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Development of a Black Fly Repellent Testing Protocol

Skyler Magnus Kerr, M.S.
Elmer Gray, M.S.
Darold Batzer, PhD
UGA Black Fly Research and Resource Center

Road Map

- Brief over-view of attraction cues
- Repellent selection
- Black Fly Repellent Testing System
- Development cycle + data

Host Attraction Cues

- Seven major influences on host location, four used in this study (1)
- Visual: Tall, dark objects are common attractants. Silhouettes of host influence attraction, as do color/shape/size. Visual cues are more important as flies approach host while chemical cues are more useful further away. (2, 3)
- Thermal: Temperature of host skin. Higher differentials in air vs membrane temperatures increased biting rates in lab (differential ≥14°C). (4)
- Chemical: Breath - primarily CO2. Other breath and body odors add significant attraction but are not fully understood (acetone/octenol in breath, compounds in sweat). Due to differences in CO2 exhalation, certain individuals are more attractive than others.(5)

Host Attraction Cues Utilized in Study

- Visual: Limit light in study. Only visible light in study is through the membrane (positive phototaxis).
- Thermal: Warm circulating water.
- Gustatory and olfaction: Sucrose through membrane, after piercing of membrane

Repellent Selection for Testing

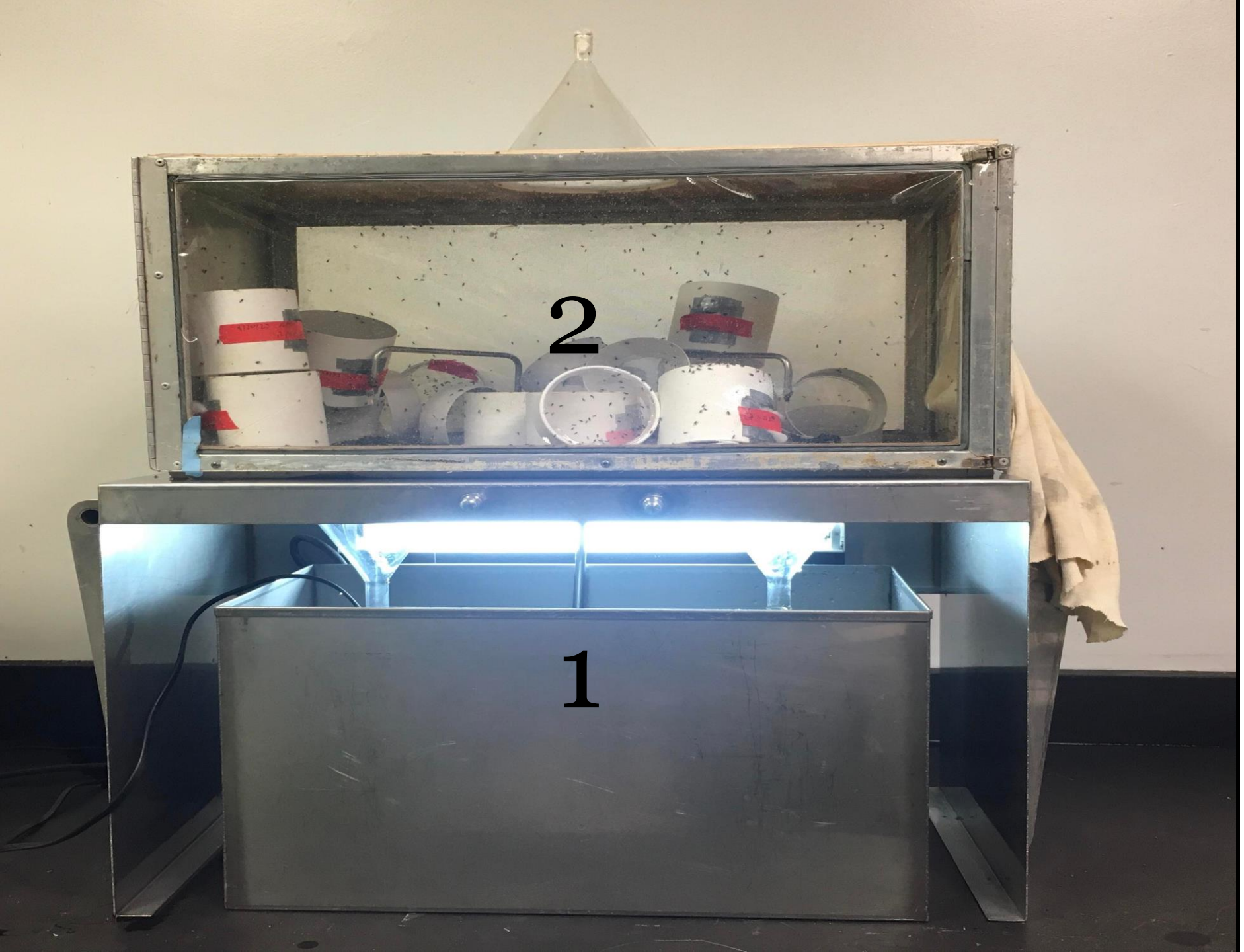
- DEET: Most common OTC insect repellent, works on most biting taxa including mosquitoes, ticks, black flies, fleas, gnats.
- IR3535: Synthetically derived active ingredient similar to β-alanine. Effective on mosquitoes, ticks, midges, headlice.
- Oil of Lemon Eucalyptus (PMD): A naturally derived chemical (p-Menthane-3, 8-diol) from plant of the same name. Effective against mosquitoes, ticks and reportedly against black flies.
- P-menthane-diol (PMD): Synthetically created active chemical in OLE
- Picaridin: Synthetically created, derived from black pepper plants. Forms a vapor barrier between skin and air, reducing the arthropods' ability to sense their target. Works against mosquitoes, ticks, chiggers.

The Black Fly Repellent Testing System

- Autogenous species
- Utilize post-oviposition females

- Water jacketed membranes – widely used
- Design based on Bernardo and Cupp (1986) [11]
- Application of repellent

- Observation of feeding rates
- Data



Oviposition Chamber

1: Reservoir to maintain flow onto egg sheets (close-up next slide). Light underneath.

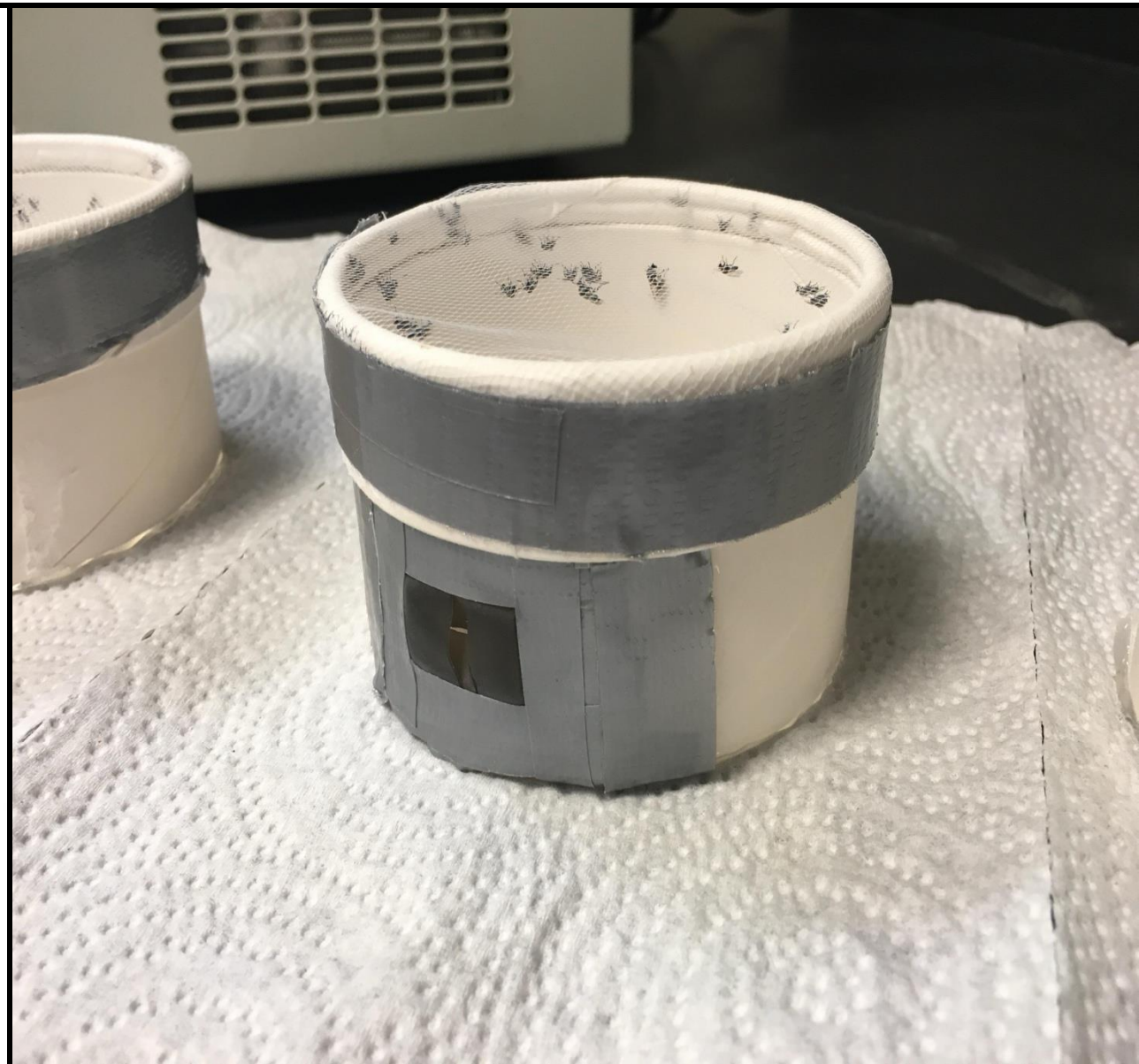
2: Holding chamber for flies, egg sheets

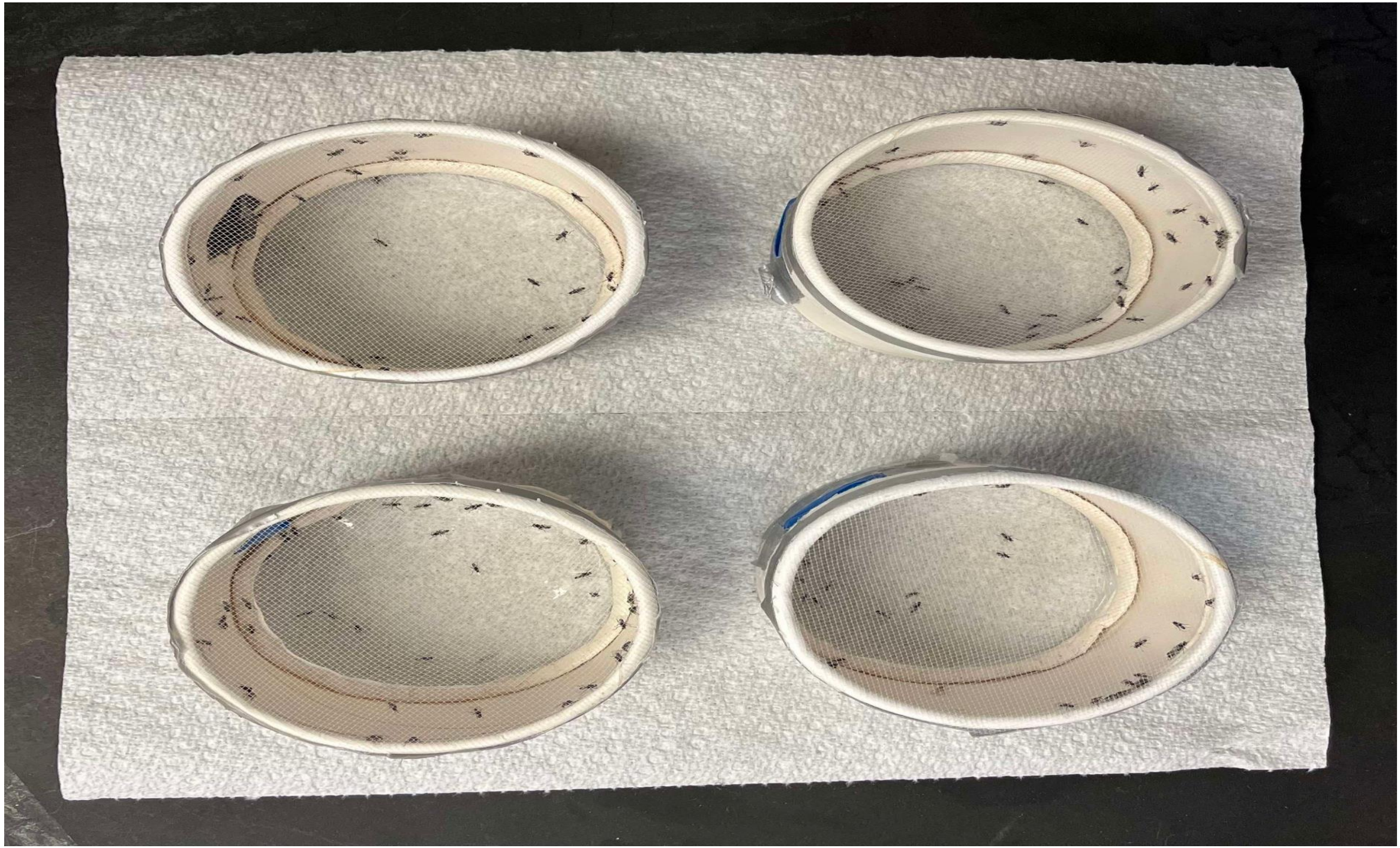
Shrouded Oviposition

Close up of Egg Sheet

Male vs Female Visual Aspiration

Example of Flies in Cup





Water Jacketed Membrane System

1. Membrane attachment site/fly bite site

2. Connection to water bath and other bells

3. Sucrose input site

Bell with Membrane Top

Bell with Membrane Bottom

Retrieval of Repellent in Fume Hood

Application of Repellent to Membranes

Repellent Test System

1. Four bells; membranes dosed, shrouded, 40 flies in cup below

2. Circulating water bath (37° C)

Assessment of Repellency

- Flies observed through clear bottom of cup

- Number of flies biting counted 5 minutes post-exposure

- Test conducted once every two hours, up to 12

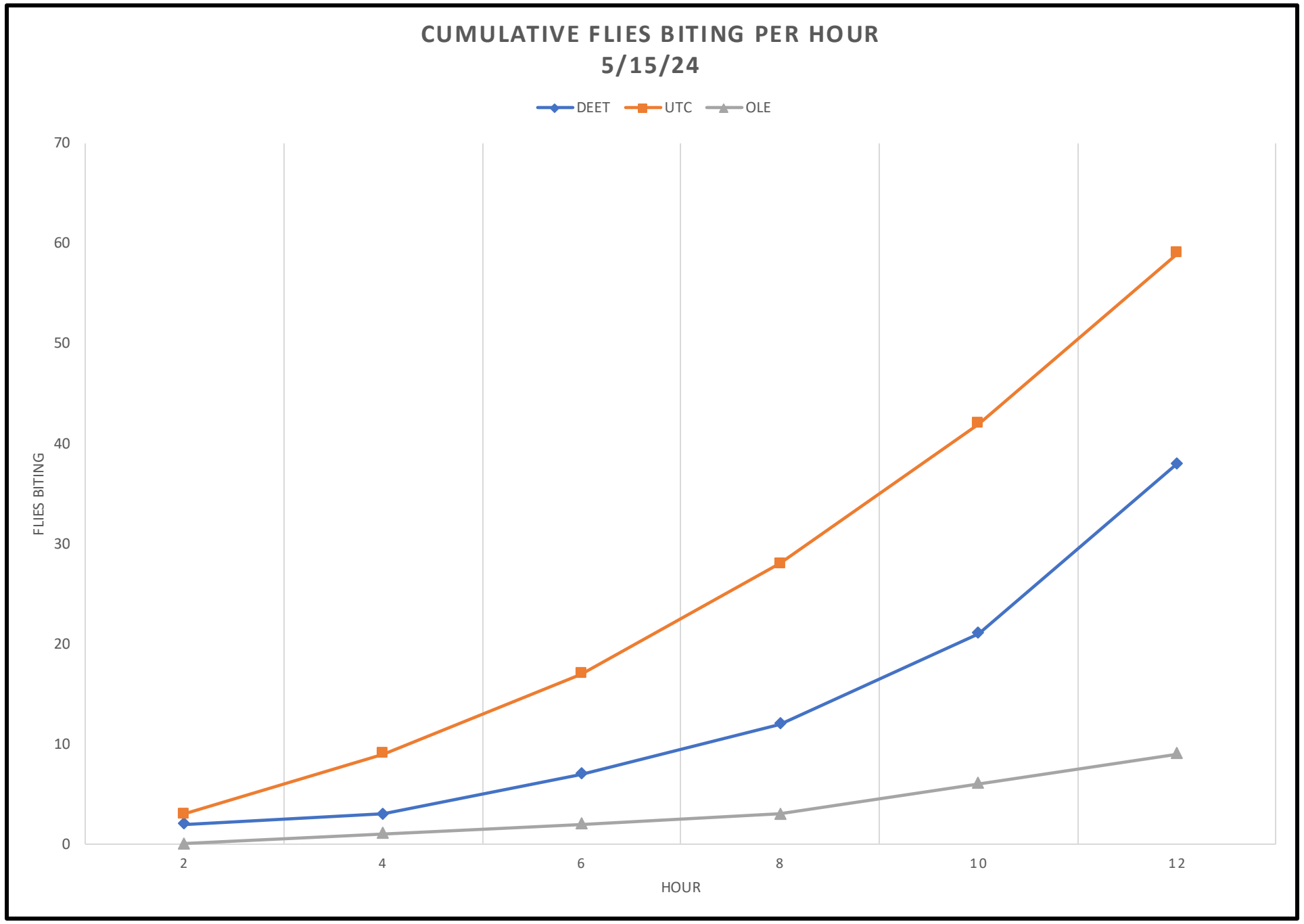
Development I: Dosage Variation

- Old protocol was varied in dosage of repellent

- Experimentation with dosage amount of product [high and low]

- Treatments used:
- * Picaridin [20%]
- * DEET [7%, 15%, 25%, 40%]
- * IR3535 [20%]
- * Oil of Lemon Eucalyptus [30%]
- * PMD [10%]
- * Nothing [control]

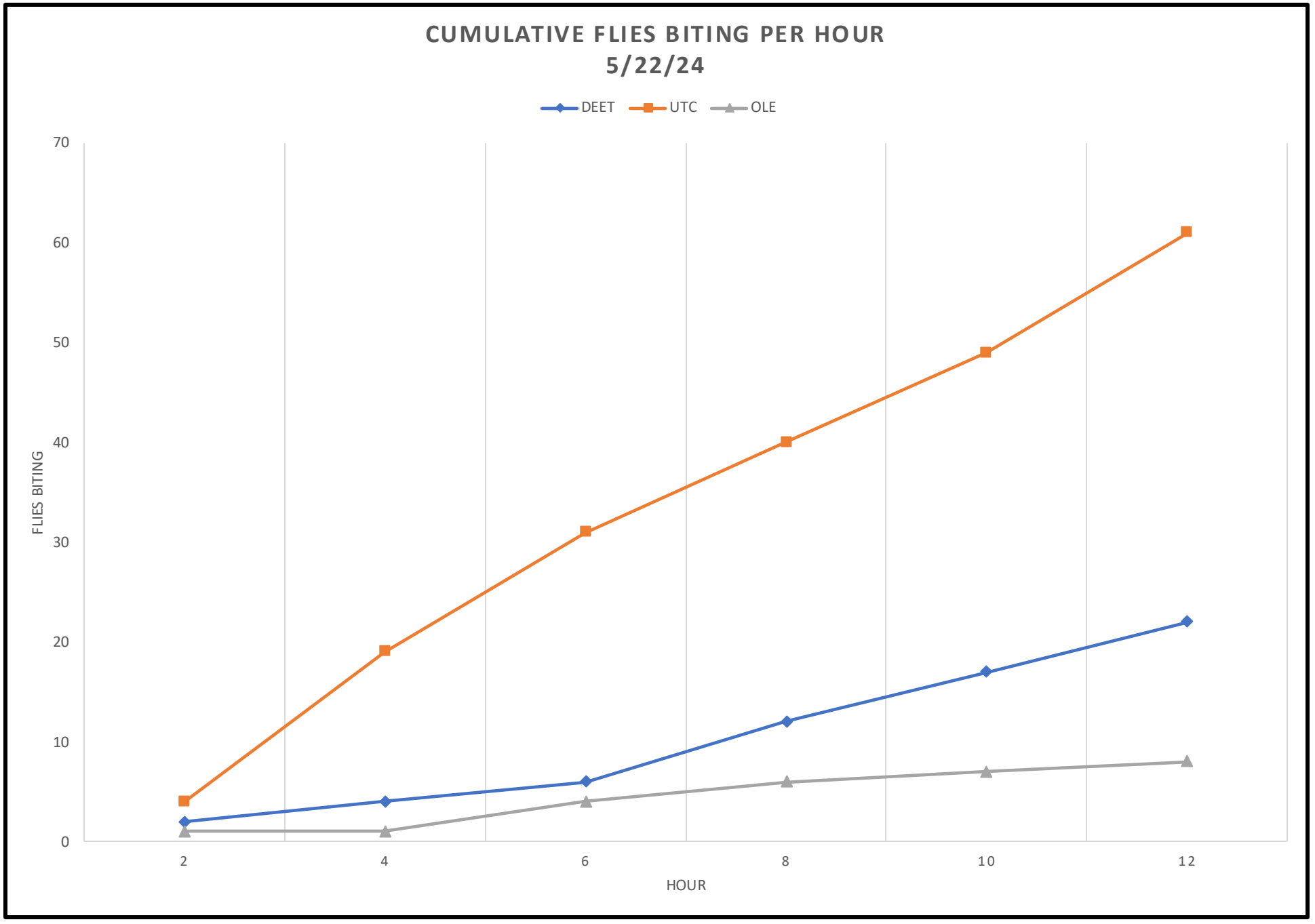
Old application method



10ul
7% DEET vs 30% OLE

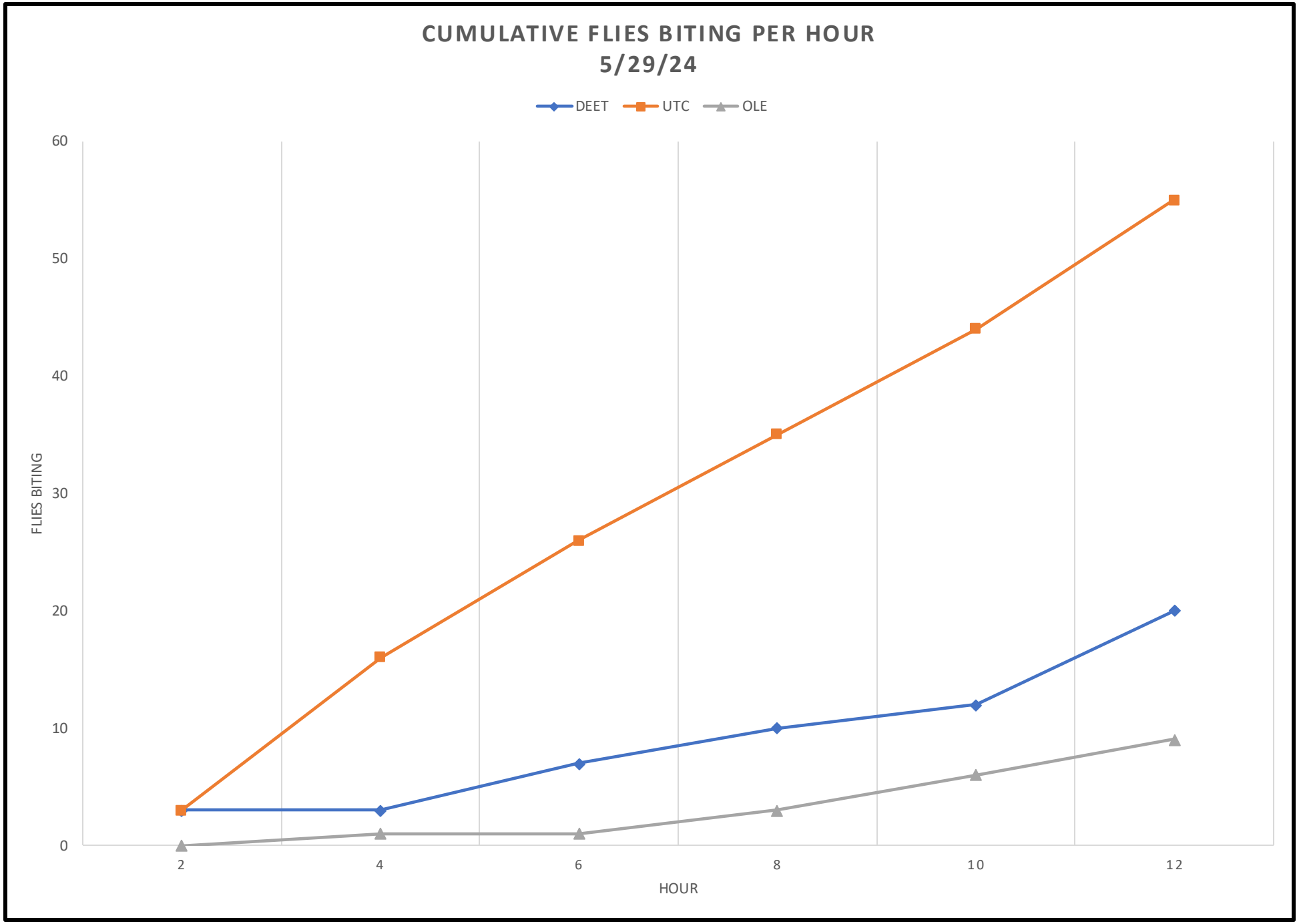
	Cumulative Flies Biting		
Hour	7% DEET	UTC	30% OLE
2	2	3	0
4	3	9	1
6	7	17	2
8	12	28	3
10	21	42	6
12	38	59	9

Next step:
Triplicate replication!



10ul
7% DEET vs 30% OLE

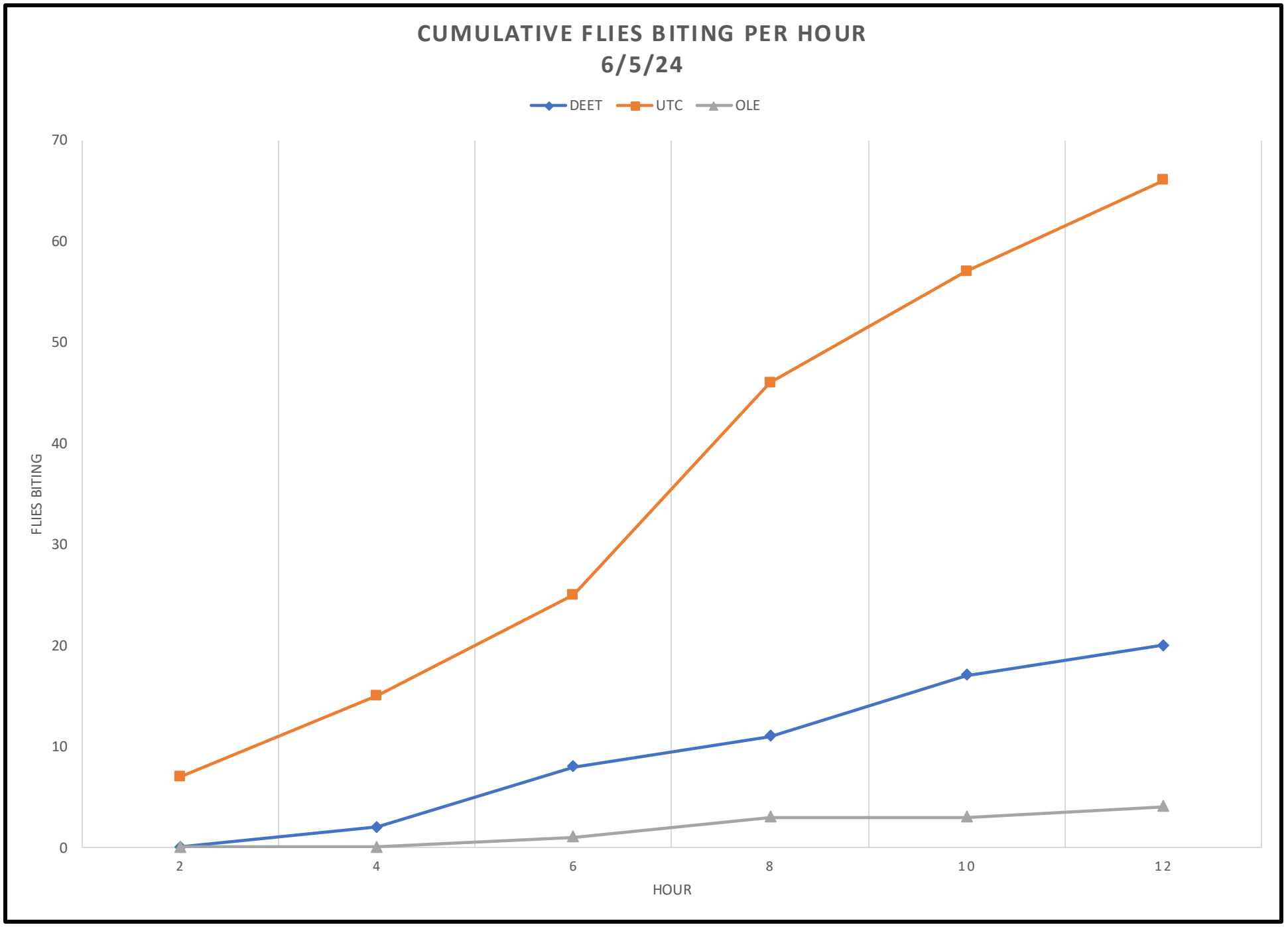
	Cumulative Flies Biting		
Hour	7% DEET	UTC	30% OLE
2	2	4	1
4	4	19	1
6	6	31	4
8	12	40	6
10	17	49	7
12	22	61	8



10ul
7% DEET vs 30% OLE

	Cumulative Flies Biting		
Hour	7% DEET	UTC	30% OLE
2	3	3	0
4	3	16	1
6	7	26	1
8	10	35	3
10	12	44	6
12	20	55	9

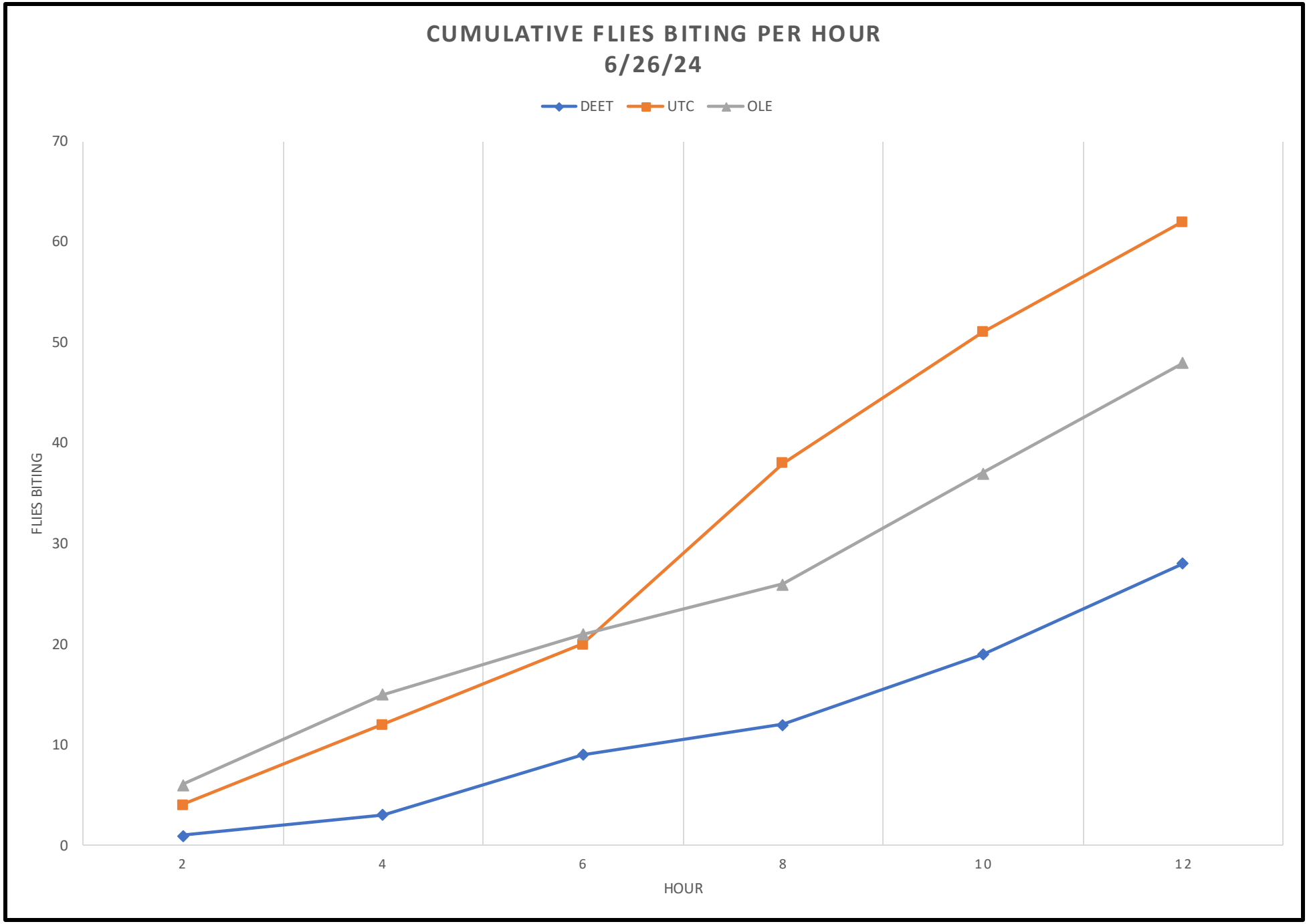
Next step:
Failure of OLE



5ul
7% DEET vs 30% OLE

	Cumulative Flies Biting		
Hour	7% DEET	UTC	30% OLE
2	0	7	0
4	2	15	0
6	8	25	1
8	11	46	3
10	17	57	3
12	20	66	4

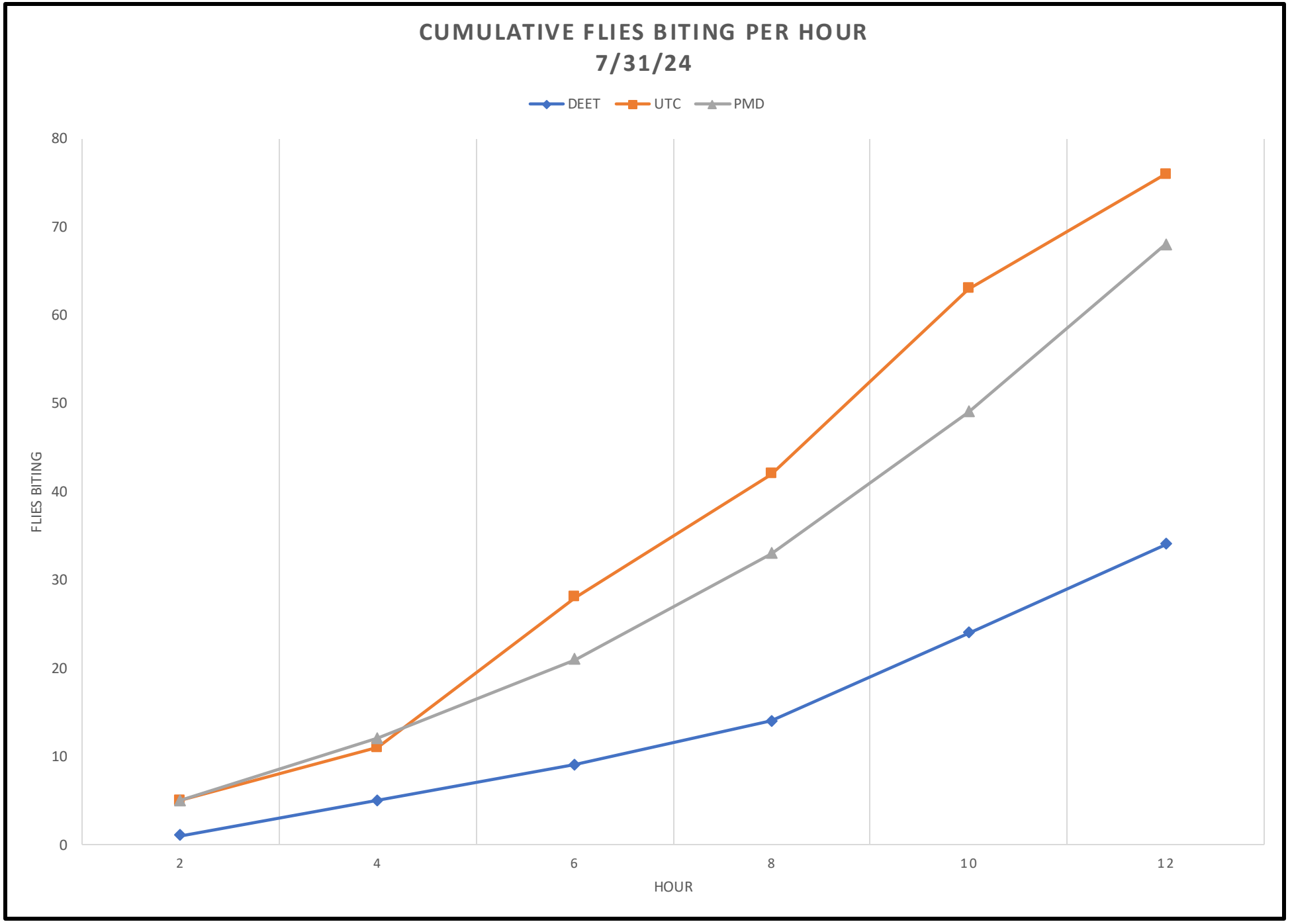
Next step:
Lower dosage



4ul
7% DEET vs 30% OLE

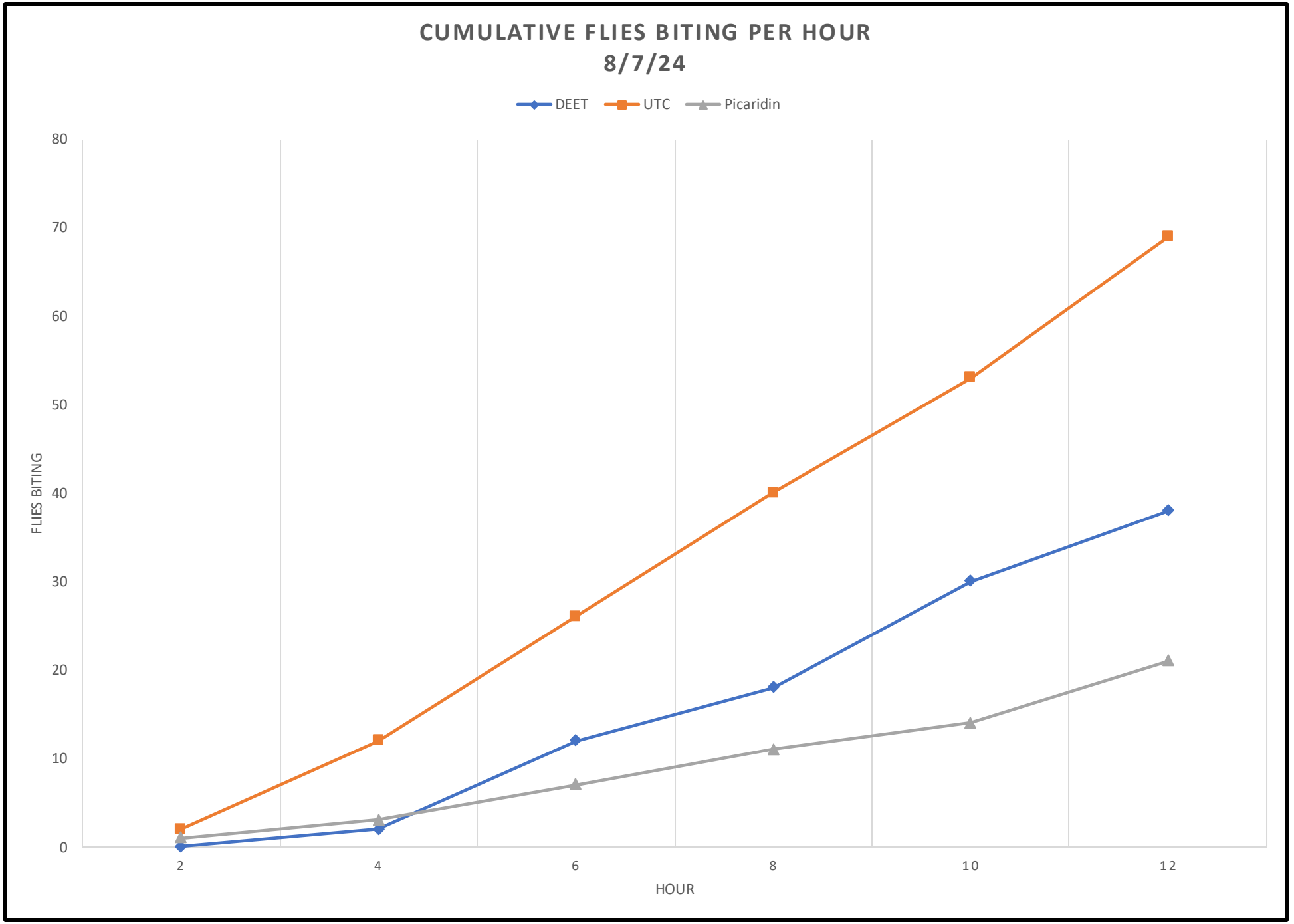
	Cumulative Flies Biting		
Hour	7% DEET	UTC	30% OLE
2	1	4	6
4	3	12	15
6	9	20	21
8	12	38	26
10	19	51	37
12	28	62	48

Next step:
Back to 5ul/other repellents



5ul
7% DEET vs 10% PMD

	Cumulative Flies Biting		
Hour	7% DEET	UTC	10% PMD
2	1	5	5
4	5	11	12
6	9	28	21
8	14	42	33
10	24	63	49
12	34	76	68



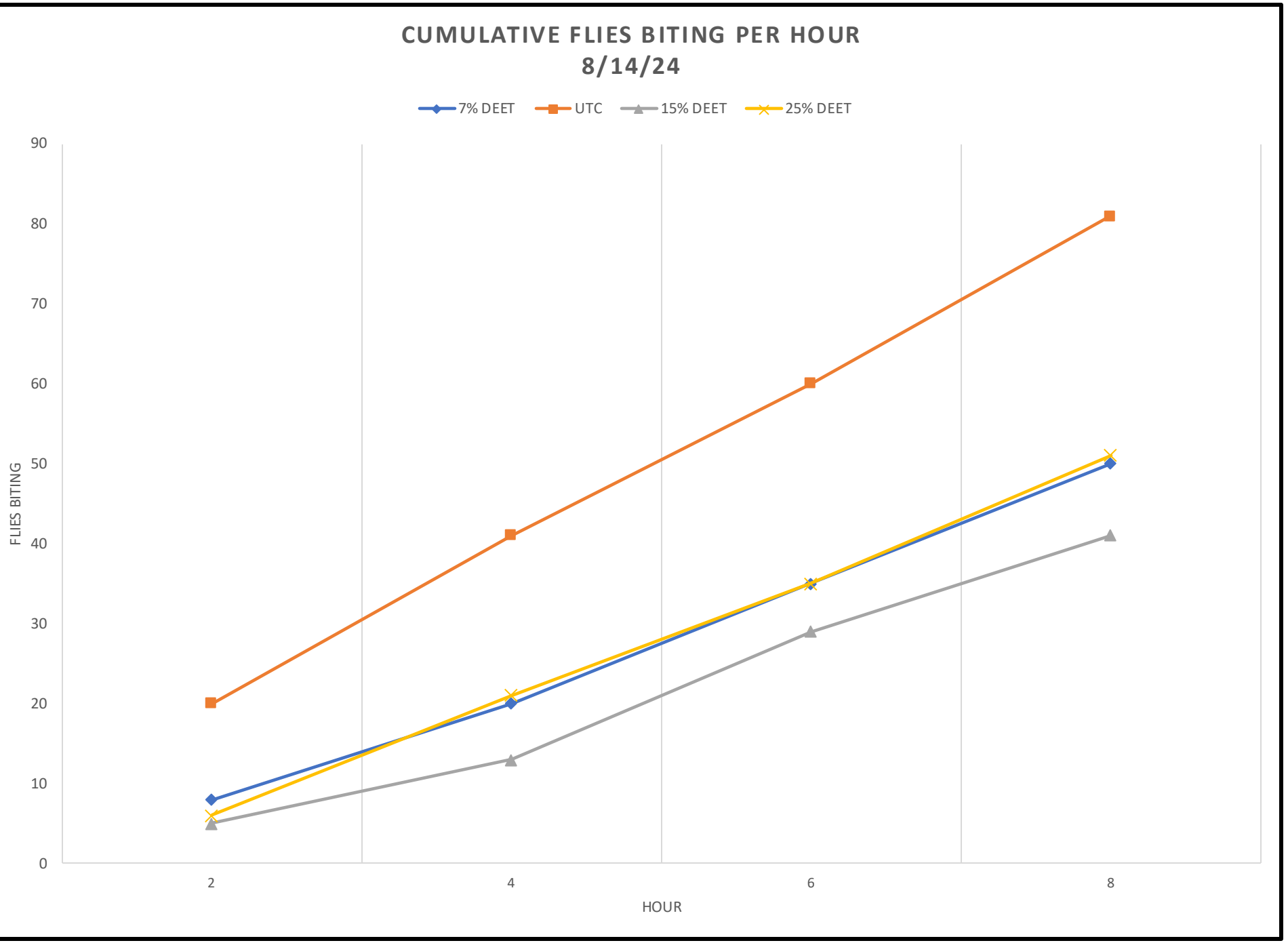
5ul
7% DEET vs 20% Picaridin

	Cumulative Flies Biting		
Hour	7% DEET	UTC	20% Picaridin
2	0	2	1
4	2	12	3
6	12	26	7
8	18	40	11
10	30	53	14
12	38	69	21

Next step: DEET standard

Development II: The Search for a Standard

- Need for a consistent baseline
- How cool would a graph showing an array of DEET concentrations look?
- Addition of 4th bell to use 7%,15%, 25%, and/or 40%
- Up dosage to account for viscosity of products

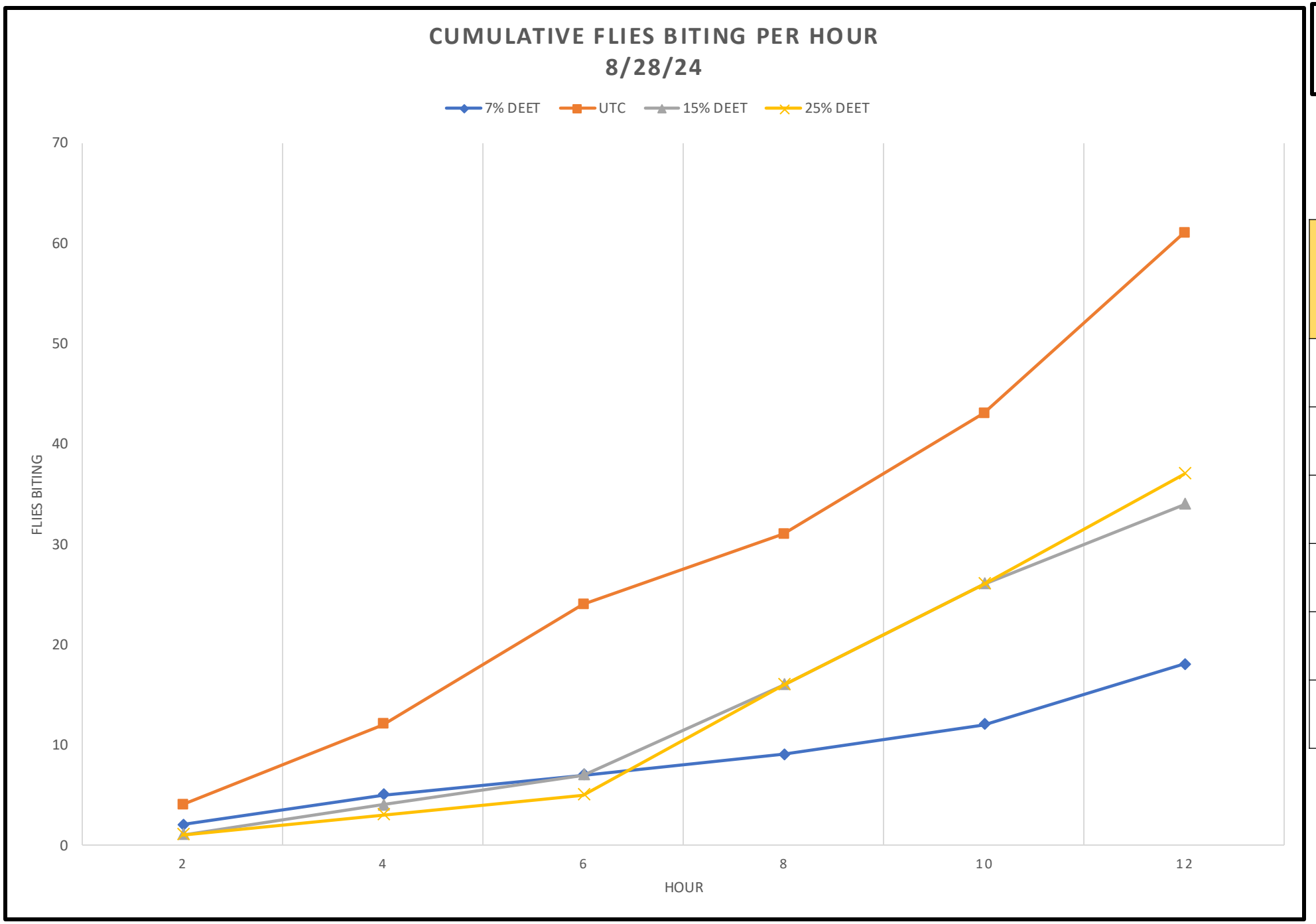


7.5ul
7%, 15%, and 25% DEET

	Cumulative Flies Biting			
Hour	7% DEET	UTC	15% DEET	25% DEET
2	8	20	5	6
4	20	41	13	21
6	35	60	29	35
8	50	81	41	51

Ended early due to a latex tear of 25% DEET.

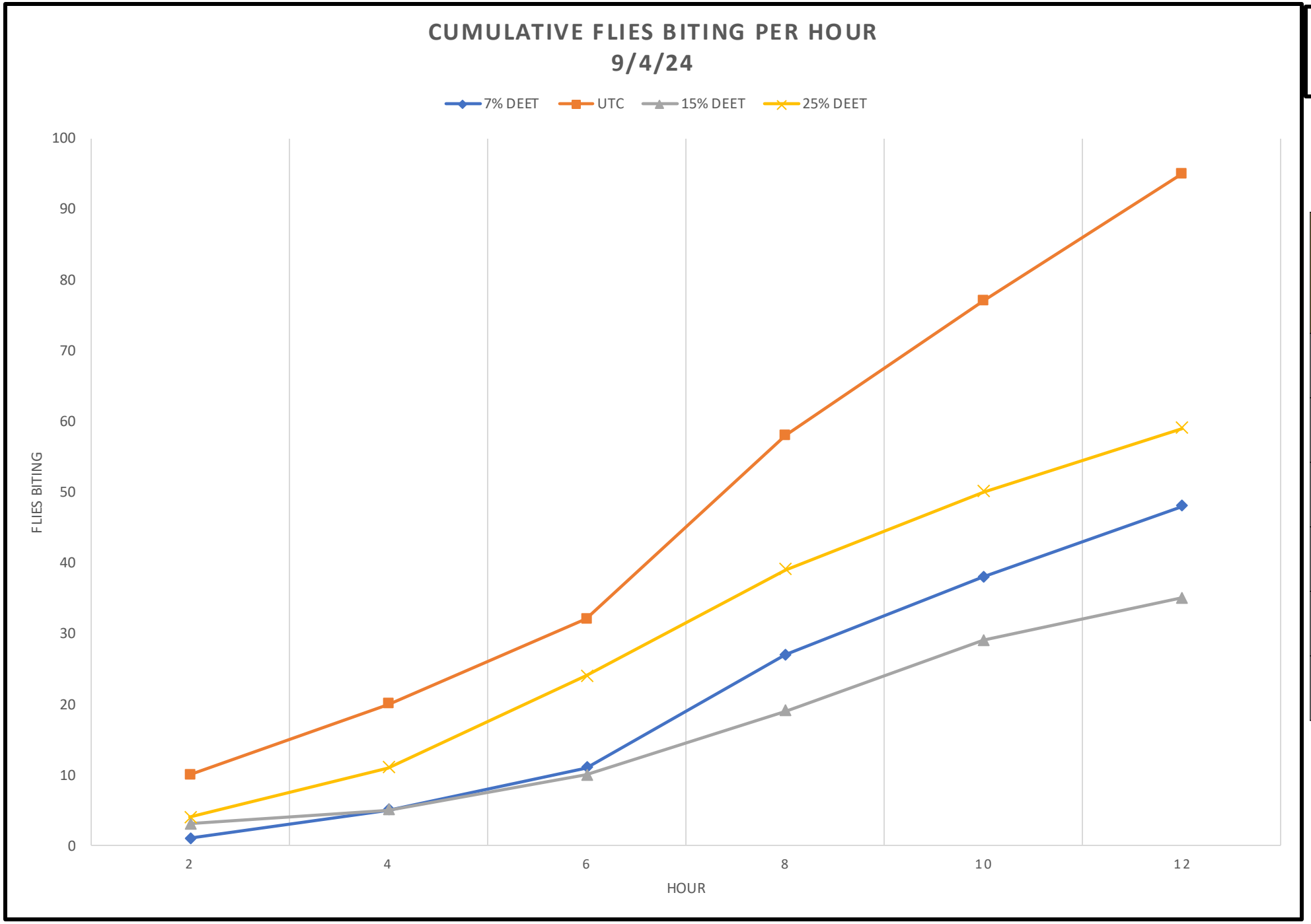
Next step:
Try again.



7.5ul
7%, 15%, and 25% DEET

	Cumulative Flies Biting			
Hour	7% DEET	UTC	15% DEET	25% DEET
2	2	4	1	1
4	5	12	4	3
6	7	24	7	5
8	9	31	16	16
10	12	43	26	26
12	18	61	34	37

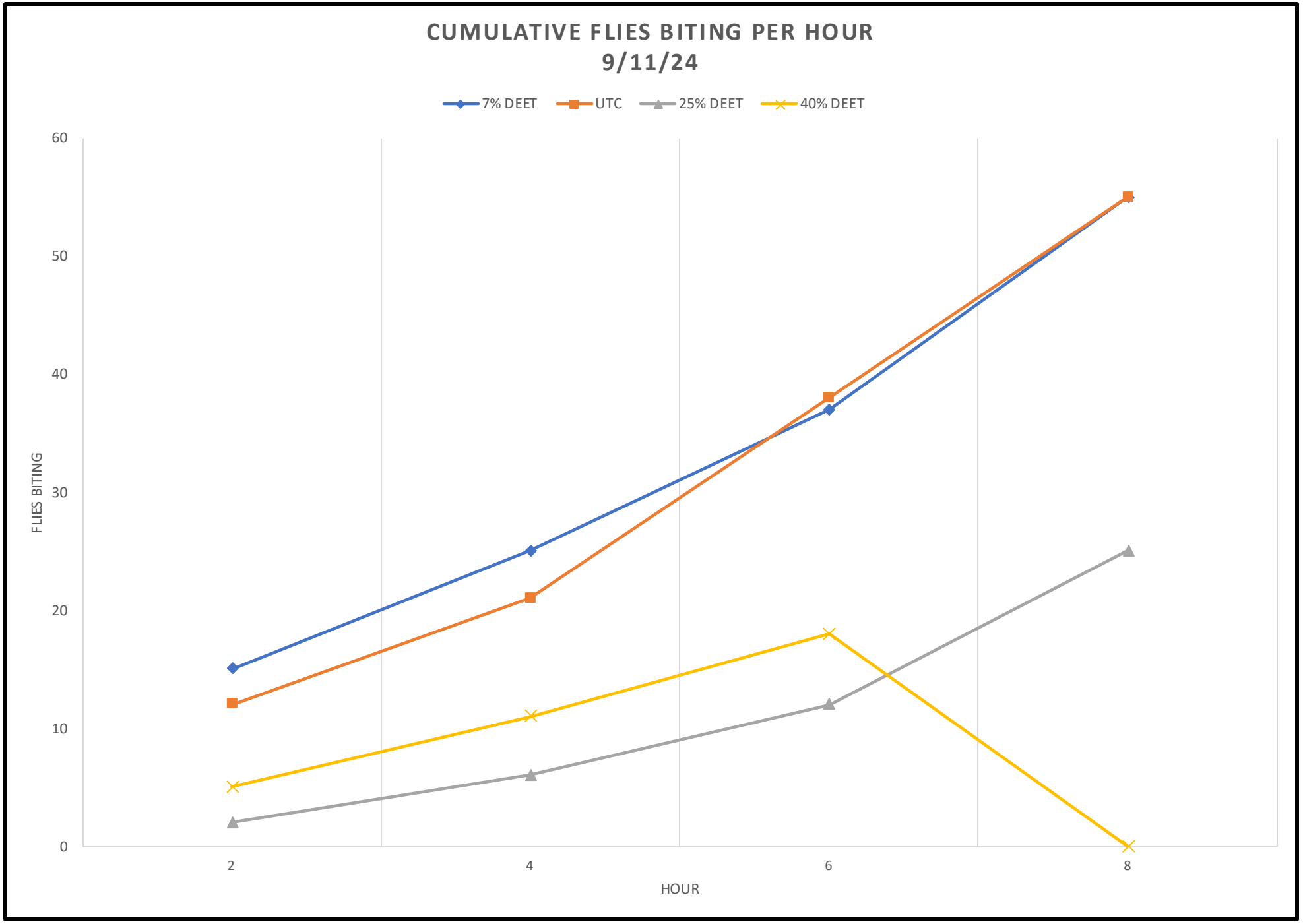
Fly congregation on a section of 25% DEET noted. Noncoverage suspected. Next step: Try again



7.5ul
7%, 15%, and 25% DEET

	Cumulative Flies Biting			
Hour	7% DEET	UTC	15% DEET	25% DEET
2	1	10	3	4
4	5	20	5	11
6	11	32	10	24
8	27	58	19	39
10	38	77	29	50
12	48	95	35	59

Next step: Try increasing DEET concentration [40%].



10ul
7%, 25%, and 40% DEET

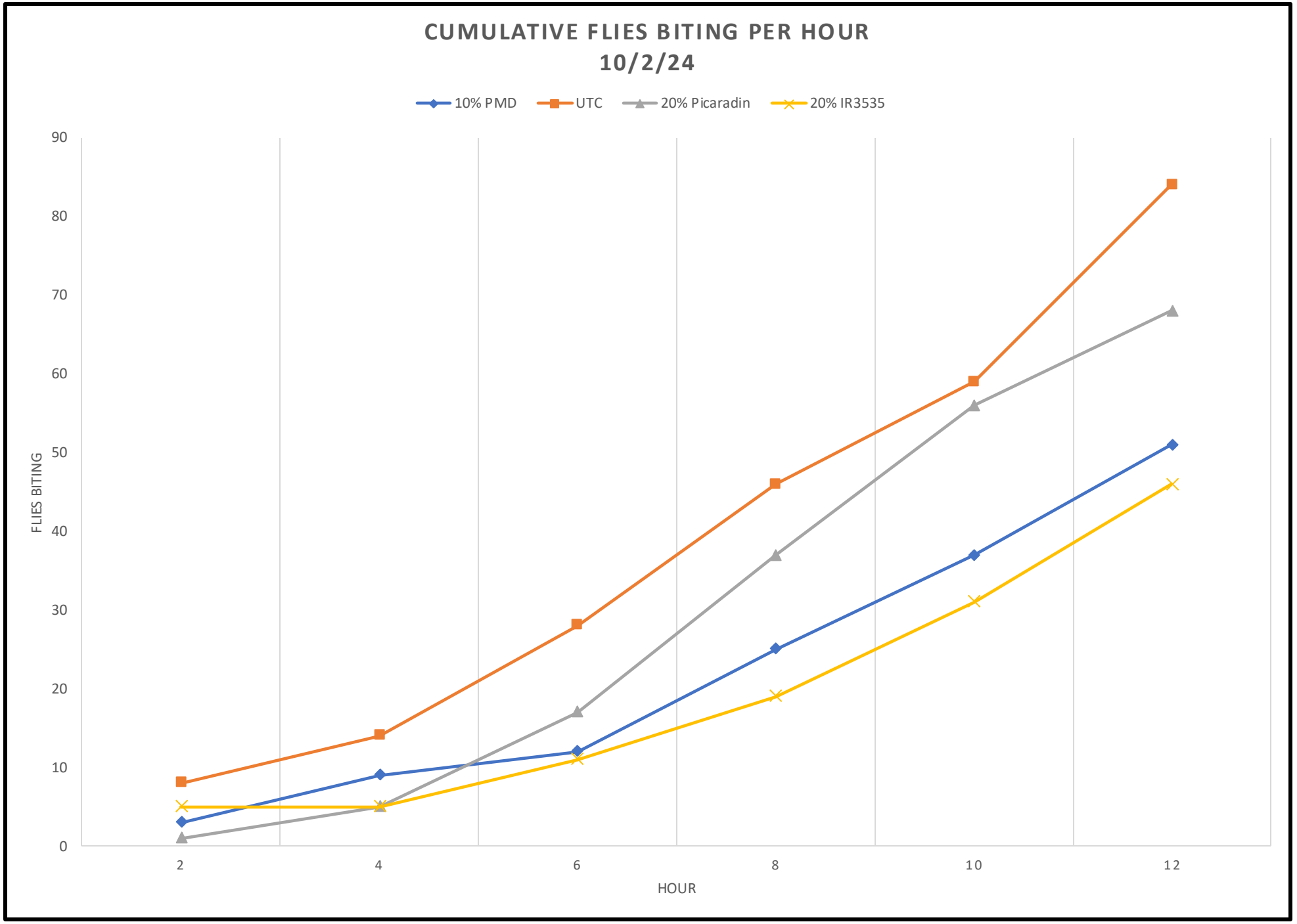
	Cumulative Flies Biting			
Hour	7% DEET	UTC	25% DEET	40% DEET
2	15	12	2	5
4	25	21	6	11
6	37	38	12	18
8	55	55	25	X

Tear on 40% membrane, experiment ended early.

Development III: DEET

"DEET is a plasticizer and must be used with care to prevent damage to plastics, rubber, vinyl, or elastics, including items such as eyeglass frames, plastic lenses and cases, contact lenses, combs, watch crystals, goggles, painted and varnished surfaces, and some synthetic fabrics (nylon is okay)."

(Technical Guide No. 36: Personal Protective Measures Against Insects and Other Arthropods of Military Significance; Defense Pest Management Information Analysis Center (DPMIAC), Armed Forces Pest Management Board (AFPMB): Washington, DC, 2002; pp 21-25.)



10ul
10% PMD, 20% Picaridin, 20% IR3535

	Cumulative Flies Biting			
Hour	10% PMD	UTC	20% Picaridin	20% IR3535
2	3	8	1	5
4	9	14	5	5
6	12	28	17	11
8	25	46	37	19
10	37	59	56	31
12	51	84	68	46

Future Directions

1. Repellent concentrations

2. Other repellent types/formulations

3. Dissections

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Thank you!

Questions?

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Products used:

10% PMD – OFF! Botanicals Insect Repellent IV

20% Picaridin – OFF! Clean Feel

20% IR3535 – Zevo On-Body

7% DEET – Cutter Skinsations

15% DEET – Repel 15%

25% DEET – OFF! Deep Woods

40% DEET – Repel MAX

30% OLE - Repel Plant-Based Lemon Eucalyptus

(6)

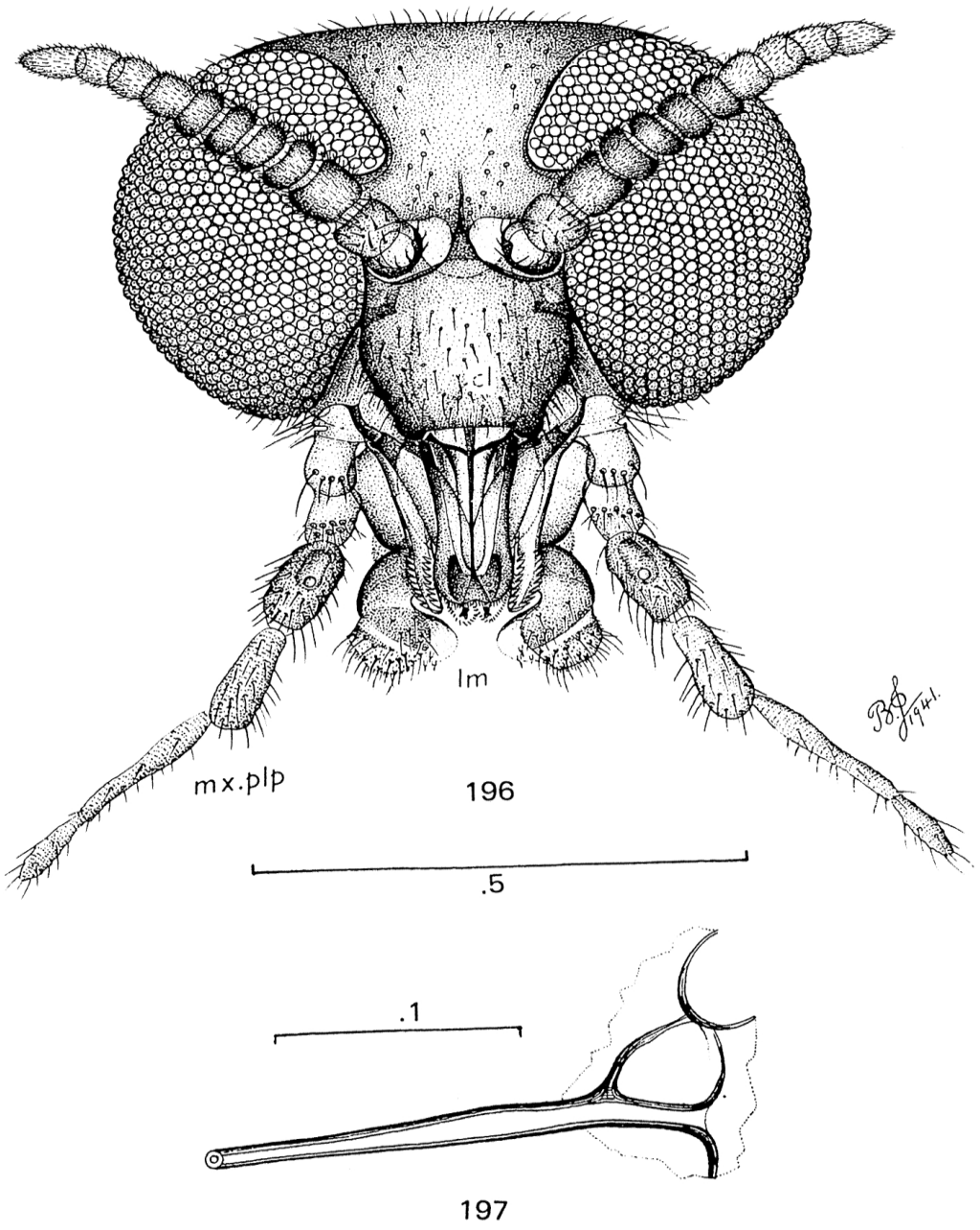


Fig 196, 197 *Simulium* (*Wilhelmia*) sp., ♀. 196, anterior view of head; 197, tentorium of same.

Anatomical Attraction to Hosts in Black Flies: Head

1. Compound eyes for determining visual cues (color and shape)

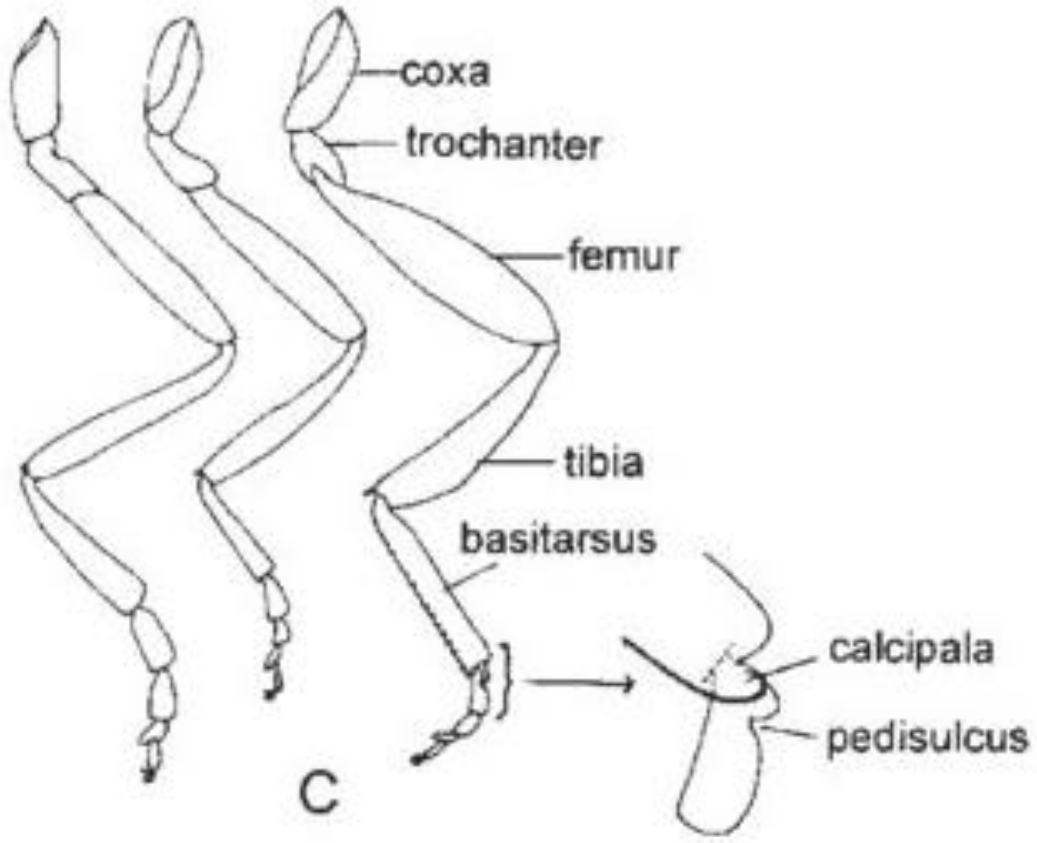
2. Capitate pegs on palps for detection of CO_2

3. Antennae for detection of host-associated odors (scape, funiculus, styloconic sensilla)

1-3, (7)

Anatomical Attraction to Hosts in Black Flies: Legs

(8)



1. Chaetica on tarsal segments and tibia for contact chemoreception

2. Peg sensilla ventrally on tarsomeres 1-4 for contact chemoreception

3. Bifurcate sensilla on mesothoracic basitarsi for olfaction

4. Sensilla placodea on femur and tibia for olfaction

1-4(7)

How do Insect Repellents Work?

- Interference with host attraction cues, primarily chemical and olfactory/gustatory

- EPA approved active ingredients for use on skin:

(9)

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