

Surveillance of Permethrin Resistance in Valdosta populations of *Aedes albopictus*

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Mosquito
Surveillance

Disease
Surveillance

Population
Control

Resistance
Monitoring

Legislature

Public Education
and Community
Outreach

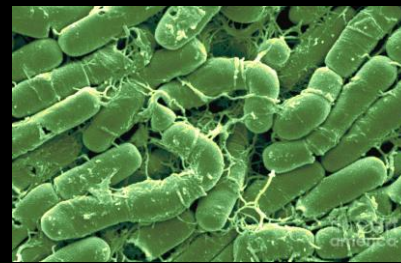
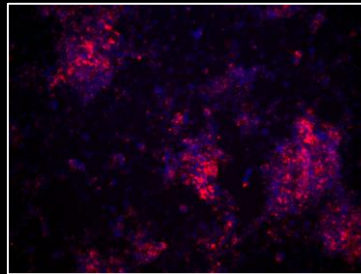
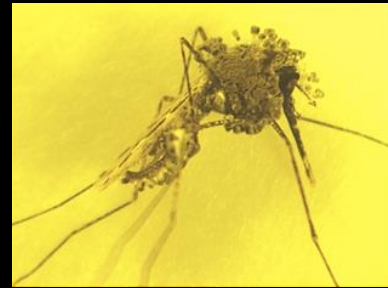
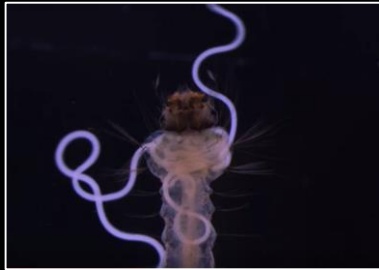
Integrated Mosquito Management

Population Control Strategies

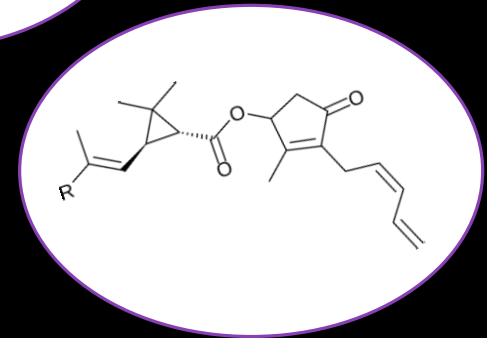
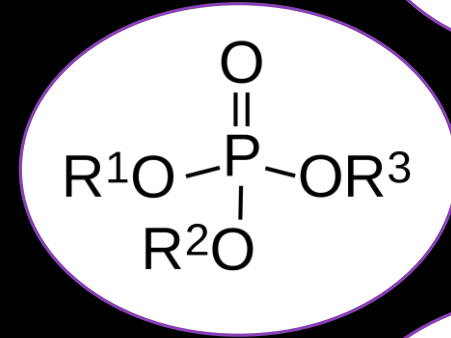
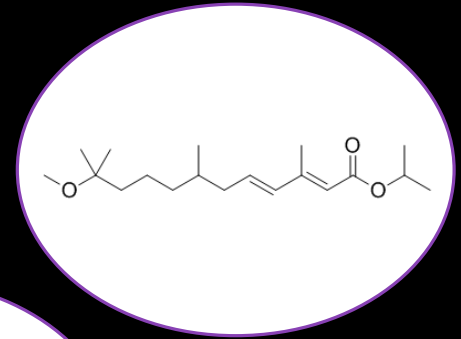
Physical



Biological



Chemical



Mechanisms of Resistance

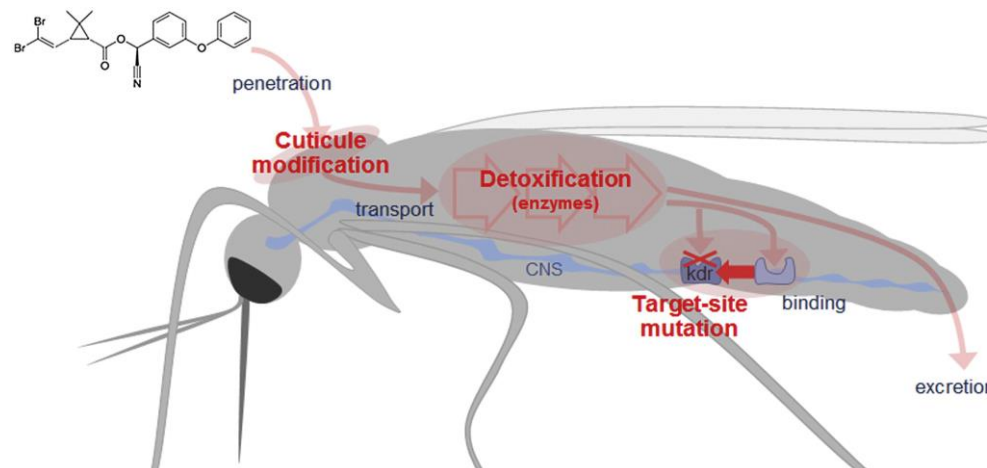
Behavioral: changes of action/habit that prevent exposure to insecticide

- exophilic (outdoor dwelling) strains avoid exposure to insecticides distributed by indoor residual spraying

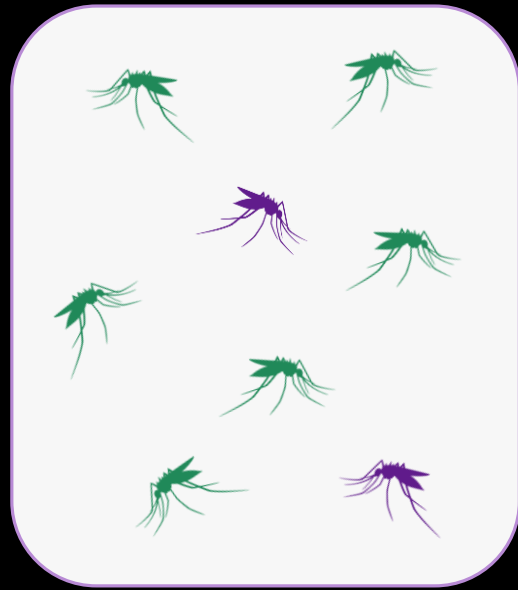
Physiological: better accommodation of the insecticide through functional changes of body parts

- increased detoxification through the possession of higher levels or more efficient forms of enzymes (metabolic)
- reduced neuronal sensitivity to insecticides (KDR)
- reduced penetration across the cuticle due to structural or compositional modifications
- increased excretion or sequestration of insecticide

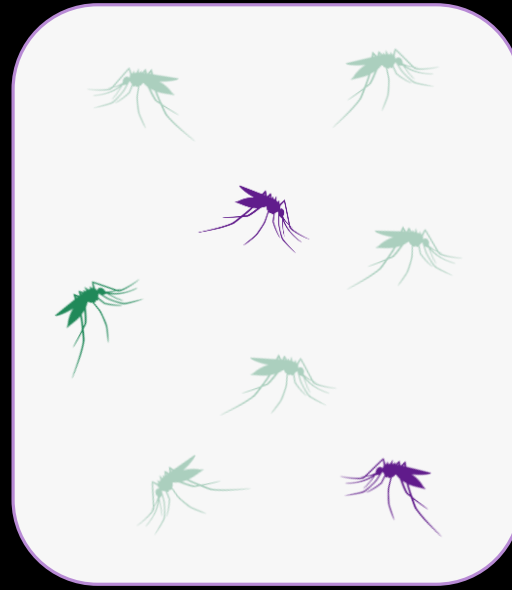
Cross-resistance: the resistance of one insecticide leading to resistance of a different unused insecticide with a similar mode of action



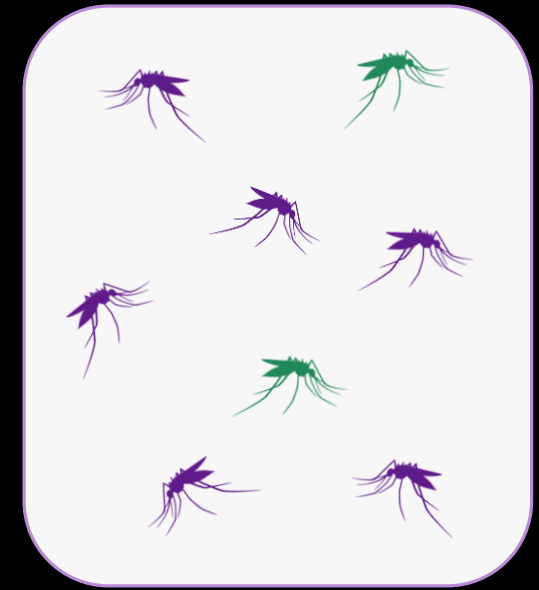
Artificial Selection of Insecticide Resistant Mosquitoes



Exposure to
insecticide



Survivors
reproduce



A few mosquitoes have
insecticide **resistant alleles**.
Most mosquitoes have **non-
resistant (susceptible) alleles**.

Mosquitoes with **resistant
alleles** have **greater
survivorship** when exposed
to the insecticide.

Since surviving mosquitoes have a
higher tendency to reproduce,
majority of the population now
consists of offspring with **resistant
alleles**.

Reproductive Cycle

Egg

- can remain dormant for months until optimal conditions arise



Larva

- develop into pupa in as little as 5 days



Adult

- lays eggs 4-5 days after blood meal



Pupa

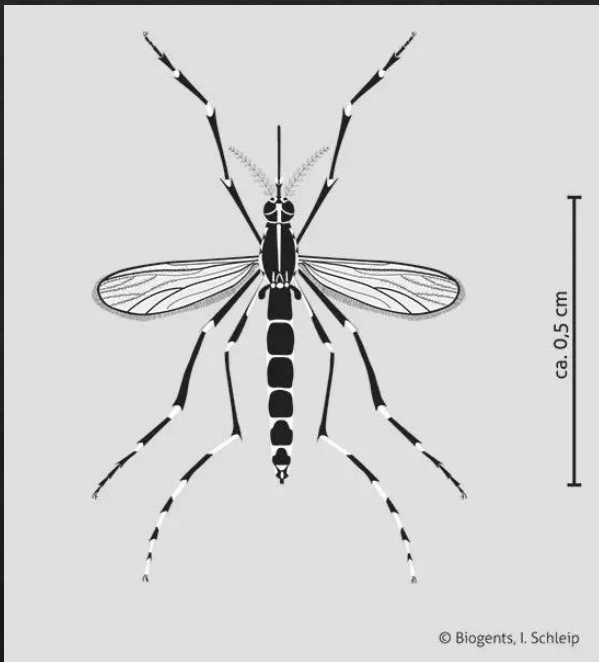
- develop into adults within 2-3 days



Objectives



- To provide baseline data of local (Valdosta) permethrin resistance in *Aedes albopictus*
- To detect presence of knockdown resistance mutations (V1016G and F1534C)



Species Description

- Originated in tropical forests of South-East Asia
First discovered in the US (Texas) in 1985
- Overwinter in the egg stage in temperate climates
- Dark colored mosquito with a silvery-white scale line extending medially on scutum and banded legs
- Short flight range; <200m from its breeding site
- Has tested positive for arboviruses such as Zika, Chikungunya, Dengue, Yellow Fever, West Nile, and Eastern equine encephalitis among others
- Potential bridge vector as it feeds during daylight hours on a variety of vertebrates (including humans)

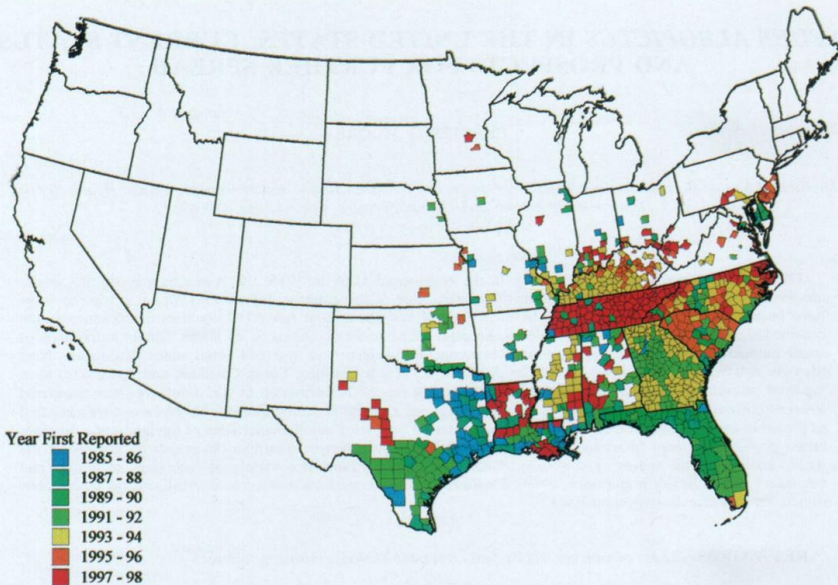















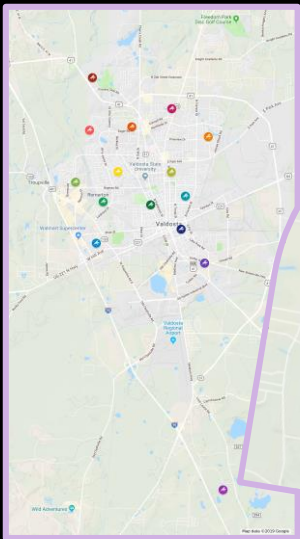
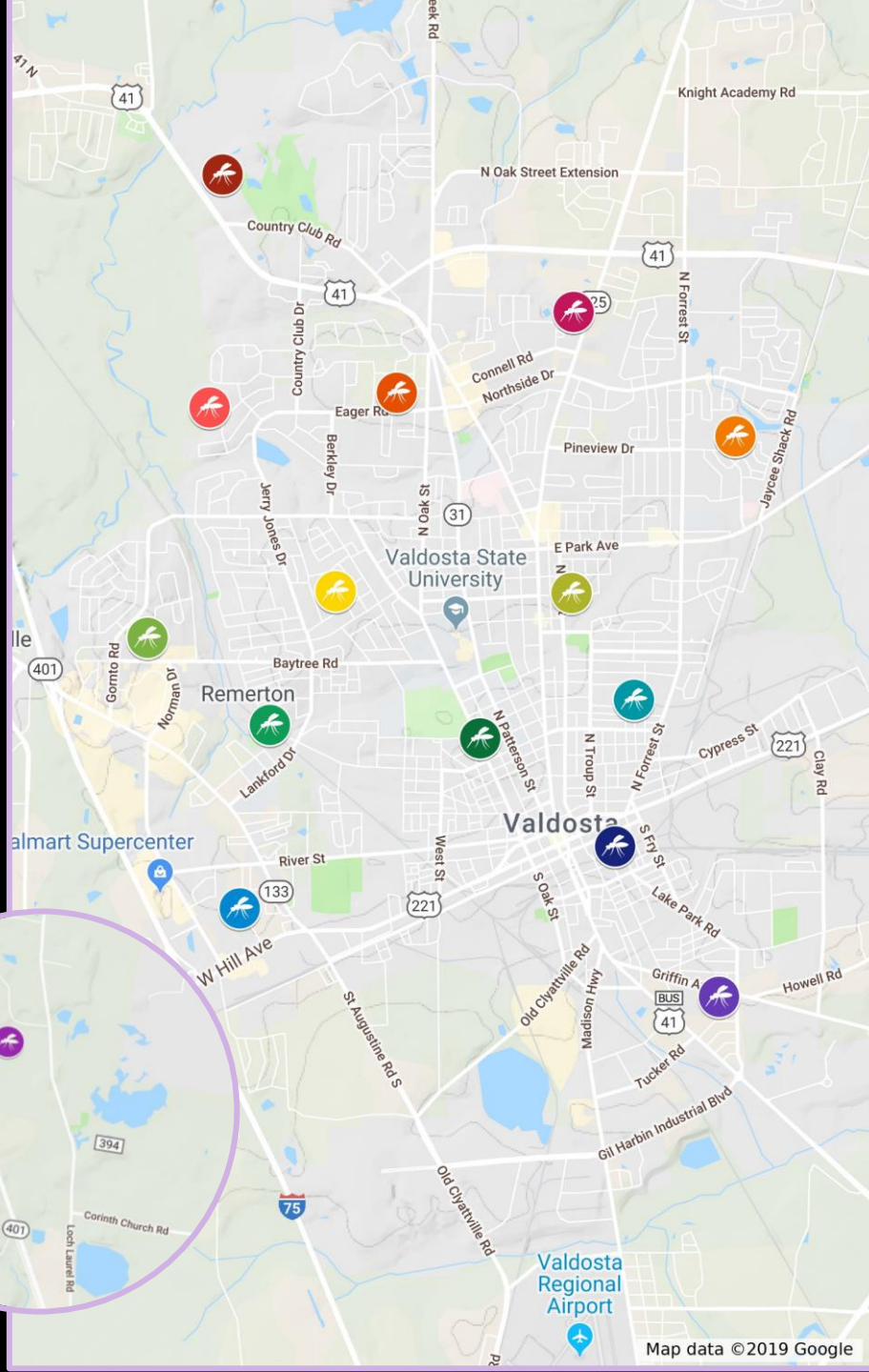


Fig. 1. U.S. counties that have reported *Aedes albopictus* infestations, by year of discovery. Two-year classes were used to simplify the map.

Egg Collection Sites

Collection Site Addresses

-  3320 Plantation Dr
-  3019 Kensington Ln
-  1201 Ravenwood Cir
-  313 Simpson Pl
-  1786 Jimmy Carter Dr
-  800-800-A E Park Ave
-  1700 Charlton St
-  1574 Baytree Rd
-  1739 W Gordon St
-  1007 N Oak St
-  1017 Myrtle St
-  204 Nan St
-  410 Wooding Ln
-  602 Conoley Ave
-  Lake Louise Field Station



Black 9 Oz cups fitted with:

- Hay-infused gravid water filled to ~1/2" from rim
- Textured paper cut to 2" X 9"
- Standard size wooden clothespin



1



2



3



4



5



6



7



8



9



10



11



12



13



14



15

Rearing

- Rearing container (14.00"L x 8.00"W x 4.88"H) filled with 1L distilled water and 2mL of bovine liver solution (40g/500mL)
- Egg collection papers gently rinsed of dirt and debris before placing into rearing container
- Container left at room temperature and exposed to a natural day/night cycle
- Mosquitoes transferred as pupa to a holding cage with a cotton ball doused in 10% sucrose solution
- Holding cage placed in an incubator set to 25°C with a 12-hour day/night cycle



CDC Bottle Bioassay Kit



“The **diagnostic dose** is a dose of insecticide that kills 100% of susceptible mosquitoes within a given time.

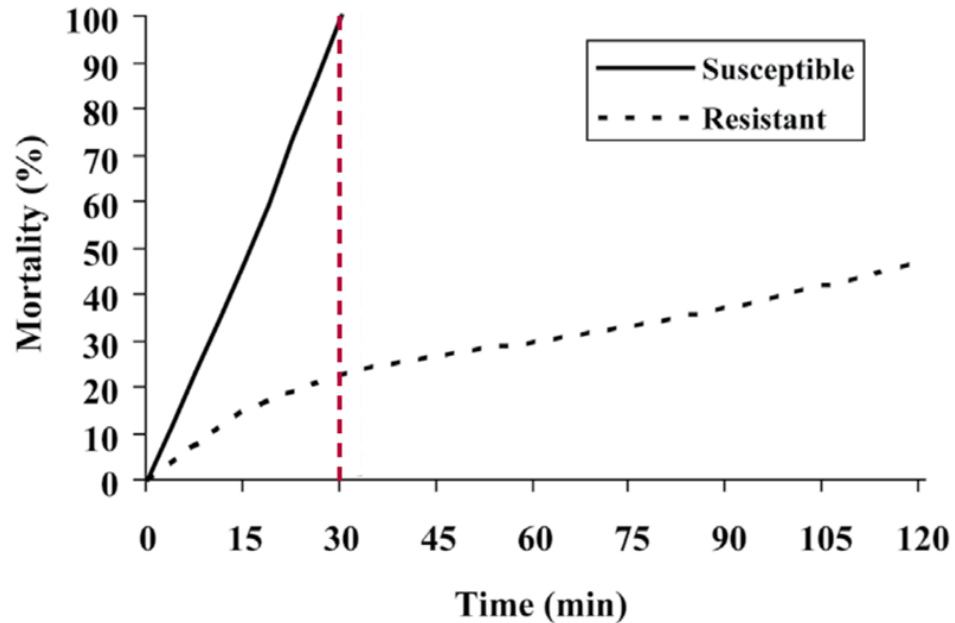
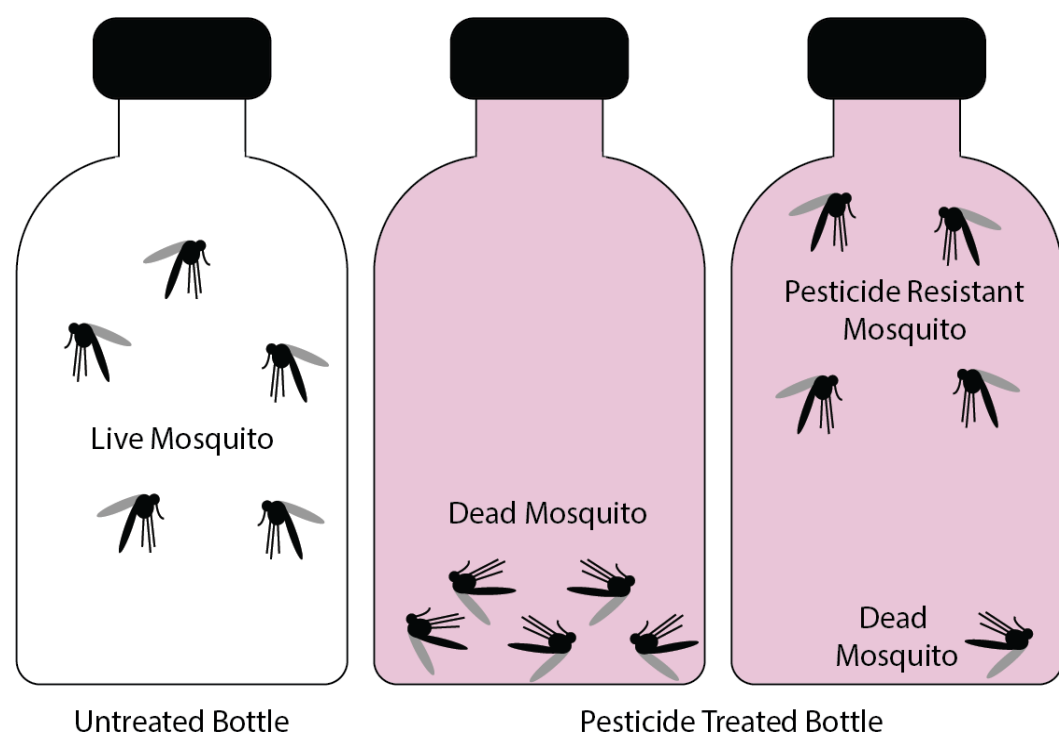
The expected time for the insecticide to achieve this objective is called the **diagnostic time.**”

Insecticide					Insecticide concentration (µg/bottle)	Diagnostic time (min)
Common name	Trade name	Chemical name	IRAC Classification	Type		<i>Aedes albopictus</i>
Permethrin	DeLice	3-phenoxybenzyl (1RS)-cis, trans-3-(2,2- dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate	3A	Pyrethroid	43	10

WHO recommendations for assessing the significance of detected resistance

- 97%–100% mortality indicates susceptibility
- 90%–96% mortality suggests the possibility of resistance that needs to be confirmed
- <90% mortality suggests resistance

Note: Where <95% mortality occurs in bioassays conducted that under optimum conditions and with a sample size of >100 mosquitoes, then resistance can be strongly suspected.


















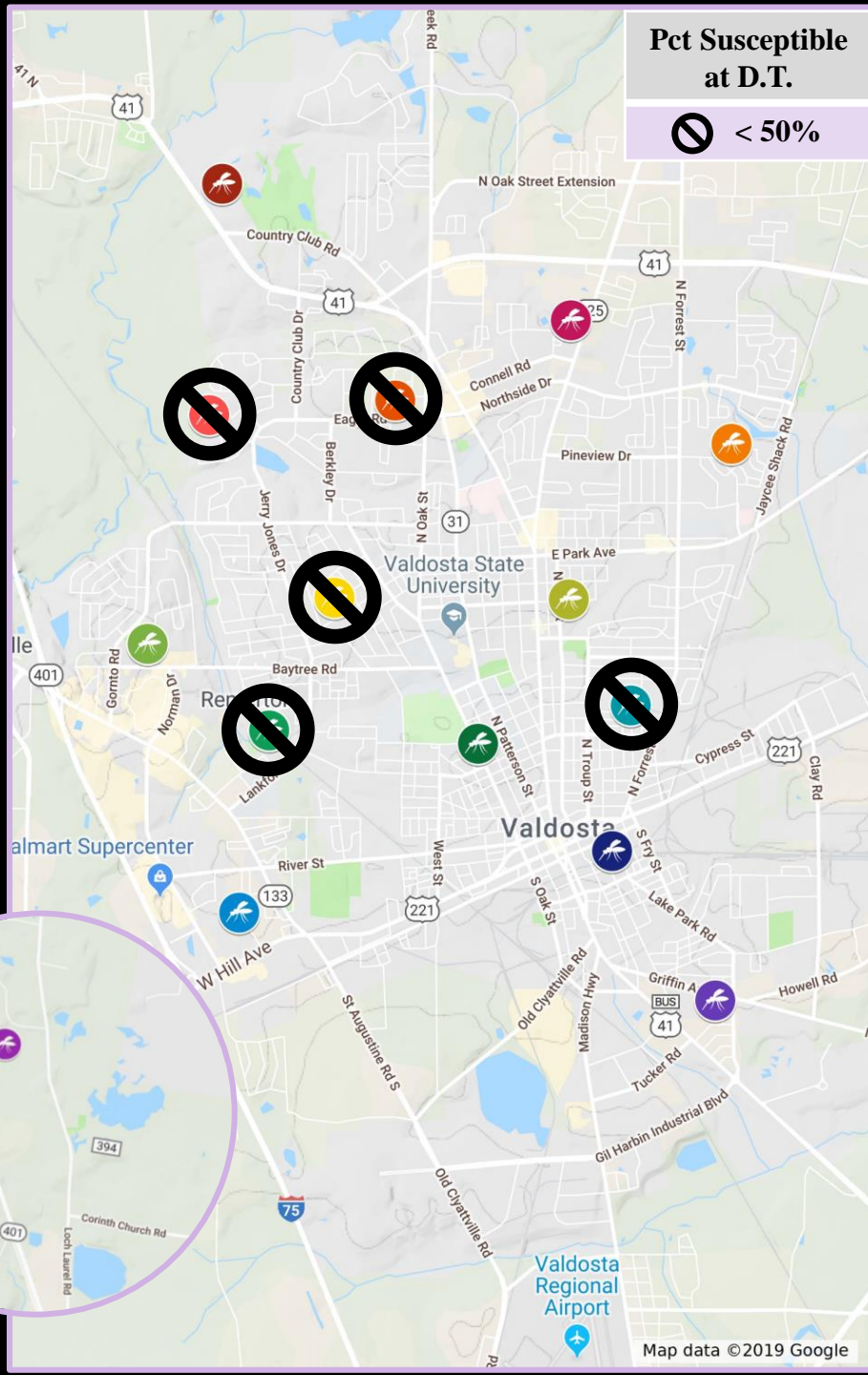
	Site Number															
Time Elapsed (min)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
5	18	2	10	4	23	2	10	4	8	18	0	5	21	2	16	0
10	43	40	56	5	65	19	48	42	23	5	3	20	11	28	14	14
15	17	8	41	10	25	11	35	16	35	14	3	5	12	7	12	14
20	7	5	27	17	23	40	10	5	9	2	1	4	4	2	2	10
25	5	4	14	1	3	7	2	13	4						3	4
30	4	1	4	12	5	4	1	0	2						0	3
35	1	1	0	3	2	3	0	2	1						1	1
40		0	1	0		0	1	0	1							0
45		1	0	1		2		0	1							0
50		0	0					0								2
55		0	0					1								1
60		1	0													1
65			1													0
70			0													1
75			0													
80			0													
85			1													
Site Count	95	63	155	53	146	88	107	83	84	39	7	34	48	39	48	51
Total Count	1140															

Pct Susceptible at D.T.
< 50%
≥ 50% – 59%
≥ 60%

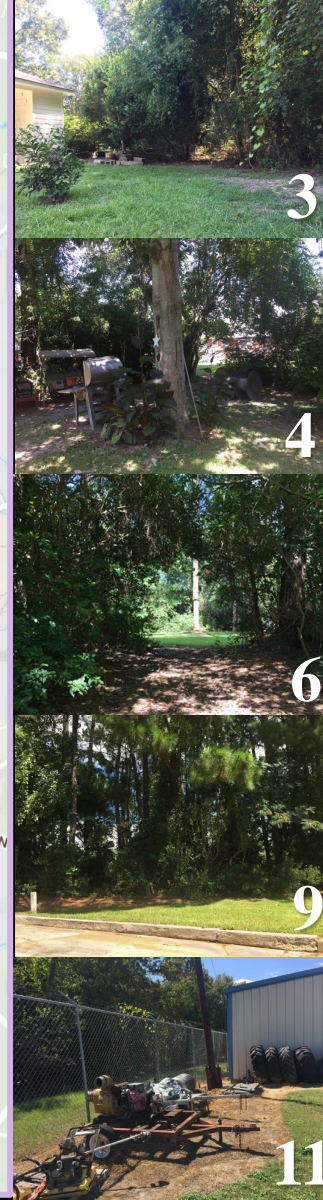
Table showing site counts of tested *A. albopictus* and distribution of mortality over 5-minute intervals

Collection Site Addresses

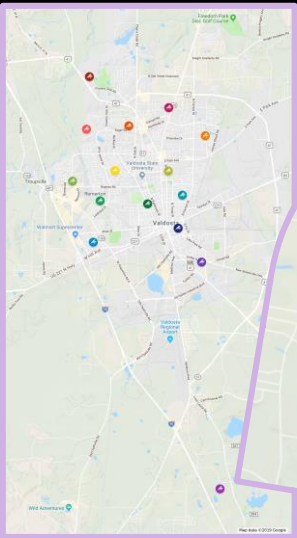
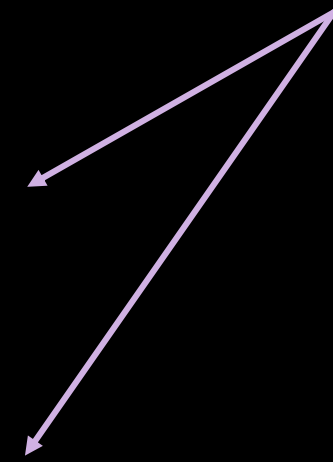
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Egg Collection Sites

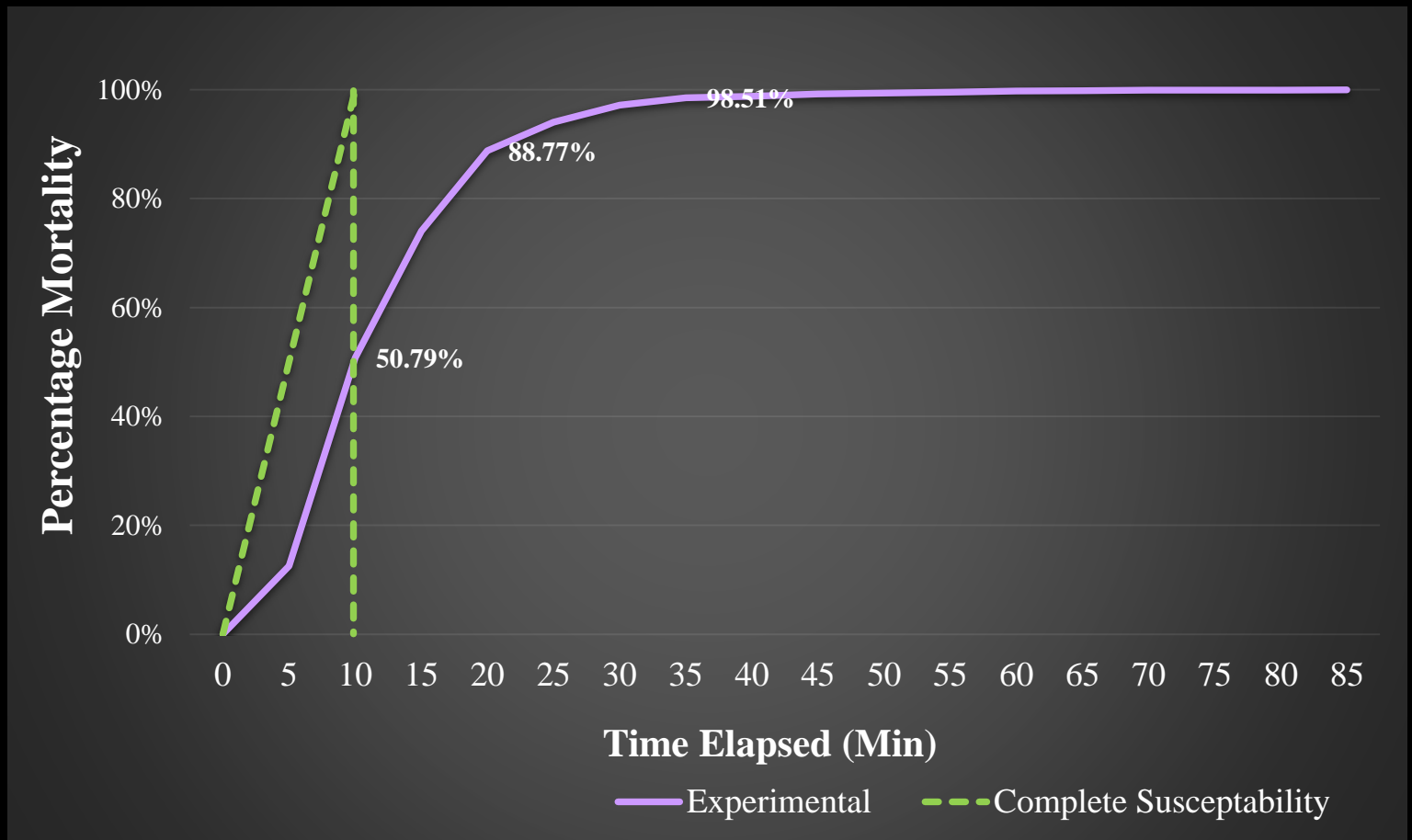


Both non-residential sites tested for less than 50% susceptibility at the diagnostic time.



Time Elapsed (min)	No. Mosquitoes	Pct. Mosquitoes
5	143	12.54%
10	436	38.25%
15	265	23.25%
20	168	14.74%
25	60	5.26%
30	36	3.16%
35	15	1.32%
40	3	0.26%
45	5	0.44%
50	2	0.18%
55	2	0.18%
60	2	0.18%
65	1	0.09%
70	1	0.09%
75	0	0.00%
80	0	0.00%
85	1	0.09%

Table showing *A. albopictus* mortality counts and percentages among all collection sites

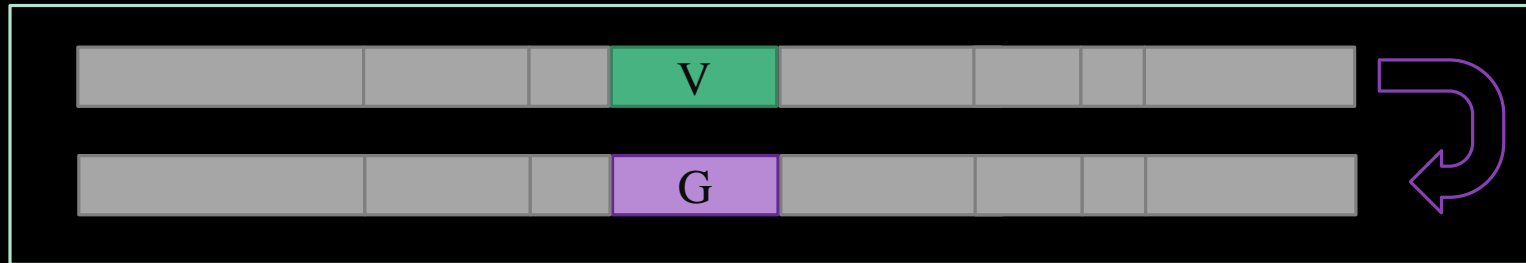


Graph showing experimental rate of mortality versus theoretical rate of mortality for completely susceptible *A. albopictus*

KDR Mutations

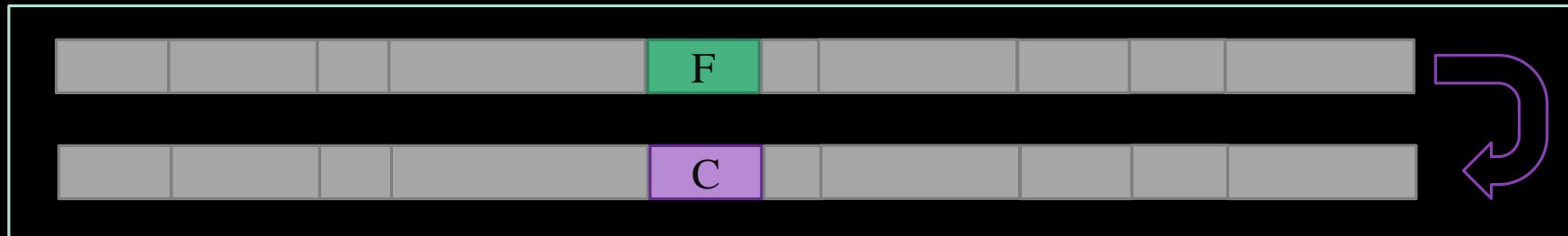
V1016G mutation: domain II of VGSC, voltage gated sodium channel gene, undergoes a valine to glycine substitution

- associated with resistance to type I and II pyrethroids



F1534C mutation: domain III at this position undergoes a phenylalanine to cysteine substitution

- associated with resistance to type I pyrethroids



Multiplex PCR: Detection of KDR Alleles

AS-PCR: allele-specific polymerase chain reaction

Possible Genotypes at 1016

VV: homozygous susceptibility

VG: heterozygous

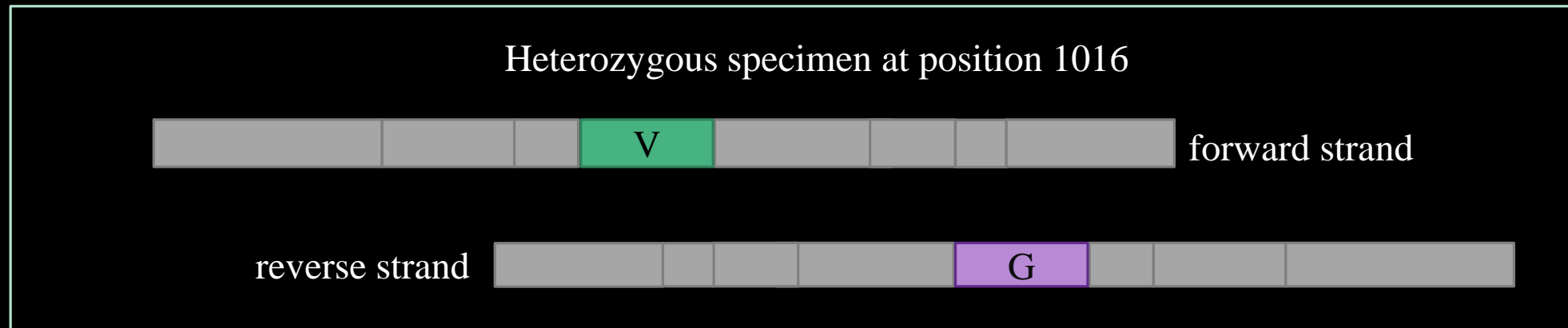
GG: homozygous resistance

Possible Genotypes at 1534

FF: homozygous susceptibility

FC: heterozygous

CC: homozygous resistance



Genotyping results amplified by **gel electrophoresis**

Questions?

