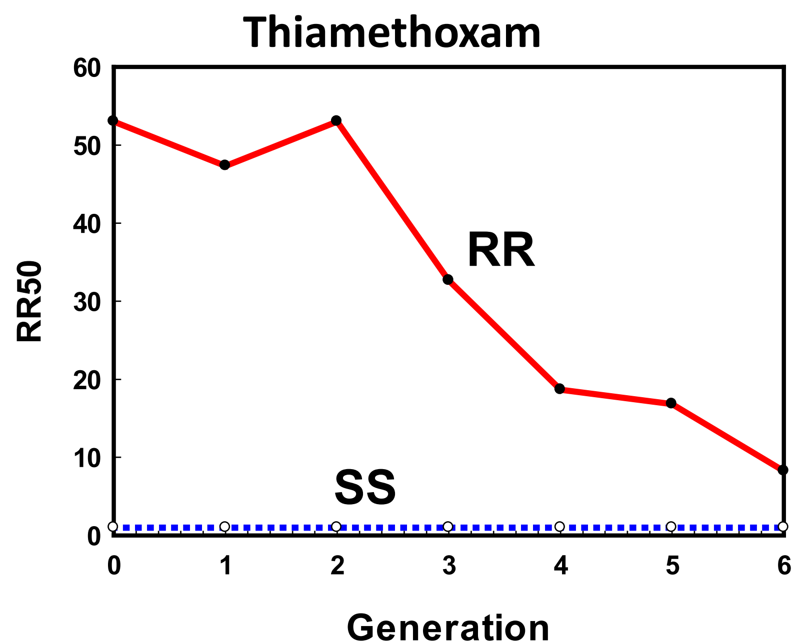
5 sequential MOAs-Protocol if known resistance to insecticide X

5 sprays every 4 weeks under ideal conditions is close to 5 ACP generations



Response of four field-sampled populations of ACP in Florida to imidacloprid and thiamethoxam after various intervals without exposure under laboratory rearing conditions (Objective 4).

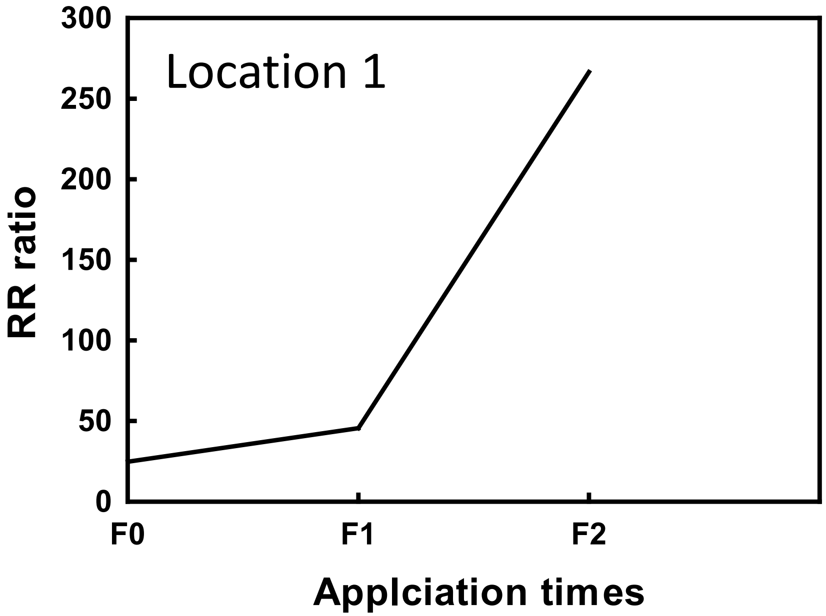




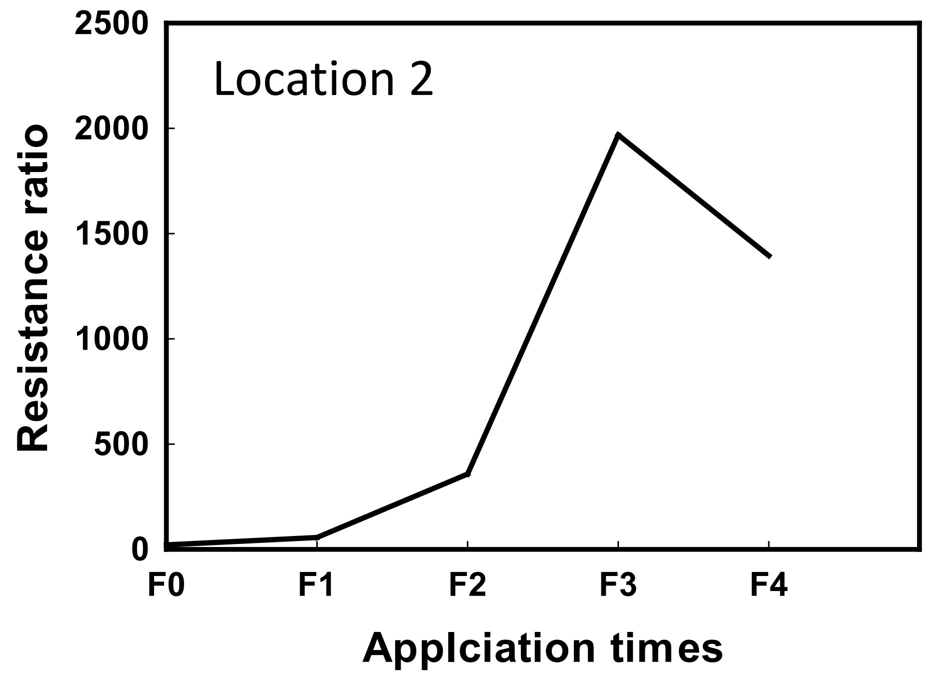
Questions that come up during the course of investigation— Is there a 'best way' when starting with a known problem with neonicotinoids?

Treatment	Selection 1	Selection 2	Selection 3	Selection 4
	Mar 24, 2019 Apr 22, 2019	May 5, 2019 May 31,2019	Jun 10, 2019 Jun 28, 2019	Jun 22, 2019 -----------------
Rotation 1	dimethoate	cyantrniliprole	fenpropathrin	diflubenzuron
Rotation 2	fenpropathrin	dimethoate	cyantrniliprole	imidacloprid
Rotation 3	thiamethoxam	clothianidin	thiamethoxam	imidacloprid

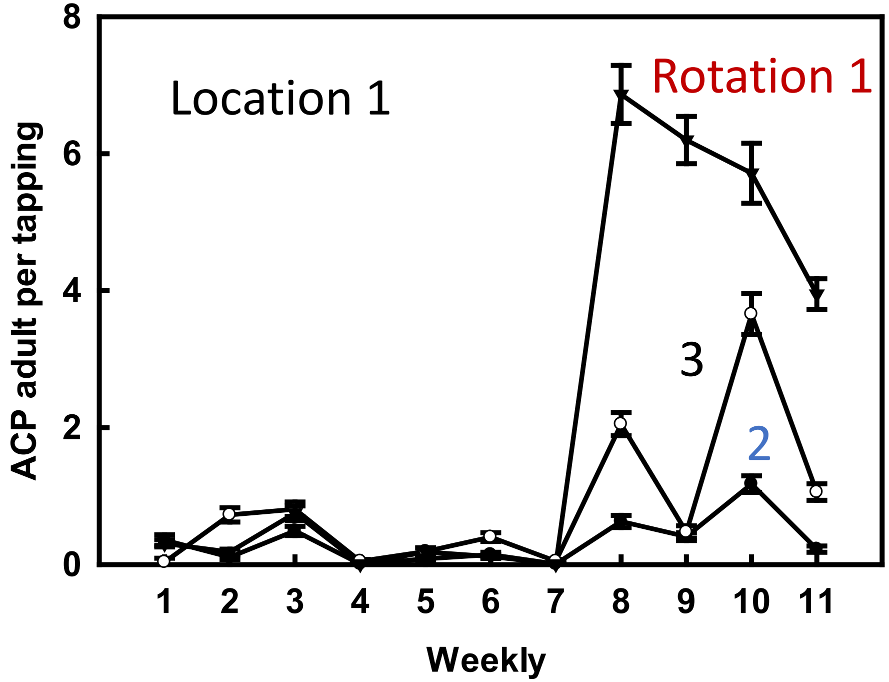
Resistance shows up fast!



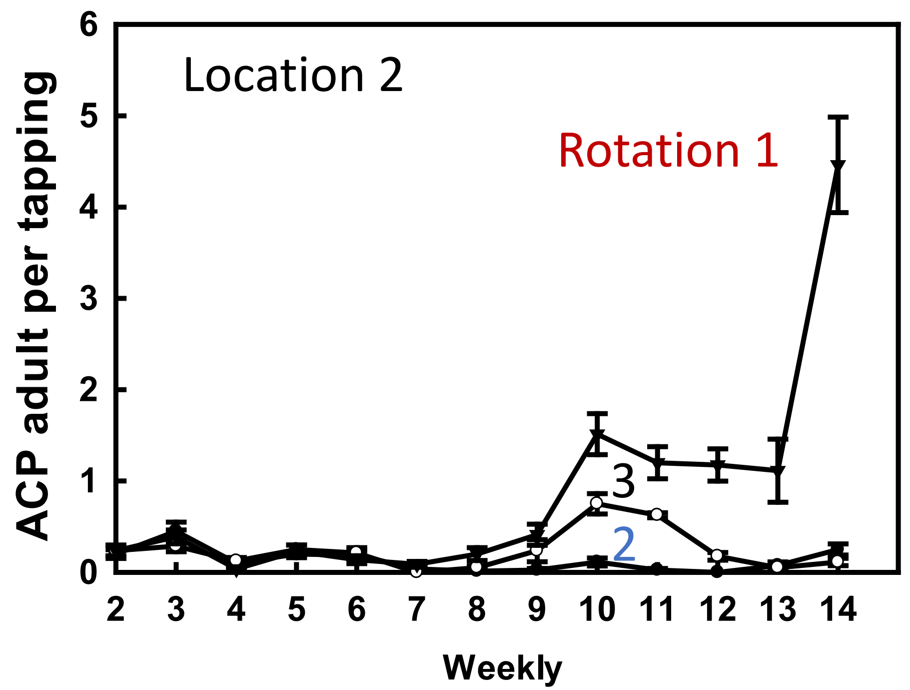
We observed 200-500 fold resistance with 2 back-to backs; ~2000 fold after 3 consecutive failures to rotate



Once we start to see > 100 fold resistance in the lab, failures in the field are evident (Objective 3).

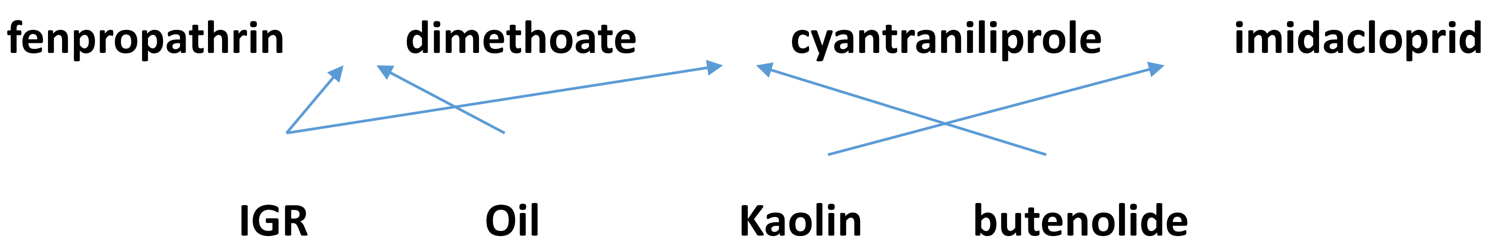


Some rotations emerging as superior in terms of population suppression and resistance management interaction



Best rotation when starting with neonic. resistance

Treatment	1	2	3	4	5
Rotation A	dimethoate	abamectin + thiamethoxam	fenpropathrin	diflubenzuron	imidacloprid
Rotation B	abamectin + thiamethoxam	diflubenzuron	dimethoate	imidacloprid	fenpropathrin
Rotation C	fenpropathrin	dimethoate	cyantrniliprole	imidacloprid	diflubenzuron



Responses of four field-sampled neonic. resistant populations of ACP to imidaclopride and thiamethoxam after various intervals under proper rotation (Objective 4).

Generation	Clermont		Lake Alfred		Ft Pierce		Davenport	
	LC50	R Value	LC50	R Value	LC50	R Value	LC50	R Value
Imidacloprid								
***Lab control value**	0.13		0.13		0.13		0.13	
Day 0 resistant	1.20	9.23	1.33	10.2	1.67	12.85	0.90	6.92
Spray 1	1.14	8.77	1.48	11.4	0.84	6.46	0.93	7.15
Spray 2	1.44	11.1	1.73	13.3	-	-	1.08	8.30
Spray 3	0.75	5.77	1.18	9.07	-	-	1.05	8.08
Spray 4	0.23	1.76	0.32	0.77	0.32	2.46	0.27	2.08
Spray 5	-	------	0.17	0.46	0.23	1.77	0.51	3.92
Spray 1	0.17	1.30	0.11	0.85	0.27	2.08	0.22	1.69
Thiamethoxam								
***Lab control value**	0.02		0.02		0.02		0.02	
Day 0 resistant	1.11	55.5	1.15	57.5	0.80	40	1.18	59.0
Spray 1	0.98	49.0	-	------	0.84	42	1.02	51.0
Spray 2	1.07	53.5	0.94	47.0	1.01	50.5	1.22	61.0
Spray 3	1.03	51.5	-	------	0.53	26.5	0.71	20.0
Spray 4	0.31	15.5	0.41	20.5	-	-	0.40	20.0
Spray 5	0.40	20.0	0.31	15.5	-	-	0.30	15.0
Spray 1	0.23	11.5	0.07	3.50	0.14	7	0.22	11.0

Conclusions:

- ACP in Florida are insecticide resistant in numerous locations and regions; primarily to the neonics. *It's sporadic*

- Soil application of neonicotinoids causes persistent sub-lethal dosages of active ingredient in plants that exacerbates resistance

- Insecticide resistance can be effectively managed by rotating 5 modes of action in sequence

- The sequence in which you apply MOAs in a rotation does not affect IRM of ACP; except if you start with a neonic. resistant population

- Six ACP generations are required to for reversal of insecticide-resistant populations to susceptibility; approximate field time of 20 weeks

The best solution: Integrated Pest management (IPM)

- Combination of cultural, chemical and biological control tactics
- Diversifying selection forces and complicating adaptation
- Resistance to one tactics can be compensated for by other tactics

--But, as mentioned earlier, we don't yet have a full proof IPM system that prevents spread of HLB. So, we need to take what we've learned from the field and combine it with the current body of knowledge to manage resistance. And, there is plenty of evidence that we are able to do it.