Insecticide Resistance: The Gene from Hell

RM Roe

North Carolina State University,
Raleigh, NC (USA)

Plant transgenic technologies which prevent insect feeding by targeting multiple target sites in the insect gut using proteins and nucleic acids

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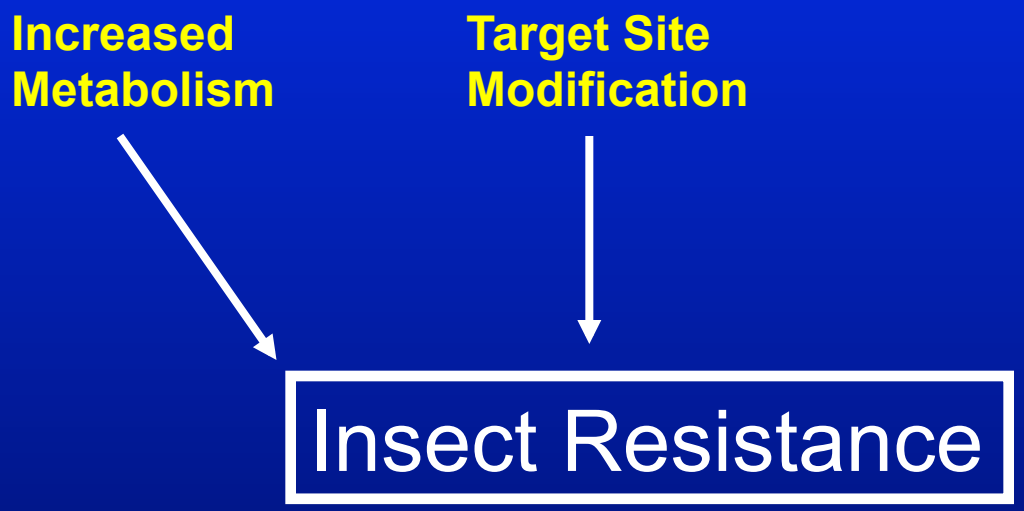
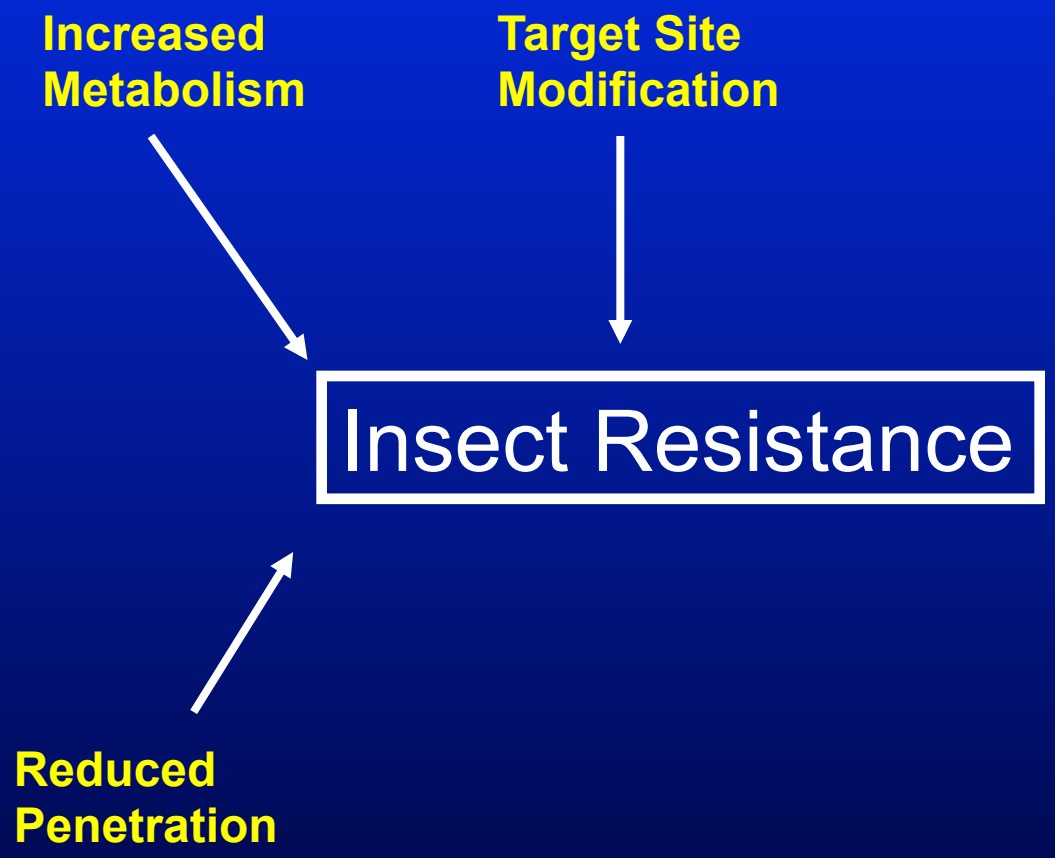
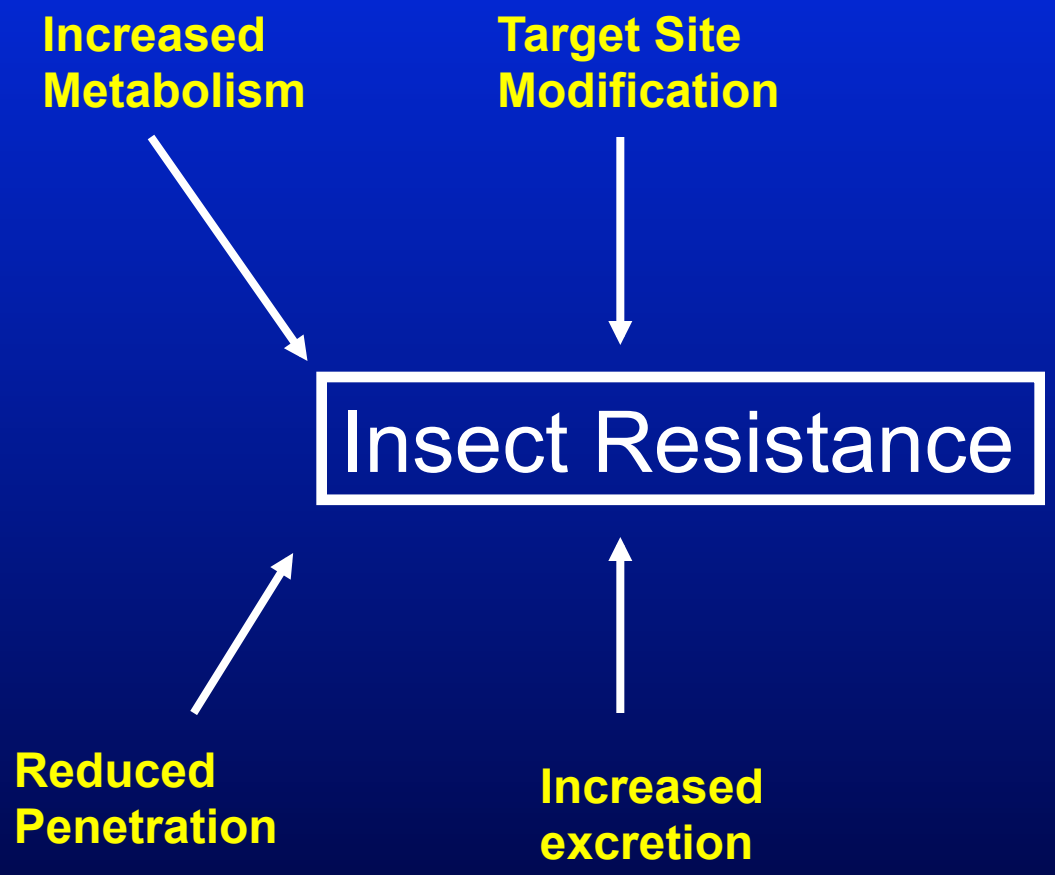
Is there a mechanism that insects could use to make any protein or nucleic acid ineffective and cause broad resistance to transgenic plant technologies?

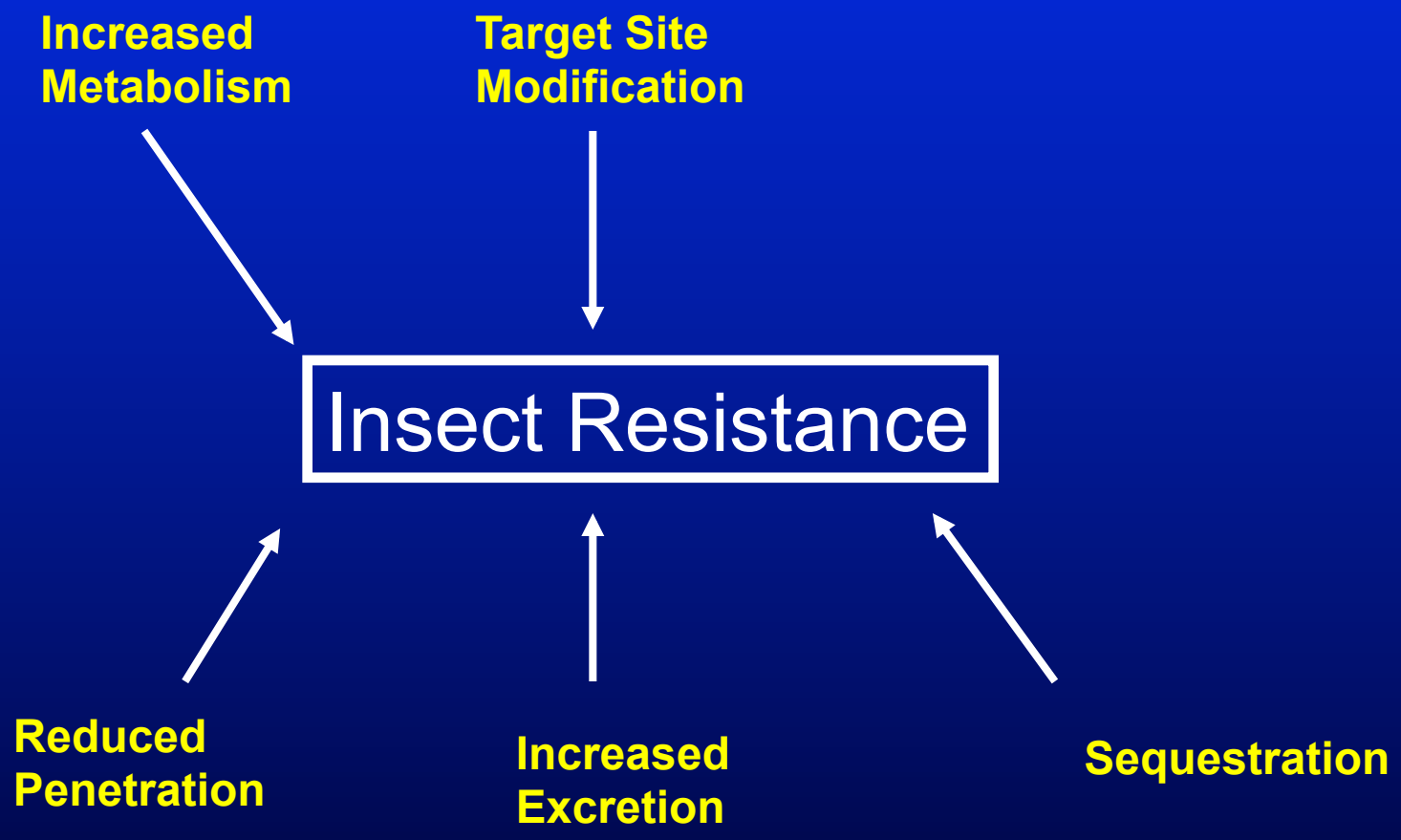
THE GENE FROM HELL

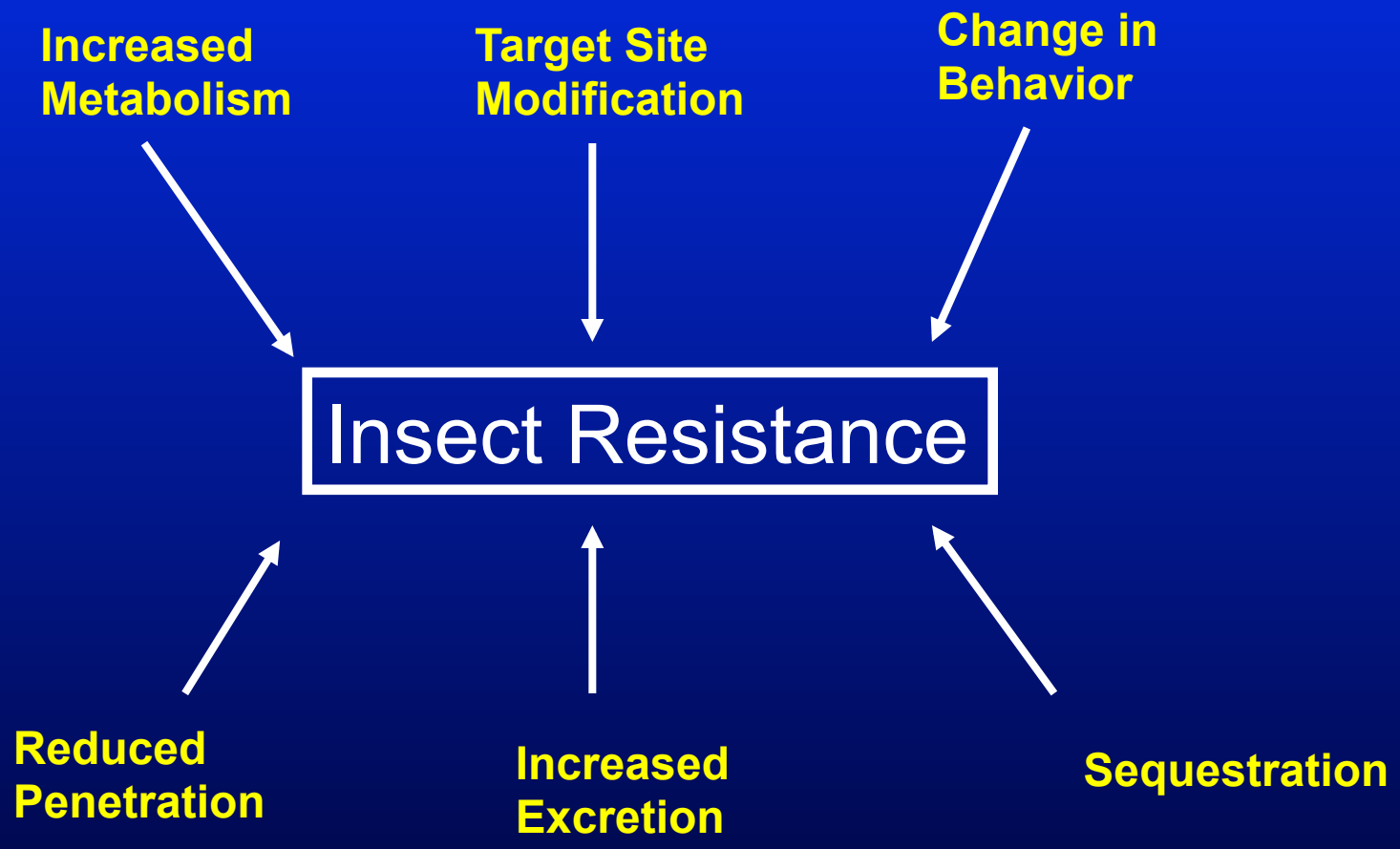
Mechansims of Insect Resistance to Pesticides


Increased
Metabolism
Target Site
Modification
Insect Resistance


Increased Metabolism
Target Site Modification
Insect Resistance
Reduced Penetration


Increased Metabolism
Target Site Modification
Insect Resistance
Reduced Penetration
Increased excretion


Increased Metabolism
Target Site Modification
Insect Resistance
Reduced Penetration
Increased Excretion
Sequestration


Increased Metabolism
Target Site Modification
Change in Behavior
Insect Resistance
Reduced Penetration
Increased Excretion
Sequestration

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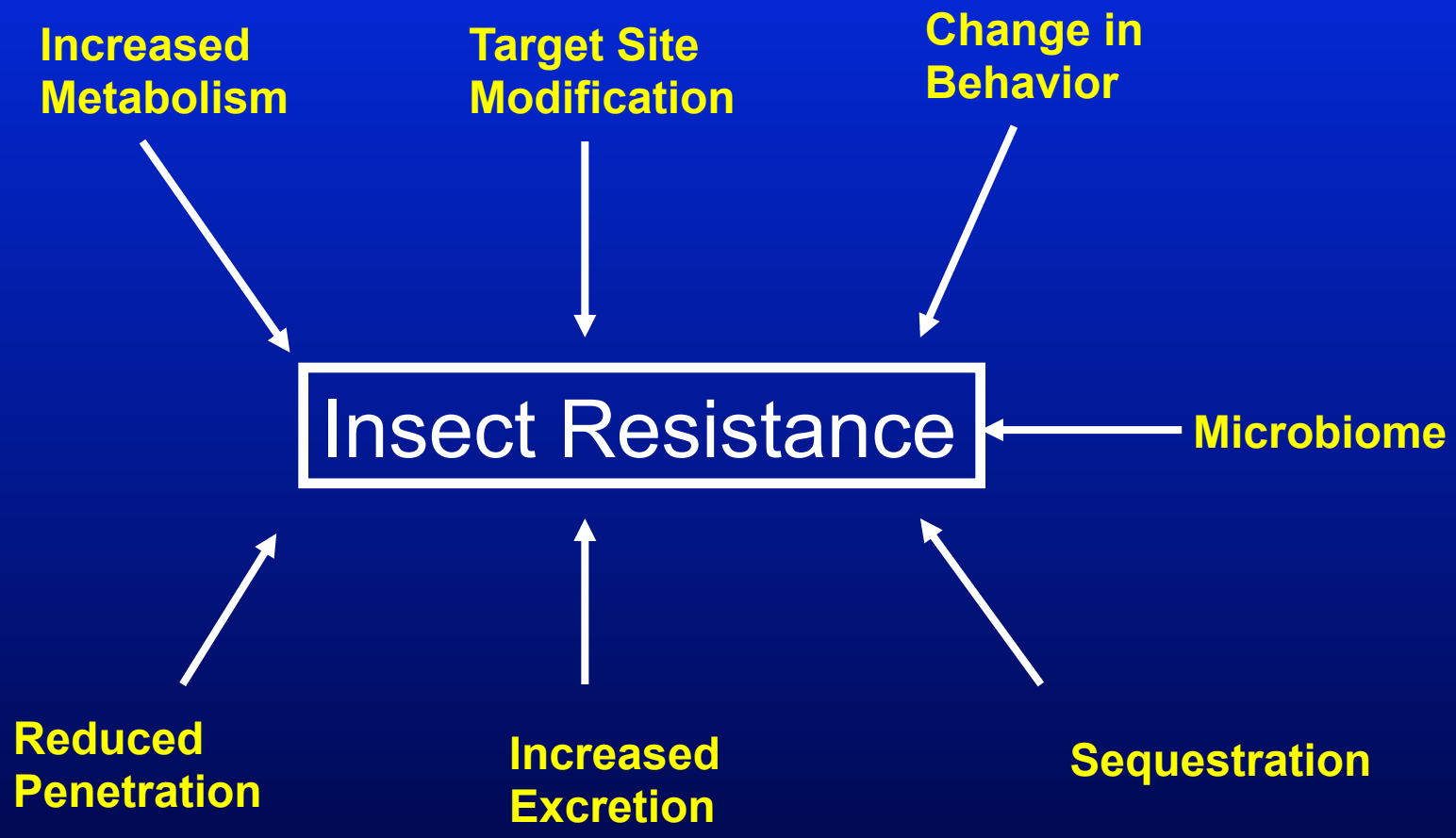
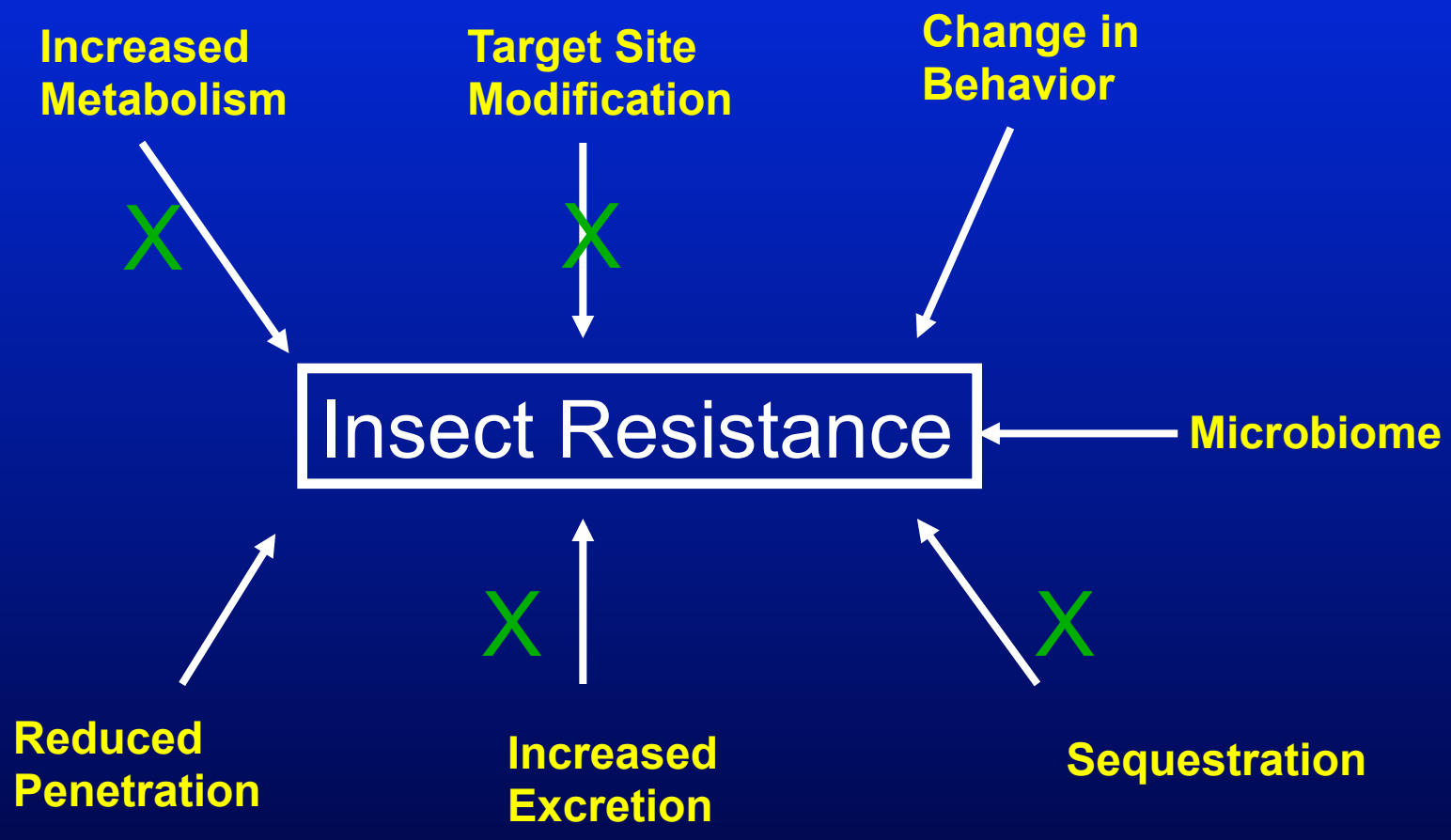
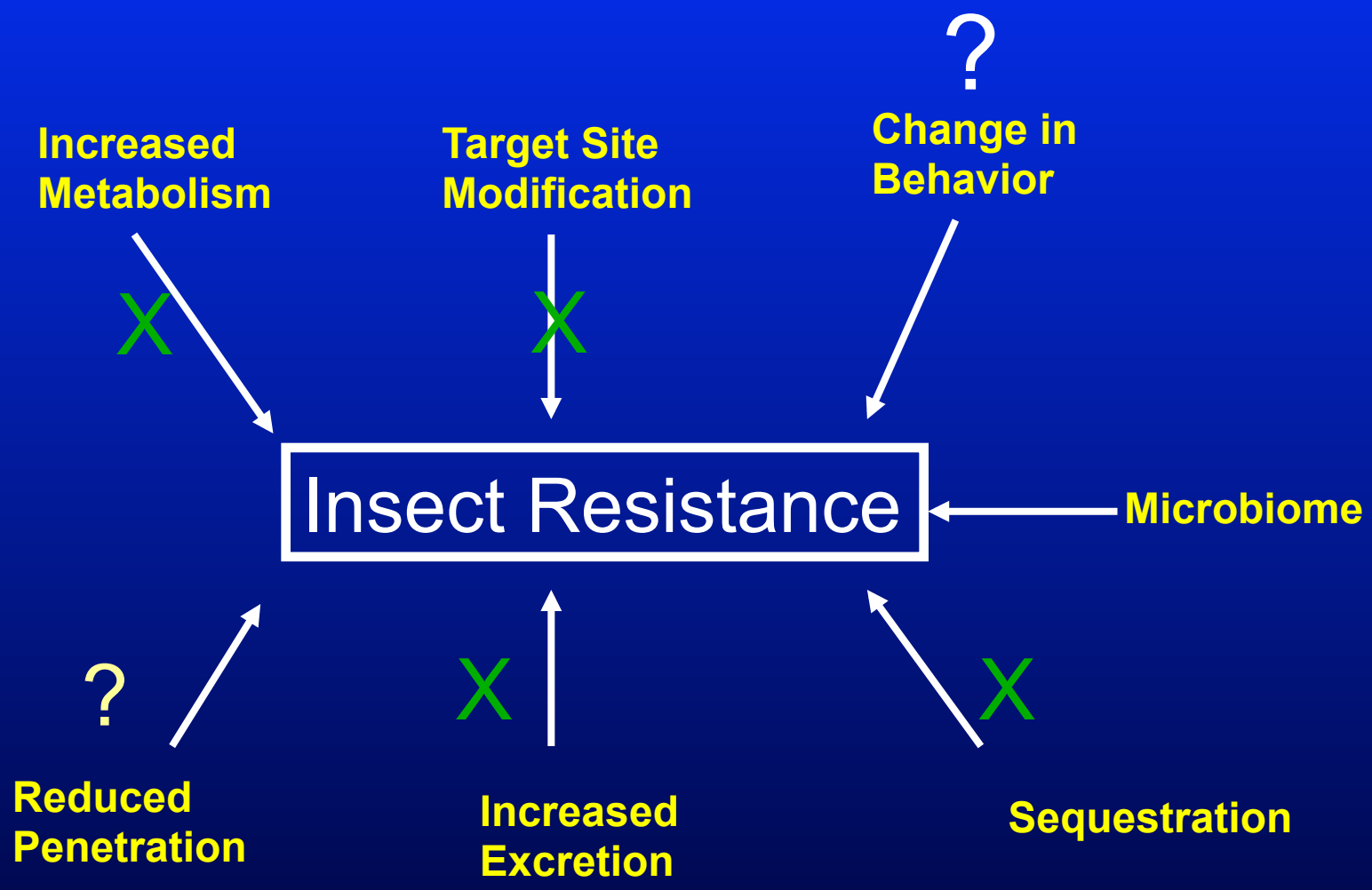


Benefit of stacked genes for Proteins and RNAi

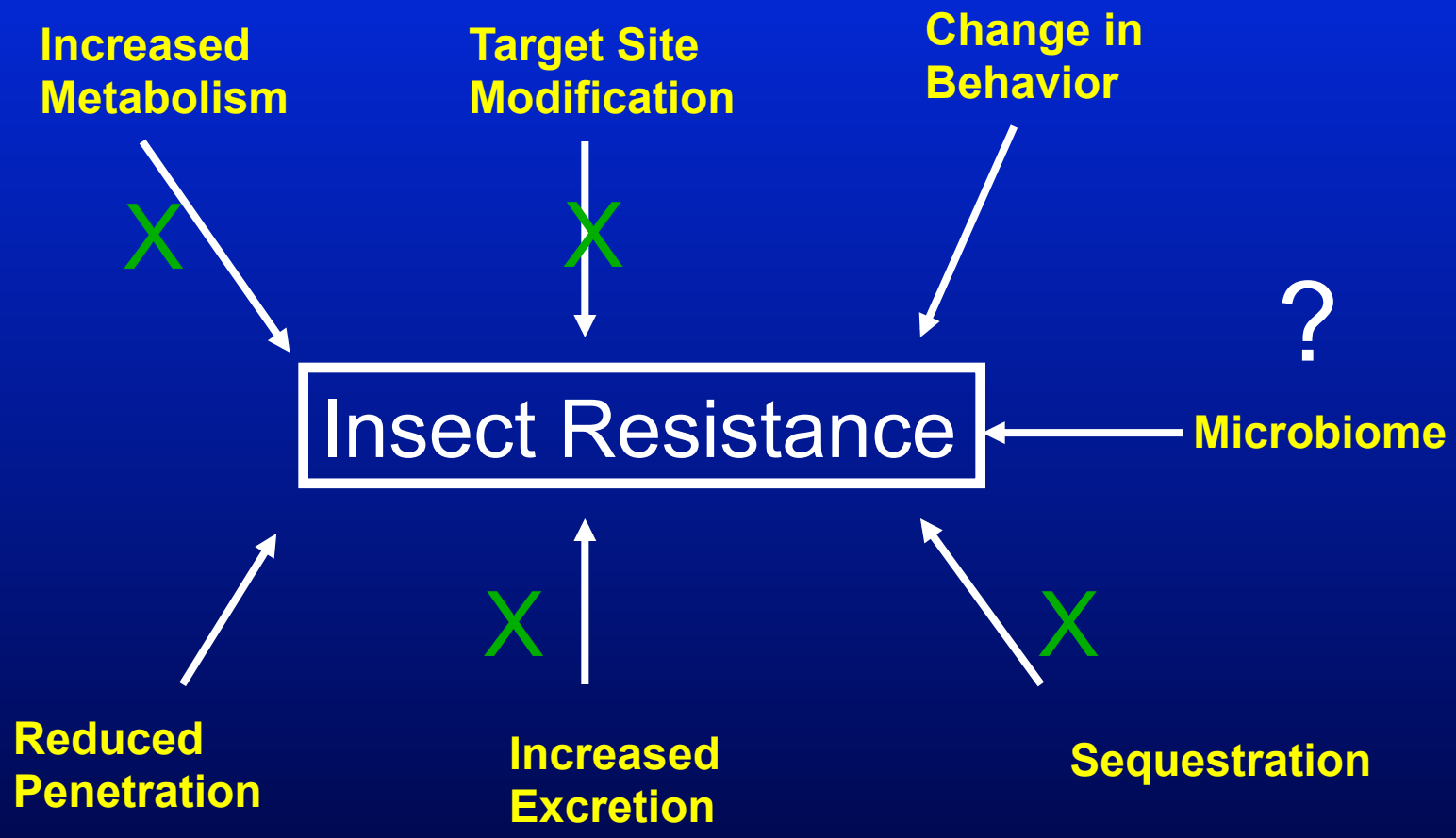
(X)



Could changes in behavior affect penetration for protein and dsRNA toxins?



Could changes in the microbiome affect susceptibility to protein and dsRNA toxins?



Methods of Insecticide Application
Advantages and Disadvantages of Each

Transgenic Cotton

- Do not have to spray
- Convenient to farmer
- Insect must eat the plant to be killed

Spray

- Have to spray
- Need to know when to spray
- Insect can not avoid spray

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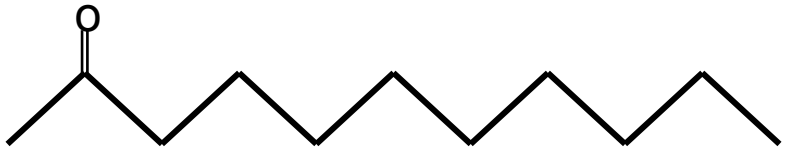
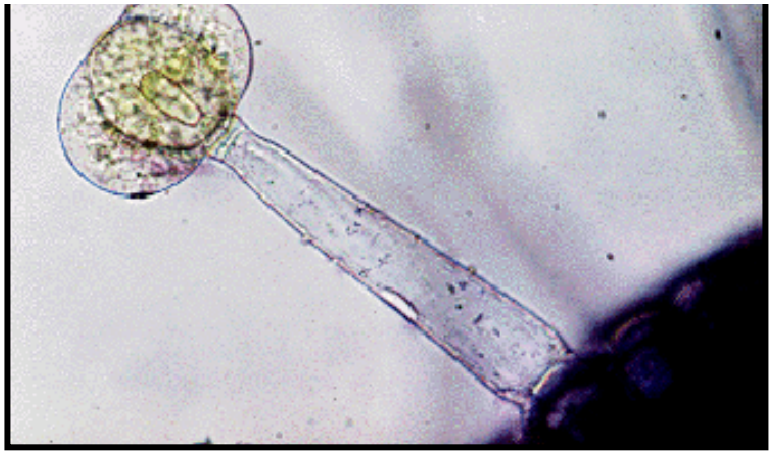
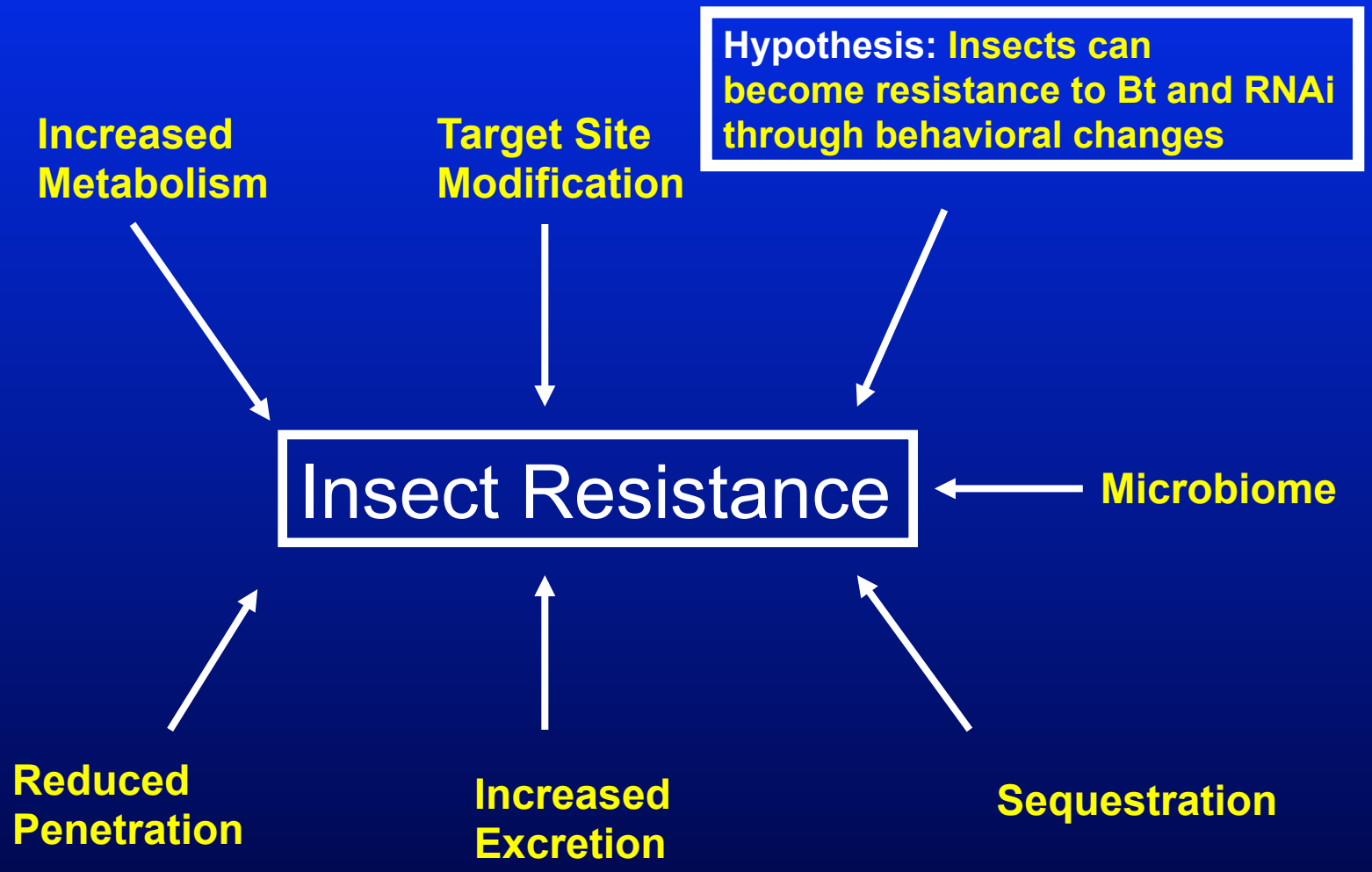
Wild tomatoes





- Undecanone makes the plant resistant to the insect.
- It is nothing new for insects to feed on plants which express insect toxins and become resistant to these plant produced toxins.
- One mechanism by which insects become resistant to multiple plant toxins with different chemistries and mode of action is changes in behavior and penetration of the toxin in the gut.

Mechansims of Insect Resistance to Pesticides



Different mechanisms for Caterpillar Resistance to Bt toxin

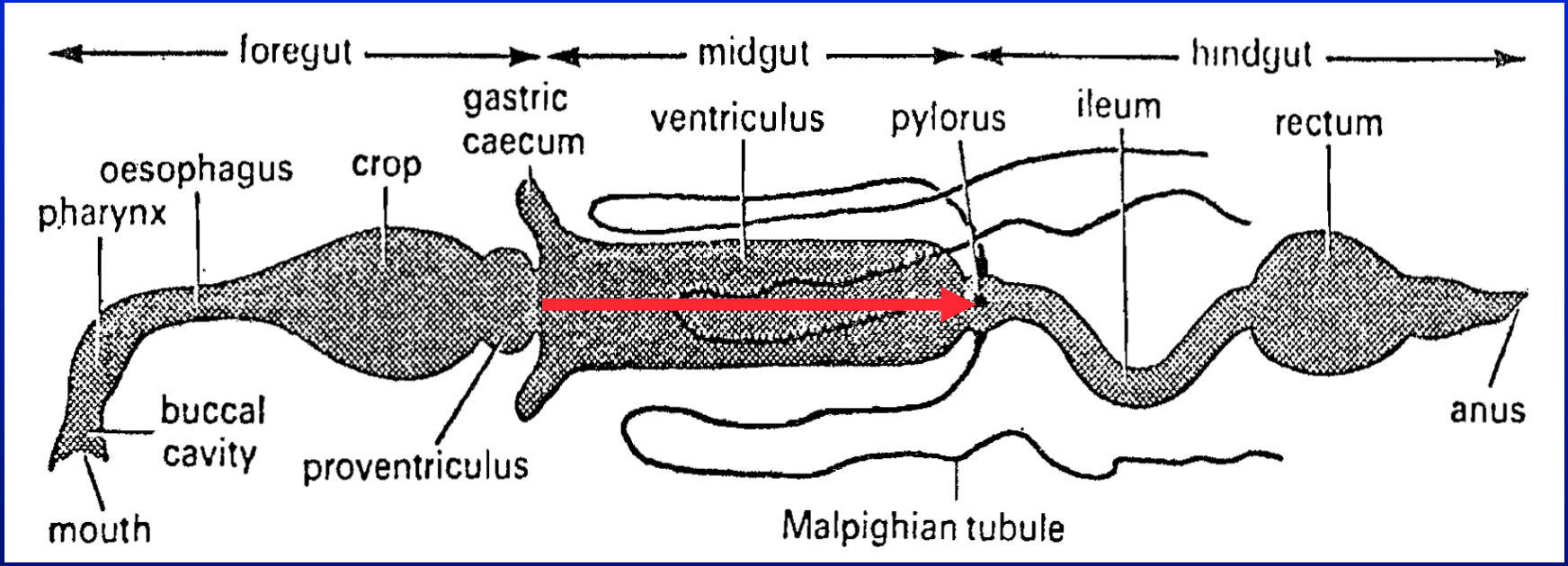
- **Choice**

  Insects can rapidly distinguish between diet with and without Bt toxin; can avoid intoxication even at low (microgram) quantities of Bt

- **Differences in feeding rate**

  Insects can reduce their susceptibility to transgenic plants and Bt absorption by increased feeding rates

Potential Mechanism of Bt Resistance by Differences in Feeding Rate



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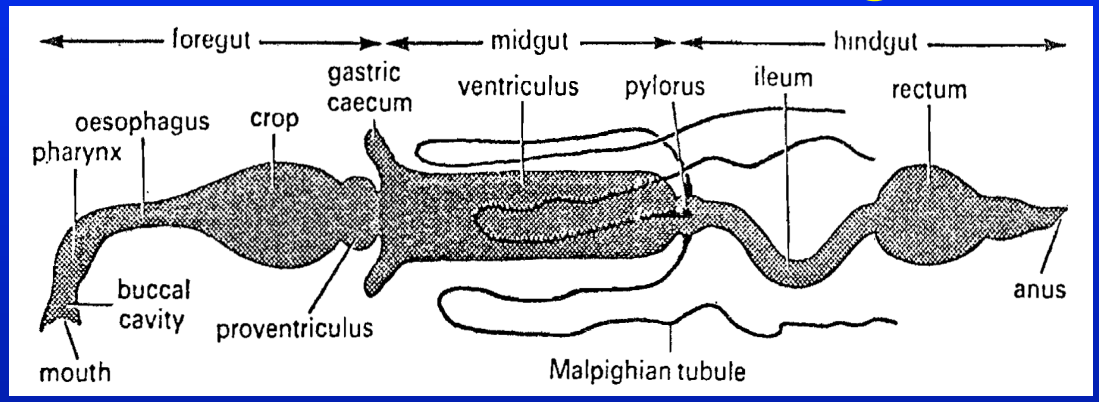

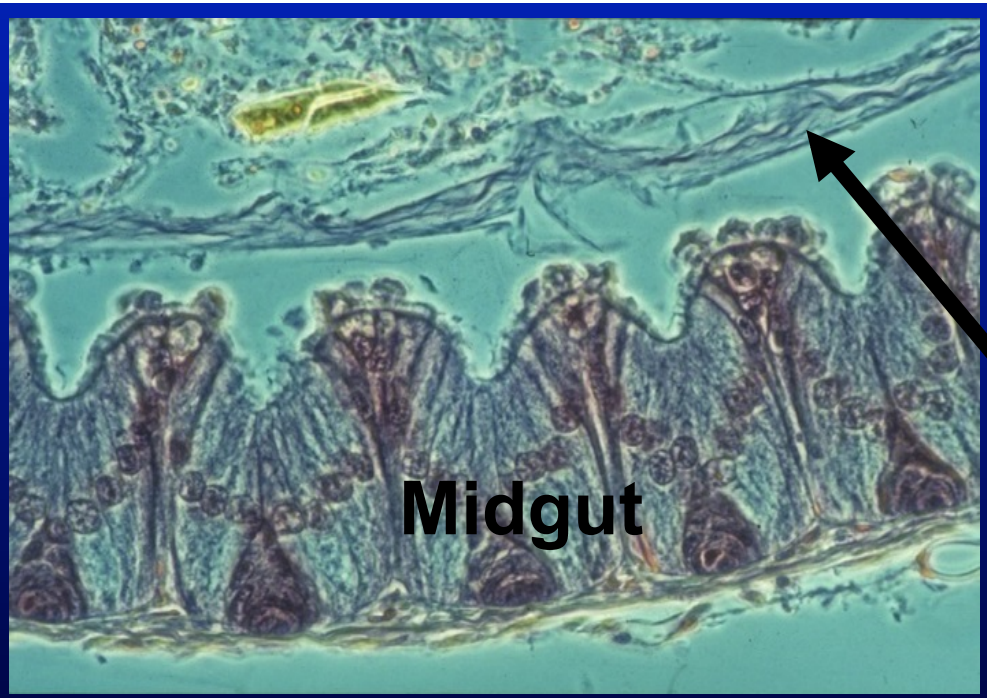

peritrophic membrane

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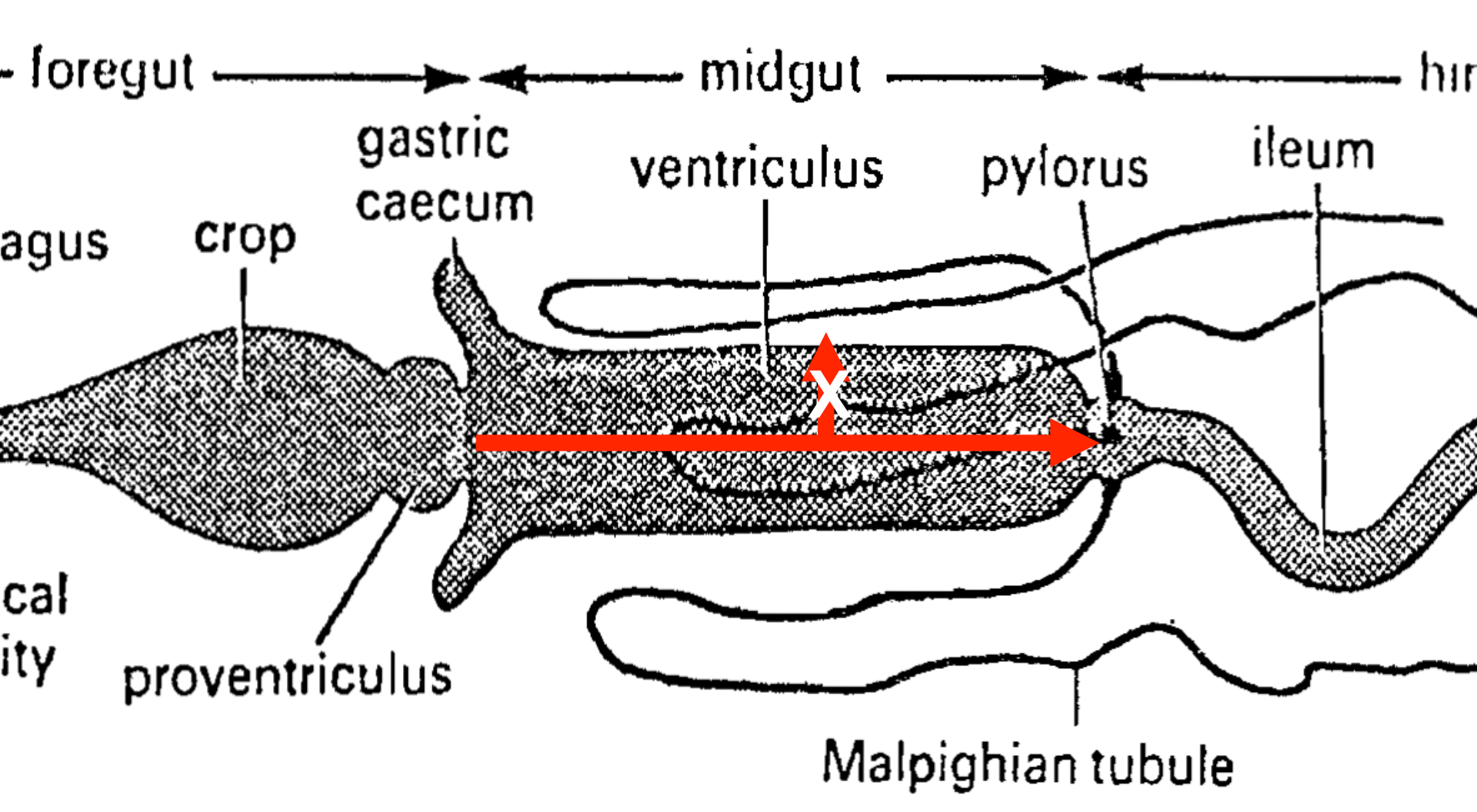
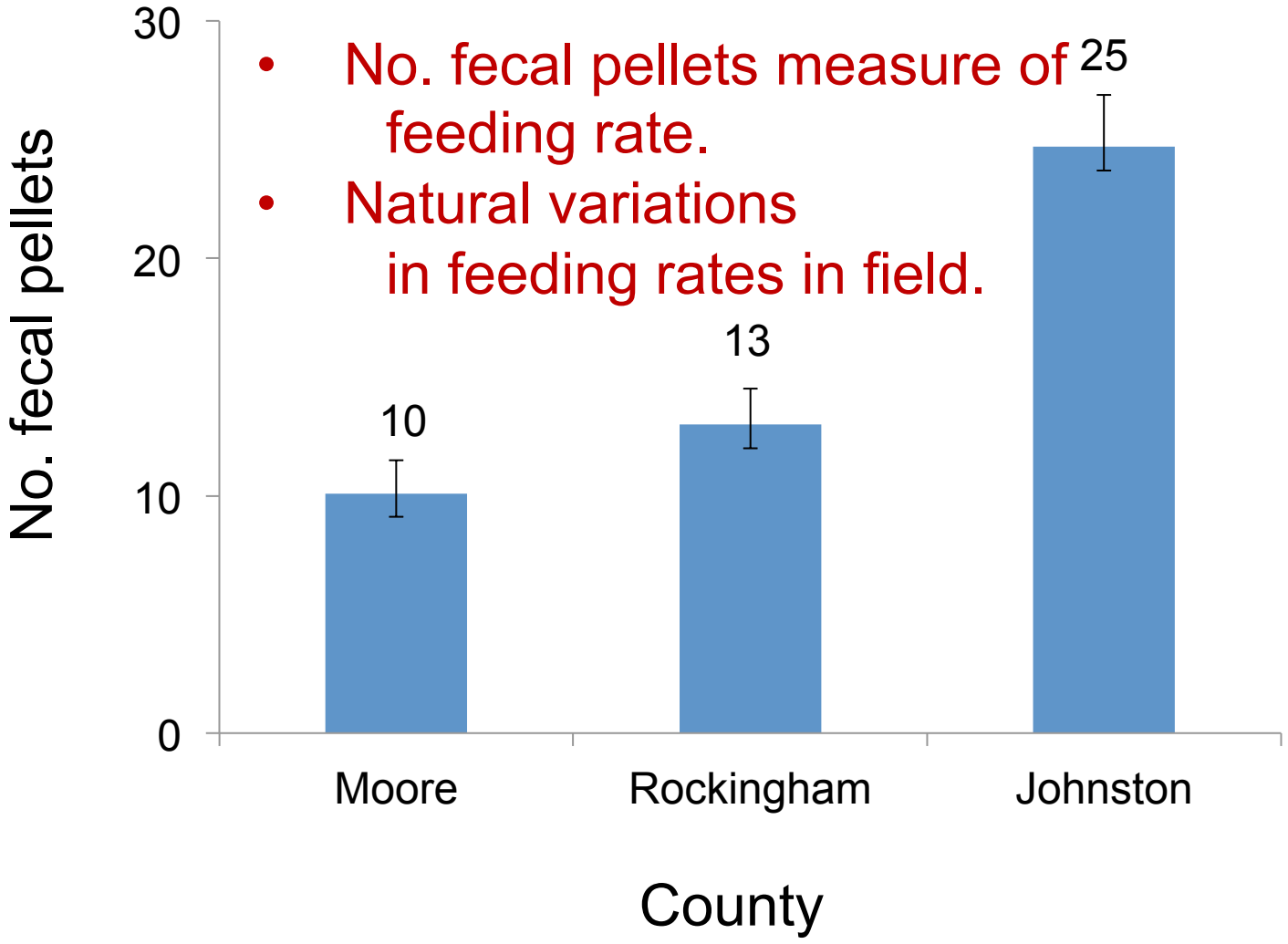
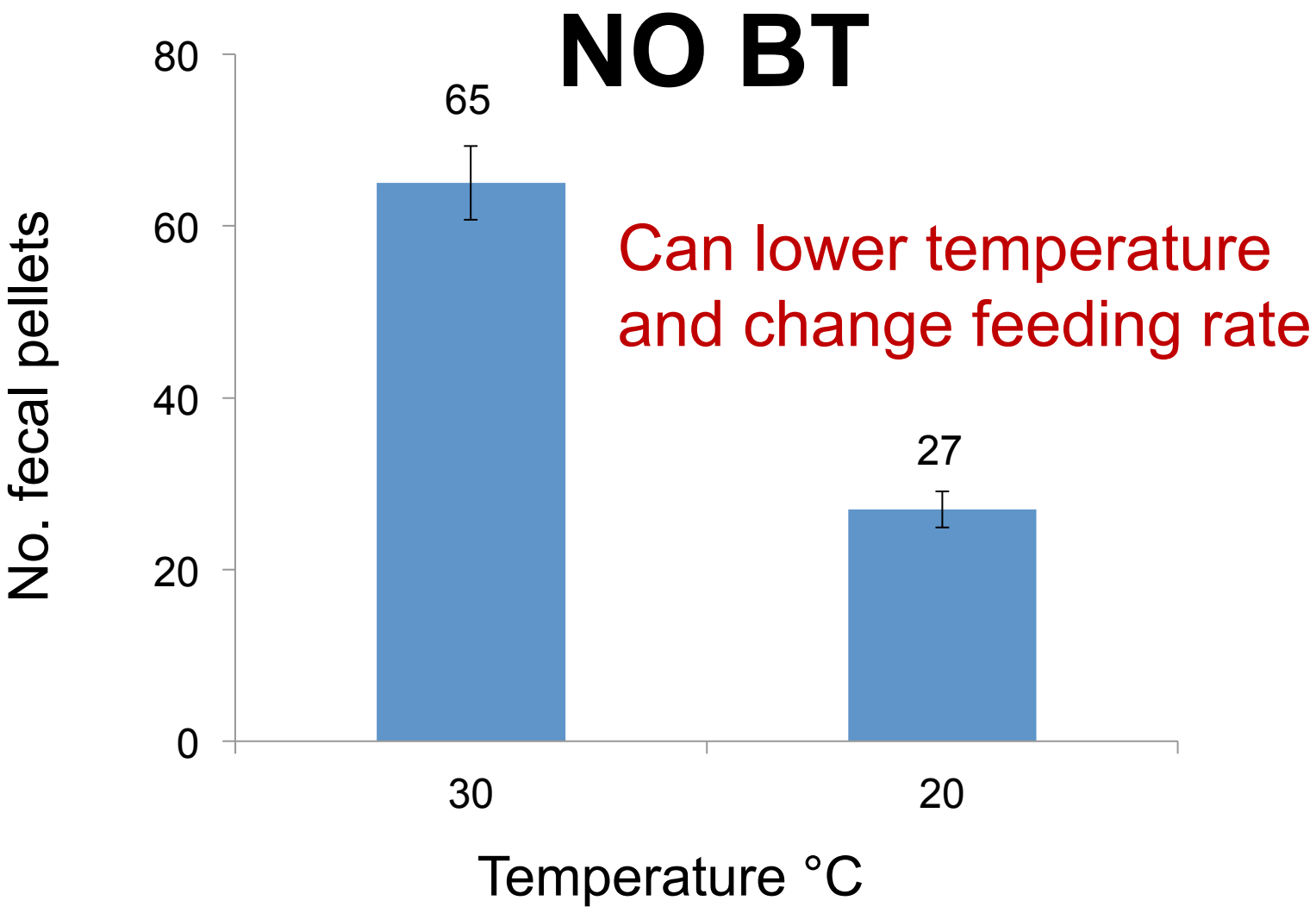


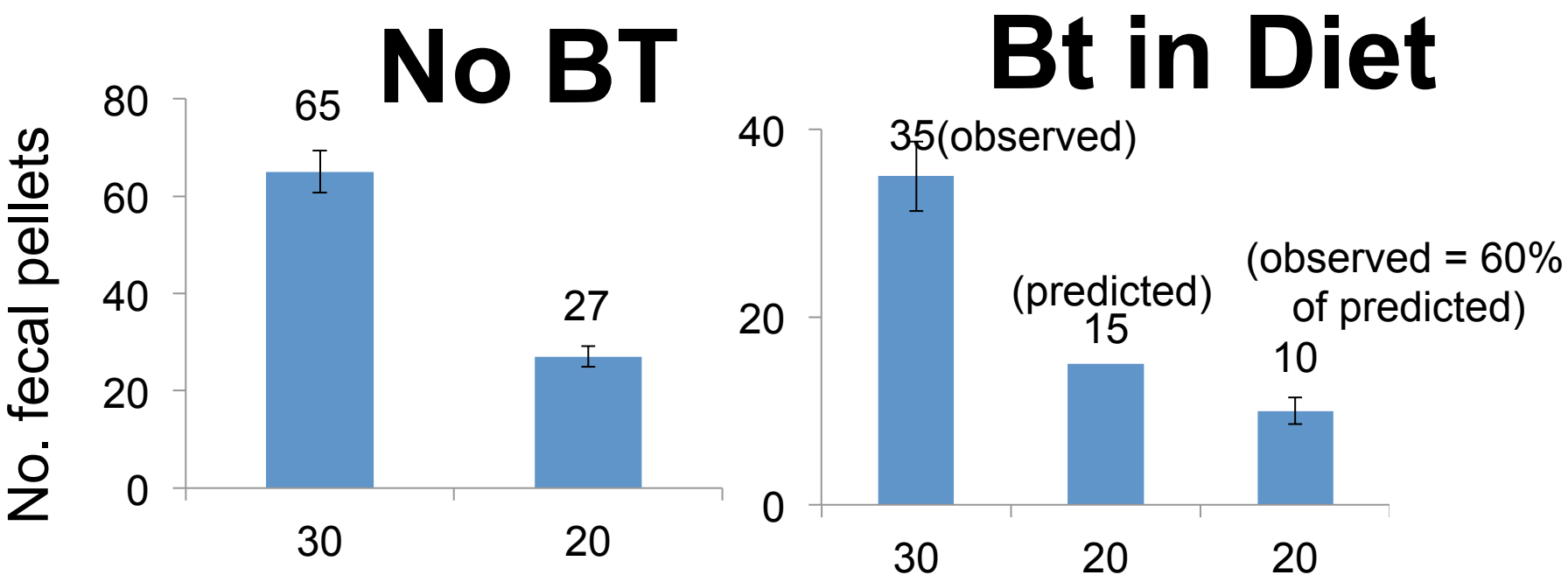
Variation in 24-hour average fecal production of TBW 1st instars (n = 64) collected as eggs from three NC tobacco fields and fed FDT meal pads



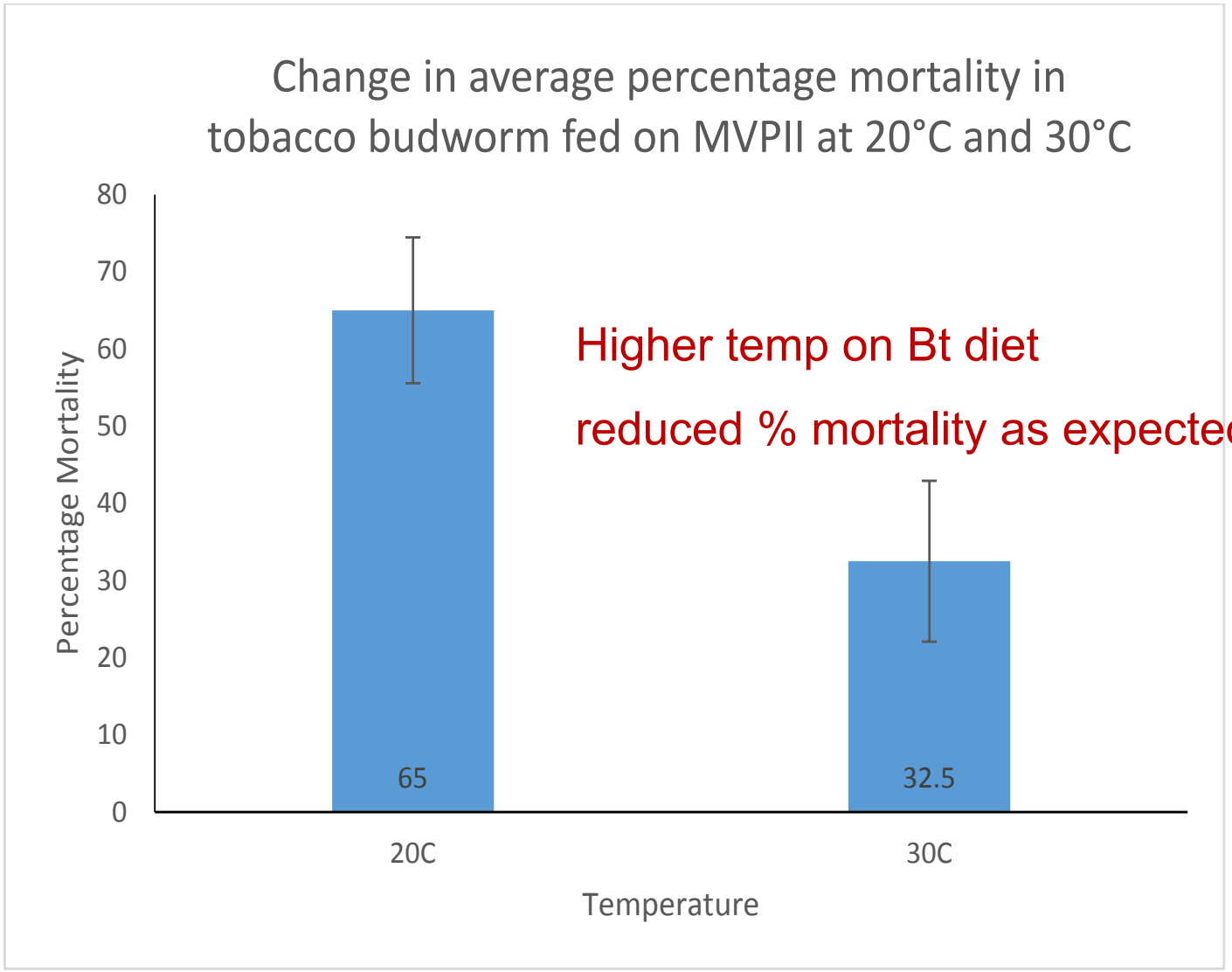
24-hour average fecal production per control TBW 1st instar (n = 45) fed FDT meal pads at 30°C & 20°C



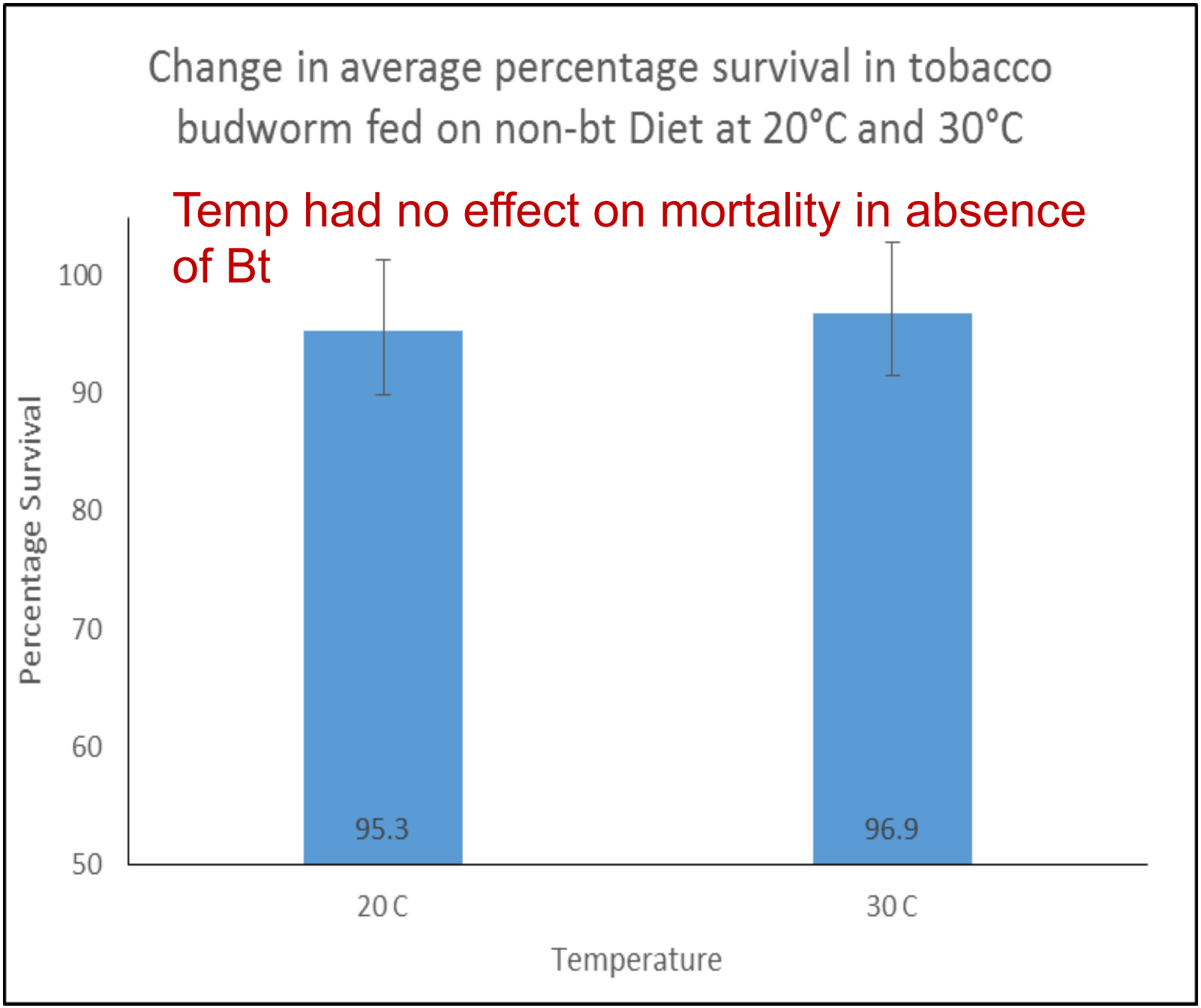
24-hour average fecal production per TBW 1st instar (n = 45) fed MVPII Bt proteins in FDT meal pads at 30°C & 20°C

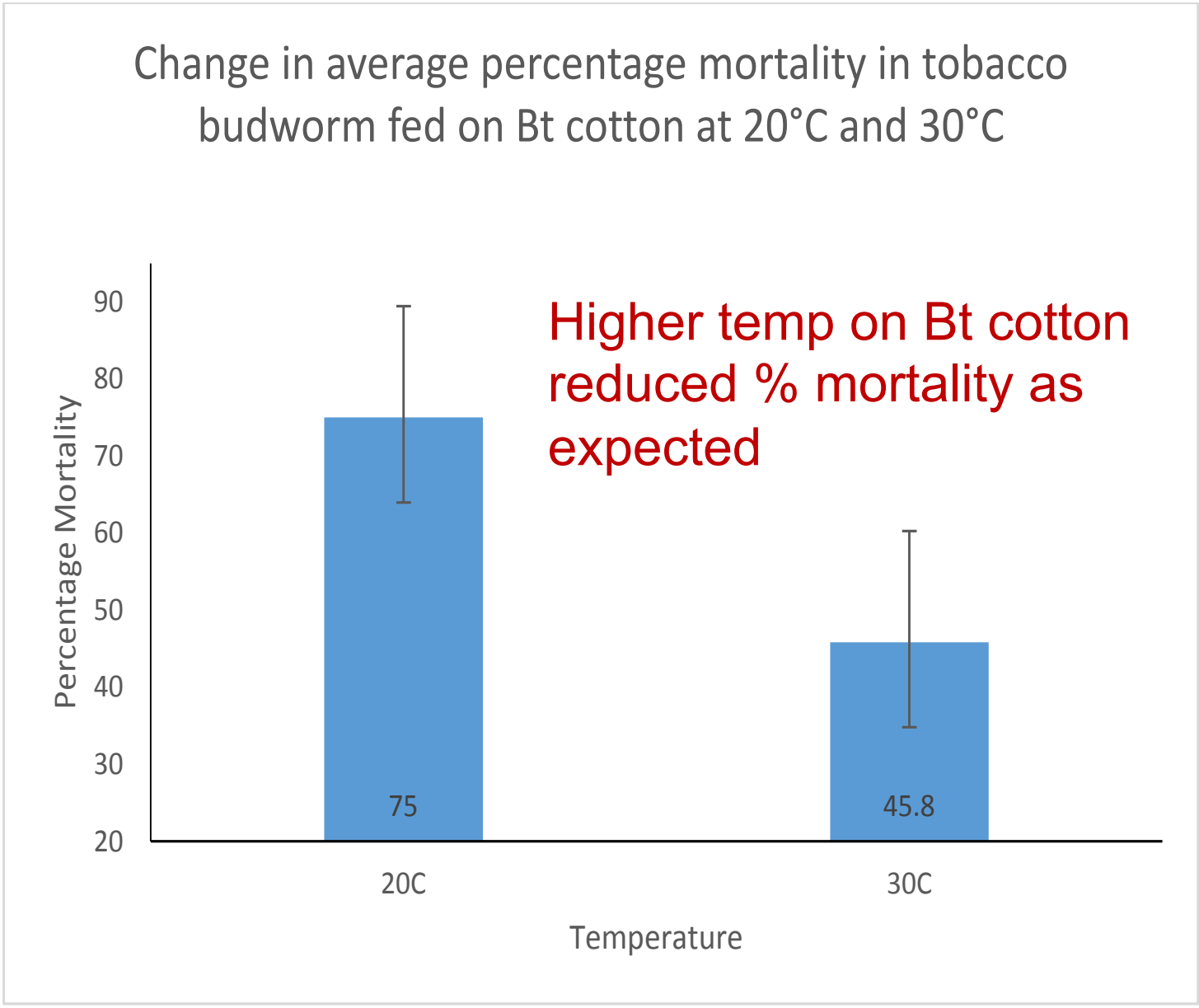


Lower temperature lowered feeding rate and increased Bt susceptibility.

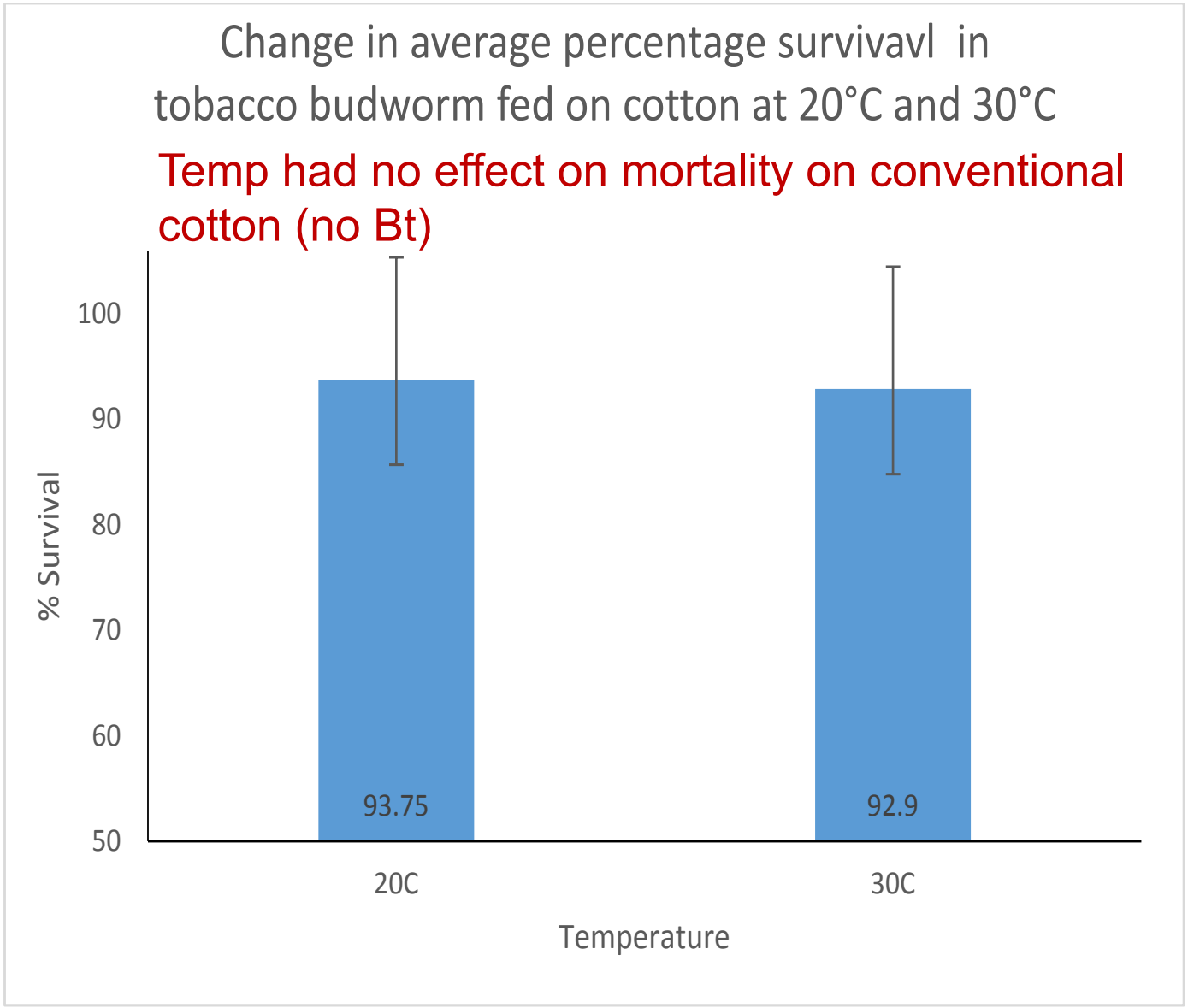


p-value = 0.0001 (Significantly different) ; n= 64


Change in average percentage survival in tobacco budworm fed on non-bt Diet at 20°C and 30°C
Temp had no effect on mortality in absence of Bt
Percentage Survival
100
90
80
70
60
50
95.3
96.9
20 C
30 C
Temperature

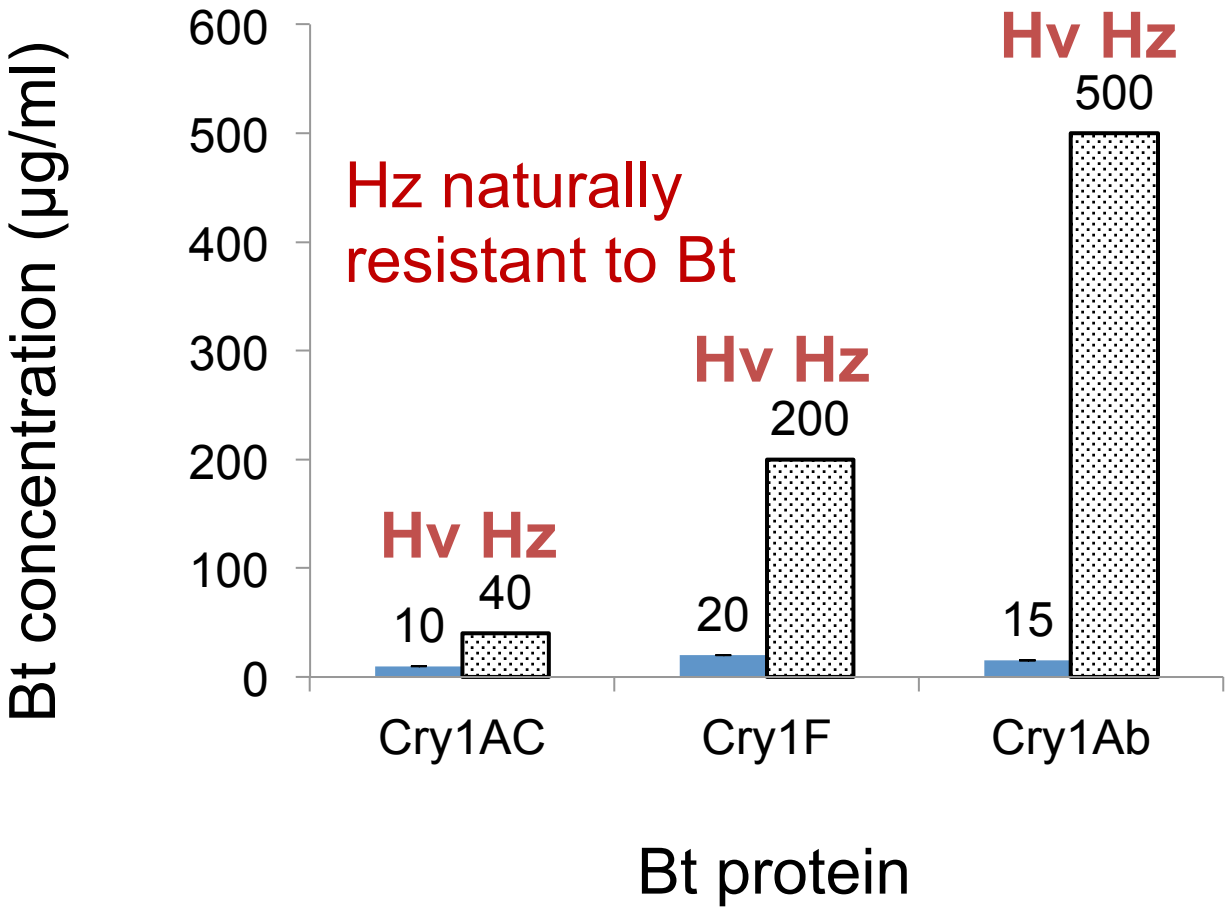


p-value = 0.0008 (Significantly different); n=48

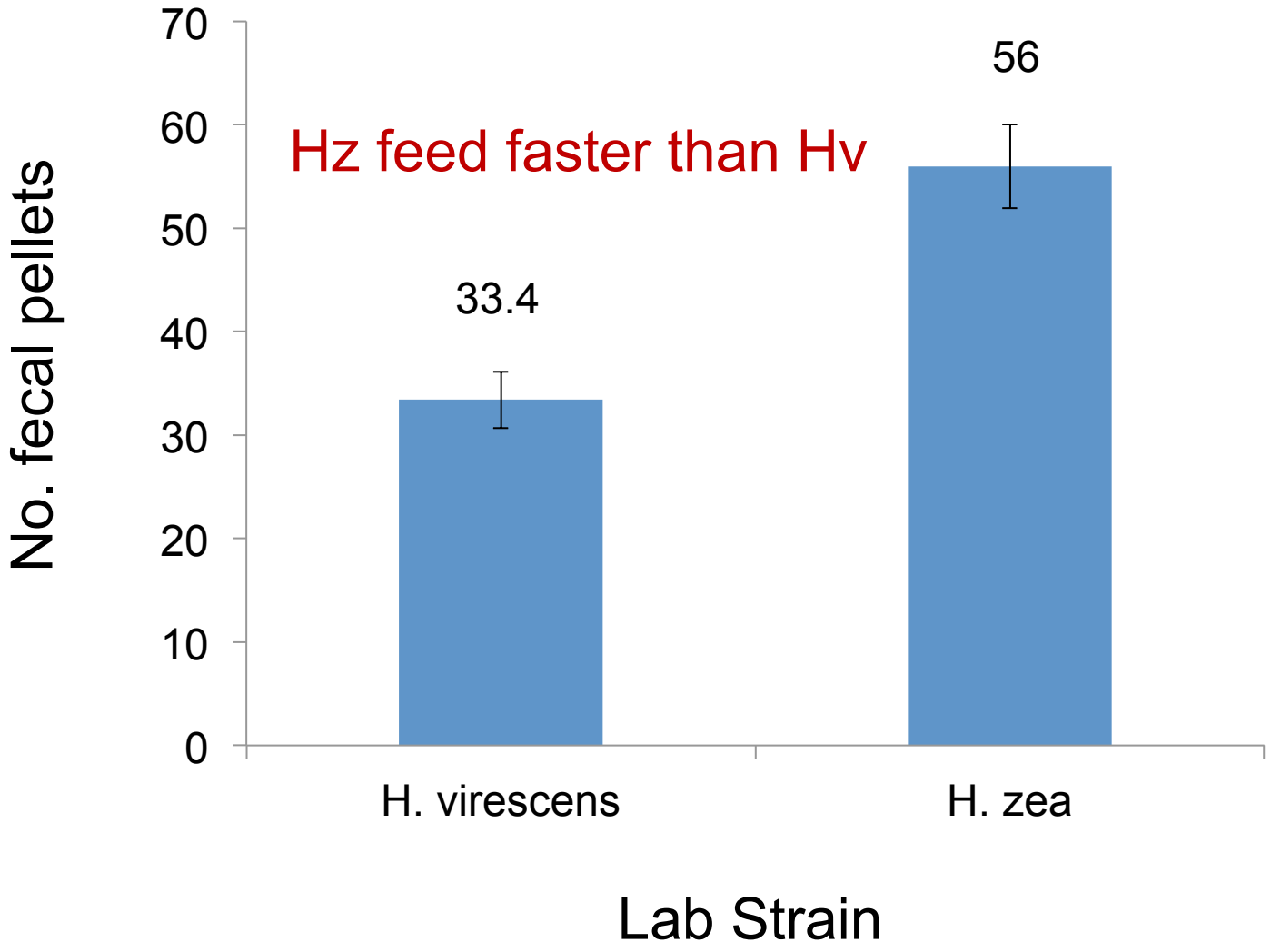


p-value= 0.6 (not-significantly different); n=48

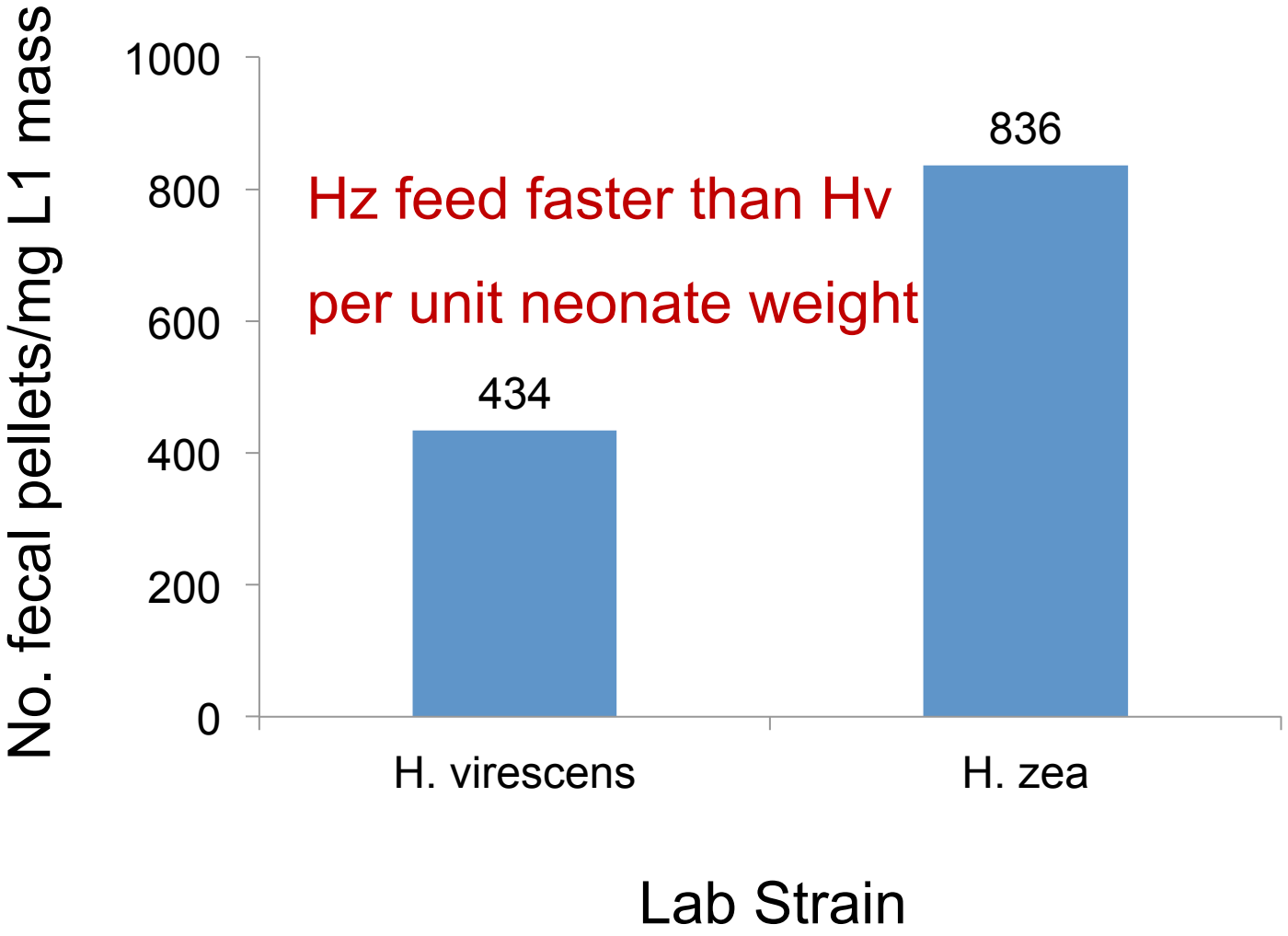
Variation in FDT diagnostic doses for *H. virescens* (blue) vs. *H. zea* (black & white) (n = 28-89)



24-hour average fecal production (n = 55-71) of *H. virescens* & *H. zea* L1s fed FDT meal pads



24-hour average fecal production (n = 55-71) of *H. virescens* & *H. zea* fed FDT meal pads expressed in terms of L1 mass



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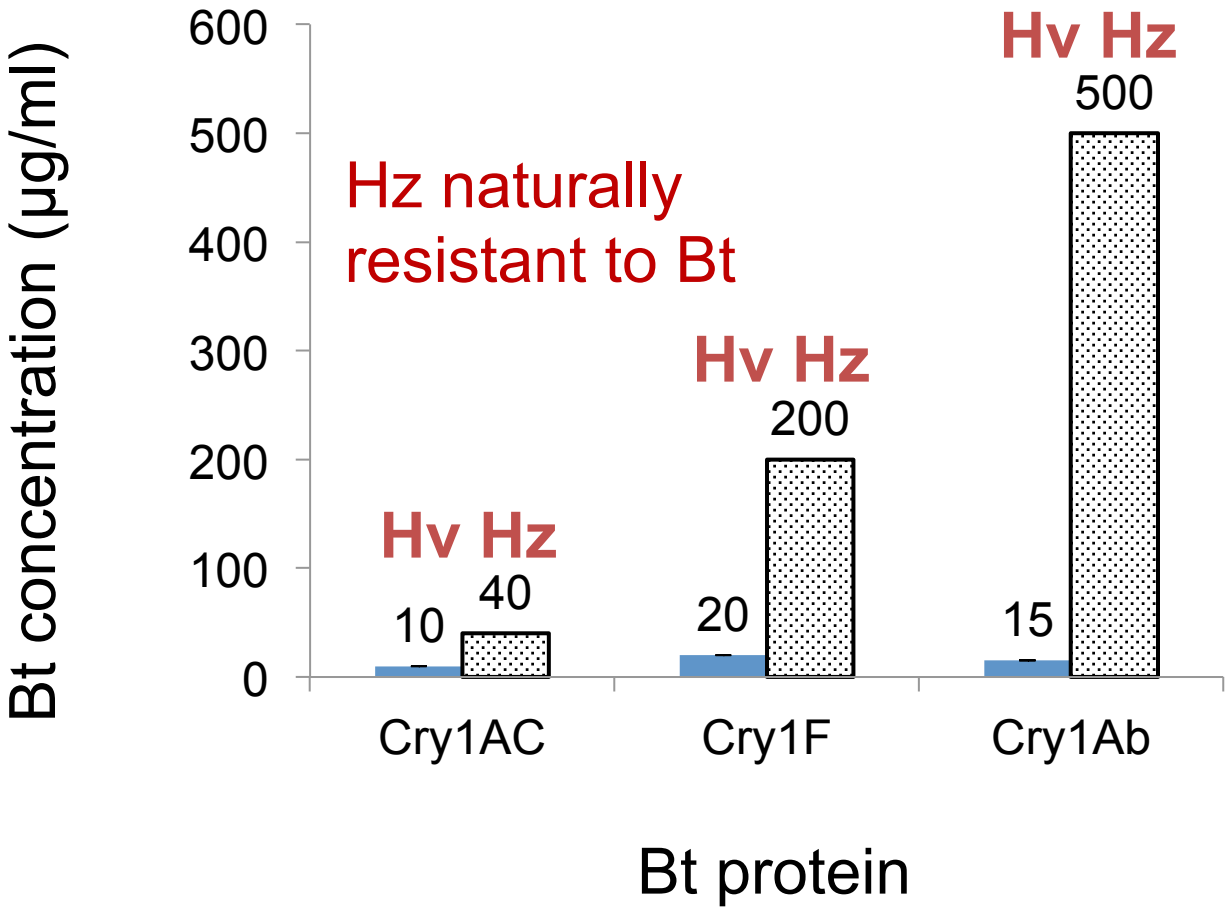
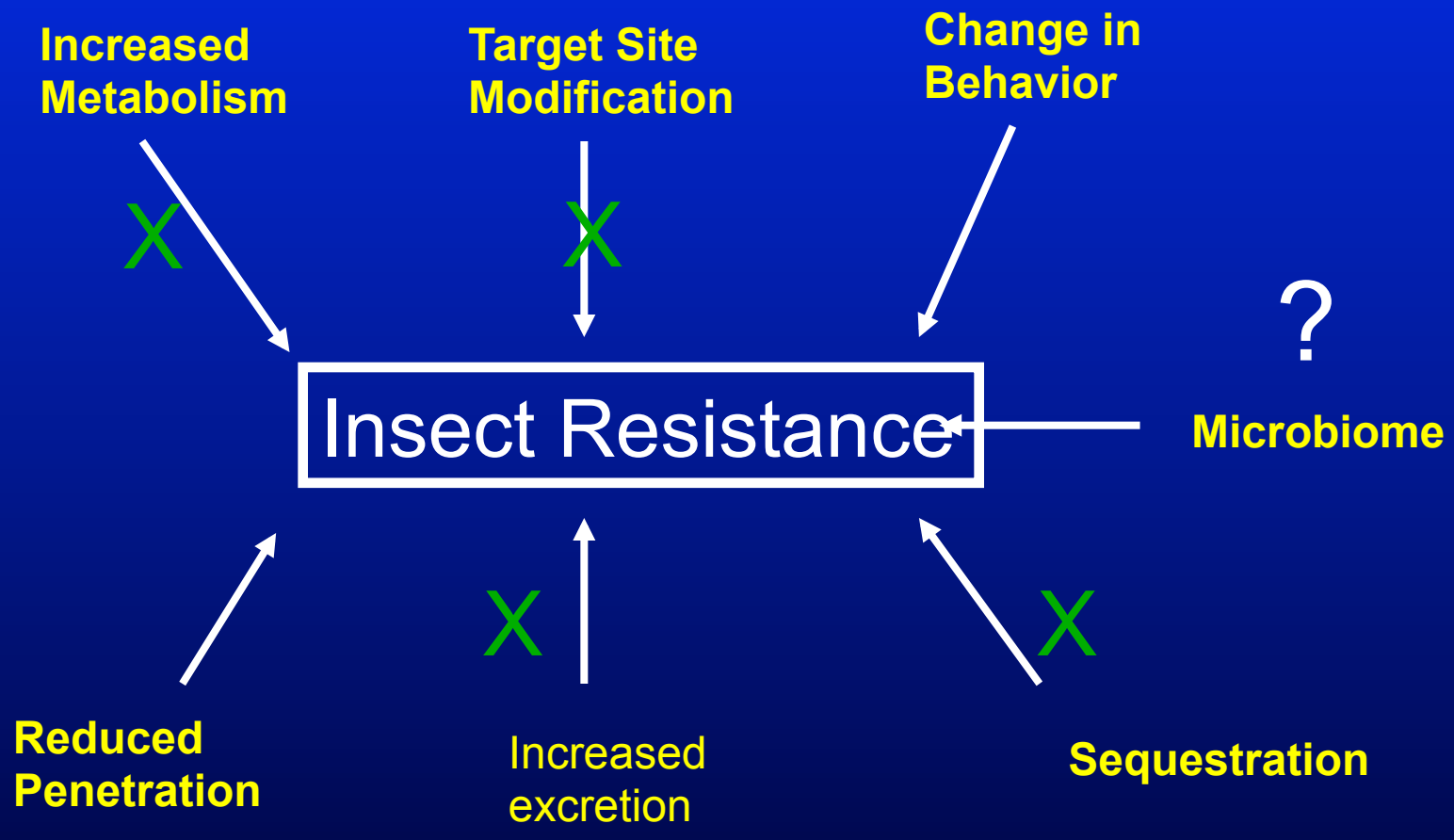
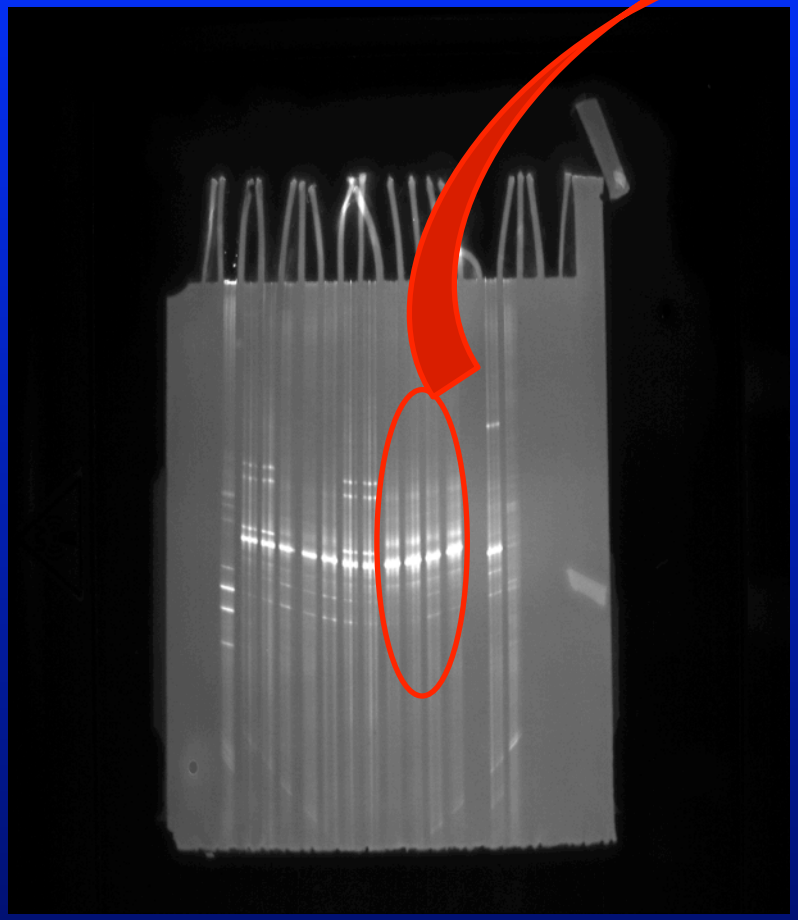


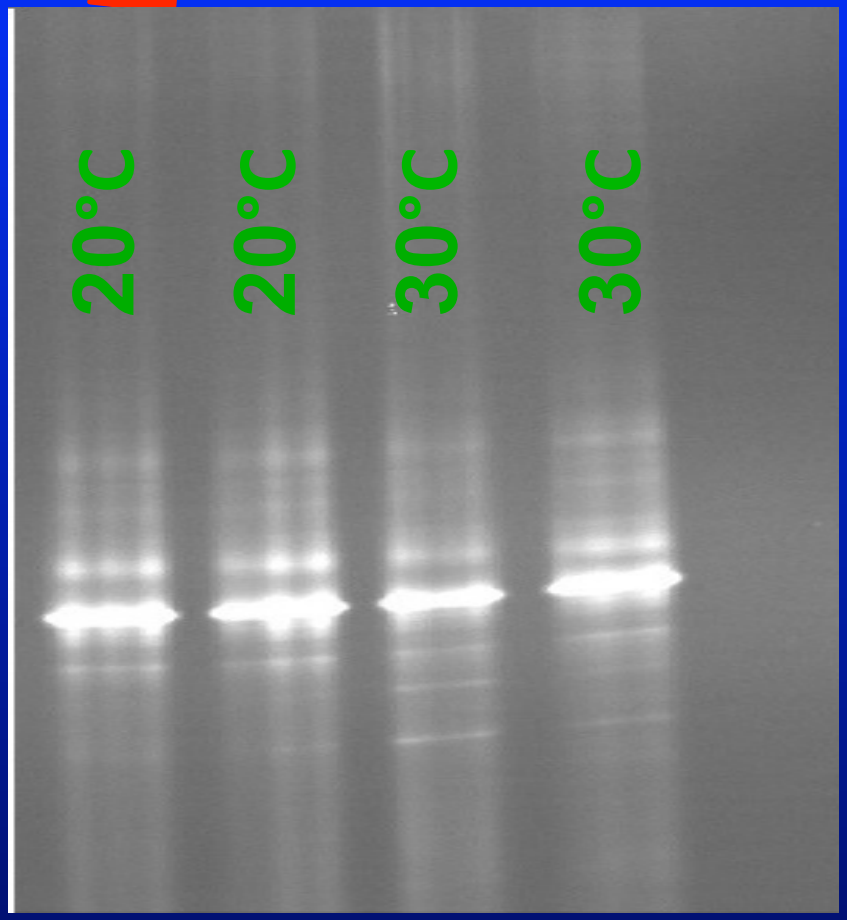
Could changes in the microbiome affect susceptibility to protein and dsRNA toxins?



How does changes in rearing temperature affect the Hv neonate microbiome?

Could differences in the microbiome explain our temperature data?





Temperature change had no effect on microbiome

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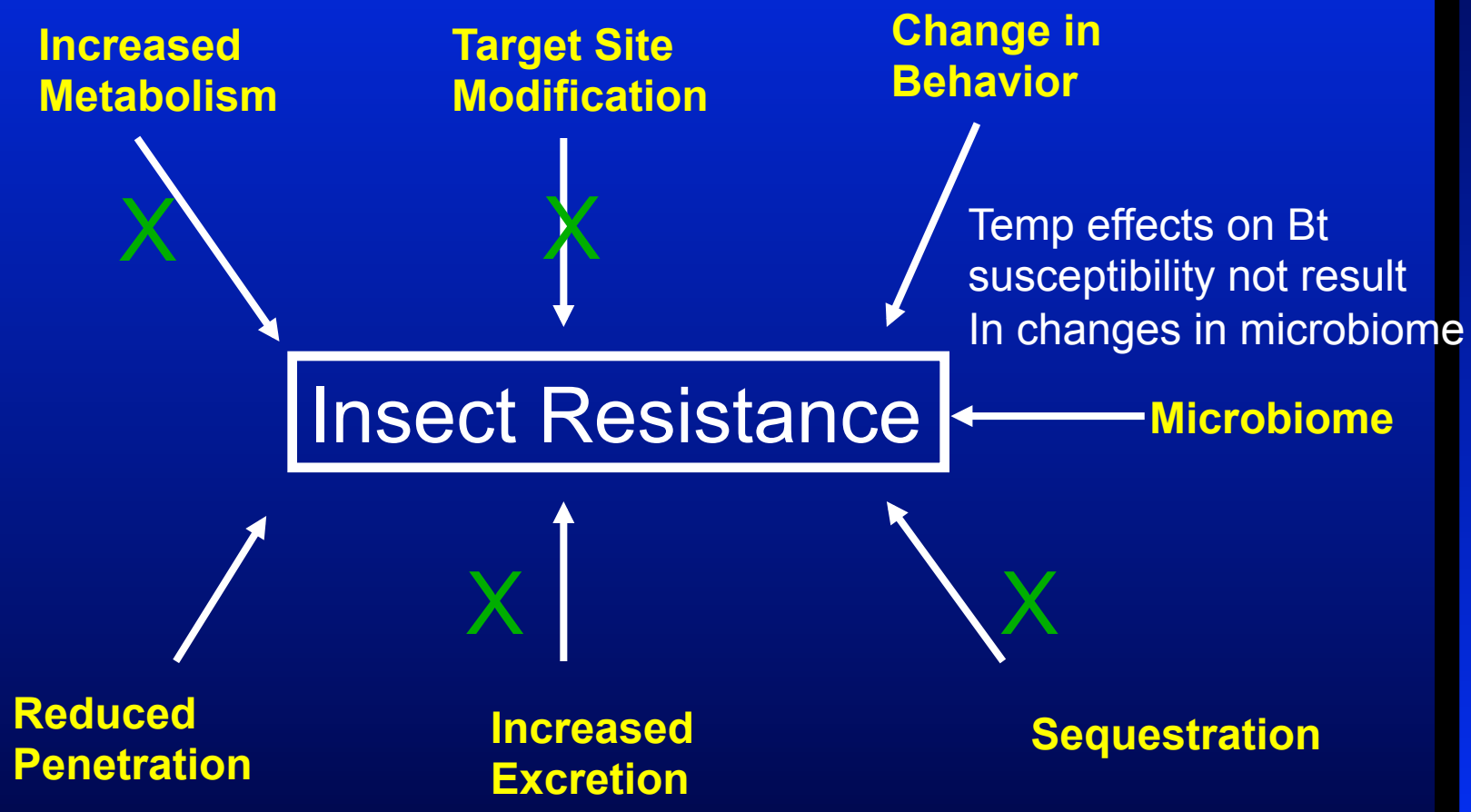
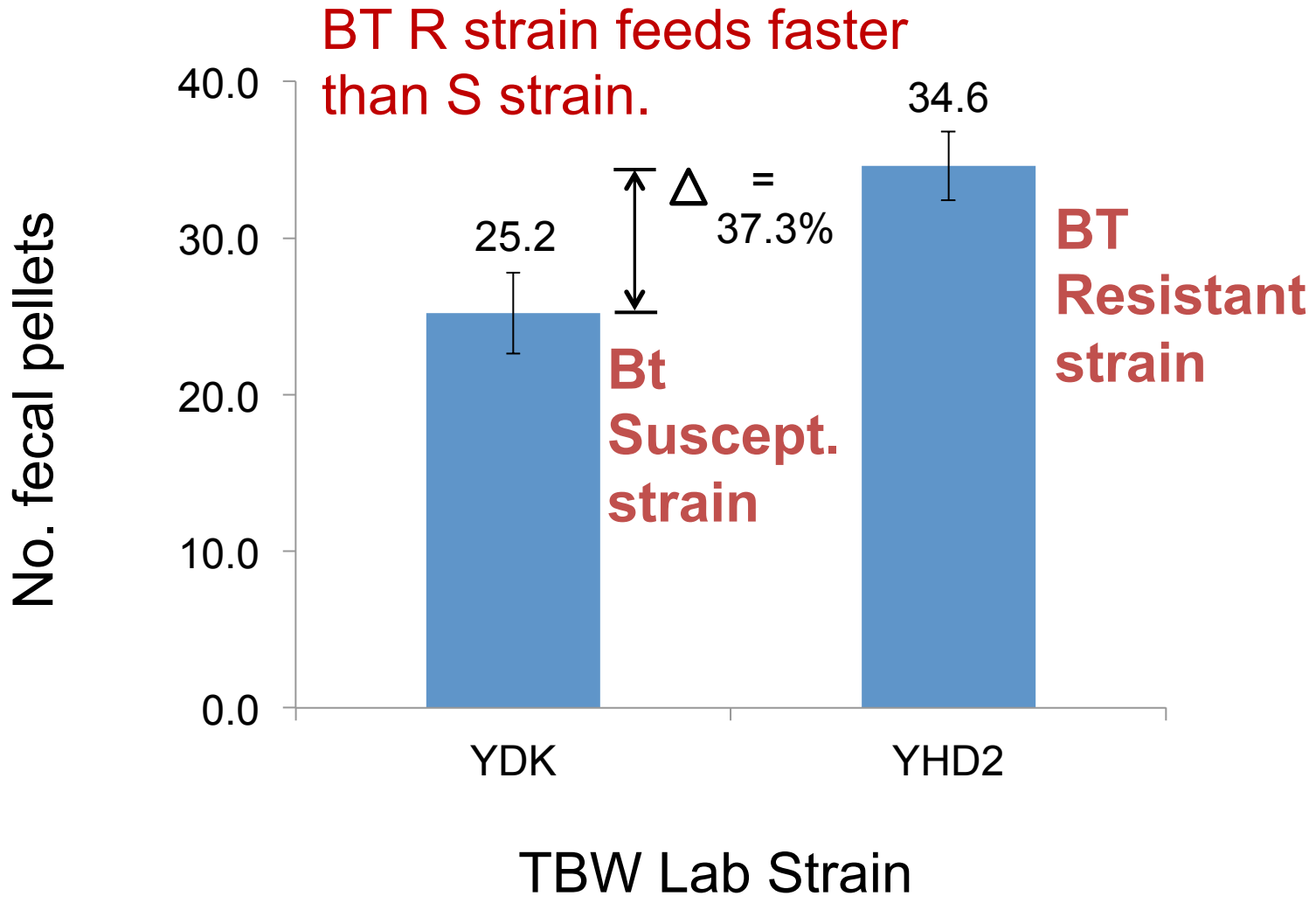


24-hour average fecal production (n = 56-64) of Bt susceptible (YDK) vs. resistant (YHD2) TBW L1s fed FDT meal pads



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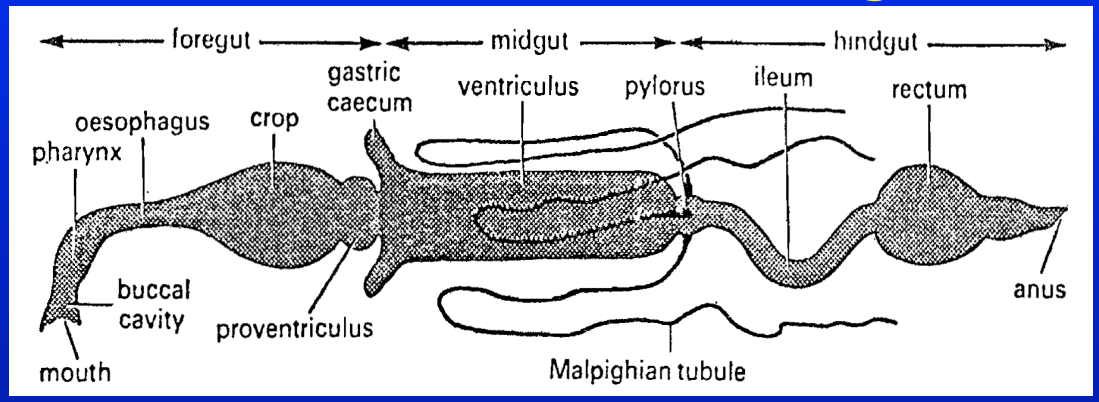

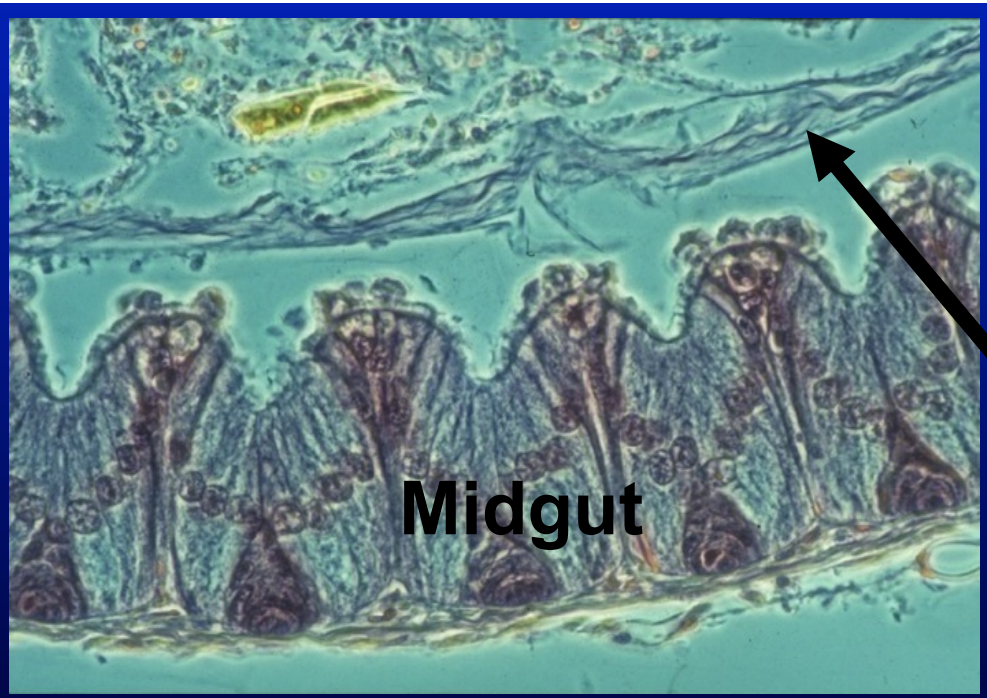

peritrophic membrane

The concern, if this is real, this could affect susceptibility of insects to multiple protein and dsRNA toxins

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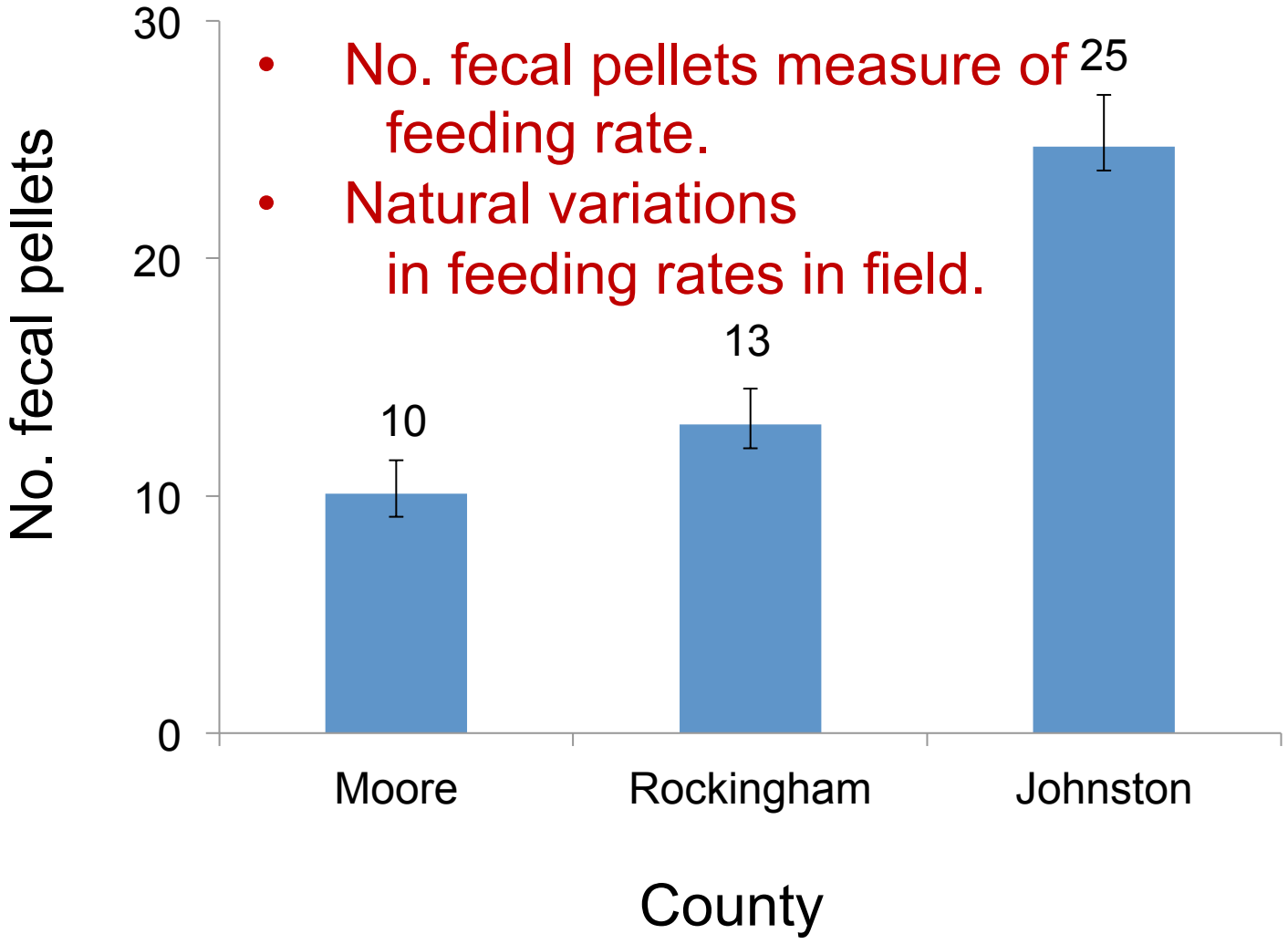


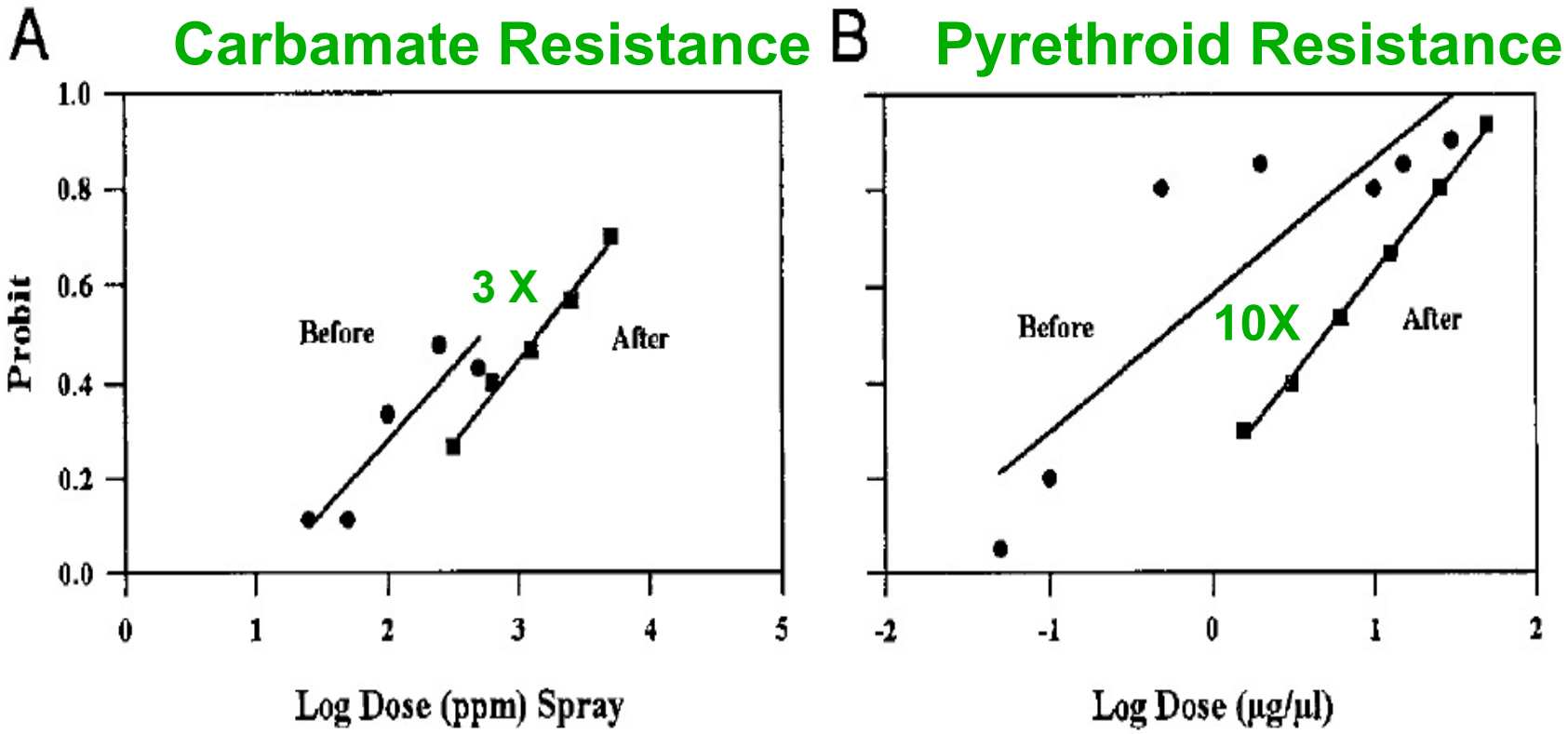
Selection for resistance to protein and RNAi could produce Insects with a higher feeding rate whether on cotton or other host plants.

Is there a

GENE FROM HELL

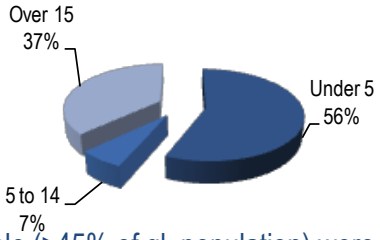
for Insecticidal Chemistries

Selection of tobacco budworms with carbamate increases resistance to pyrethroid faster than the carbamate at LD50 (CORRELATED WITH CYP9A1 IN BOTH STRAINS)

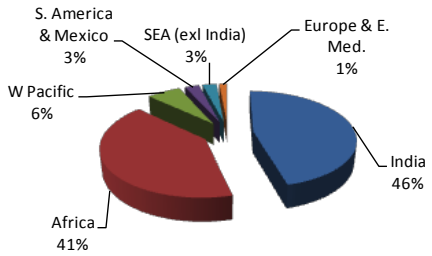


Malaria Facts & Insecticide Resistance

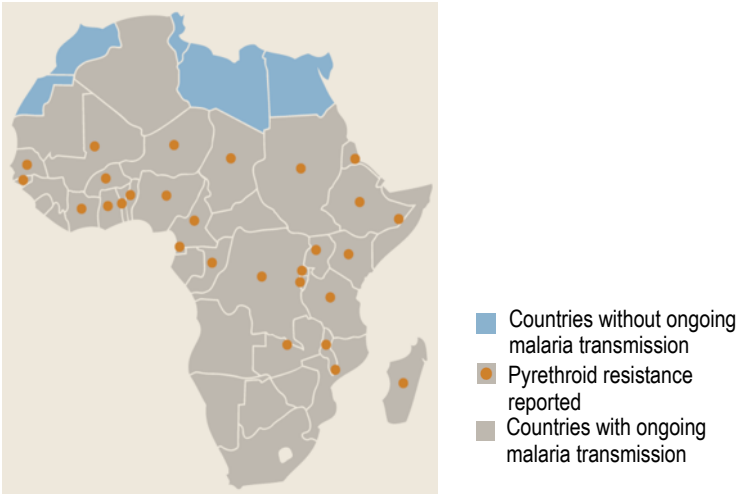
- There were ~1.2 million malaria related deaths in 2010 (IHME)
  - ~2x the WHO reported deaths in 2010 which understated fatalities in children <5 years old in Africa and all ages outside Africa
  - 2010 death by age group (IHME)
  - Children <5 years & pregnant women are most at risk



- 3,3 billion people (>45% of gl. population) were at risk of malaria in 2011
  - Populations living in sub-Saharan Africa are associated with the greatest risk
    - 80% of cases and 90% of deaths occur in WHO Africa Region
- There were >250 million suspected malaria cases in 2010
  - > 226 million cases India & Africa alone



- 2015 WHO global coverage target
  - Global/national mortality caused by malaria is near zero for all preventable deaths and global incidences of malaria are reduced by 75%
- An alarming pattern of mosquito resistance to pyrethroids is rapidly emerging across Africa
  - African countries reporting pyrethroid resistance in 2011 in at least one malaria vector and one monitoring site



- In 2010 the proportion of mosquitoes resistant to deltamethrin was reported to be 37%
- The genetic resistance of Anopheles gambiae to one type of insecticide rose from 8% to 48% between 2007 and 2010

Source: Institute for Health Metrics and Evaluation (IHME)
www.guardian.co.uk/news/datablog/2012/feb/03/malaria-deaths-mortality#data
World Malaria Report 2011 & 2012
Prevention and Management of Insecticide Resistance in Vectors of Public Health Importance
Mosquitoes 'developing resistance to bed nets, BBC World Service, 08/172011

So what can we do about

A GENE FROM HELL

Innovation Critical

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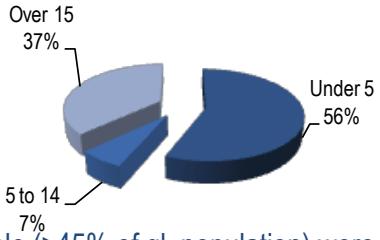

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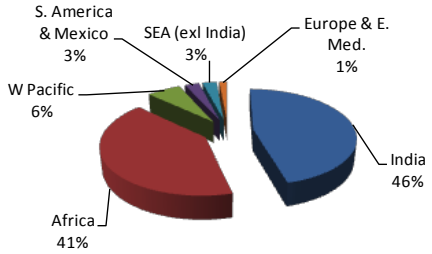

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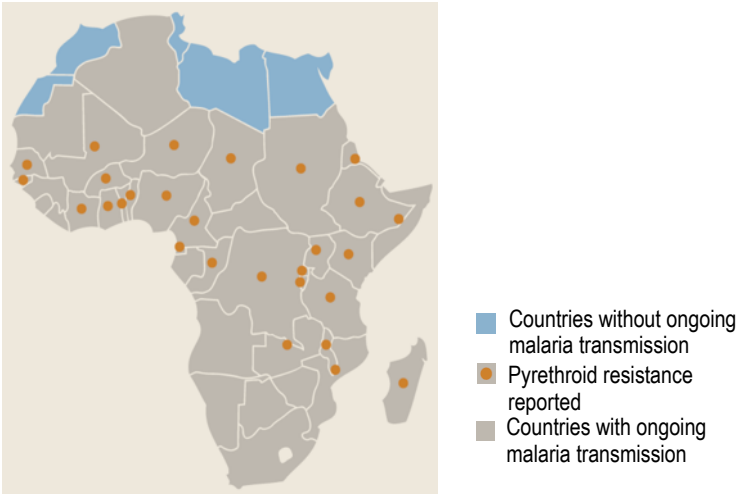

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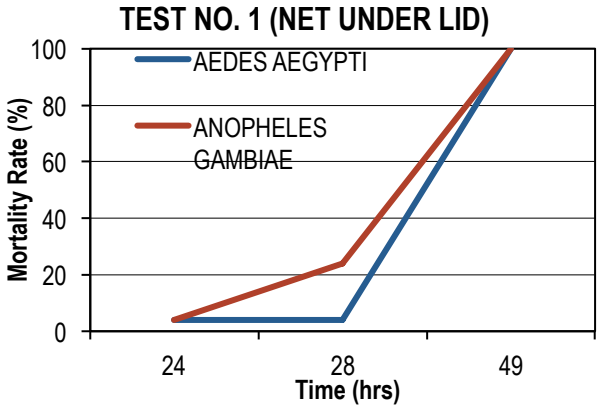
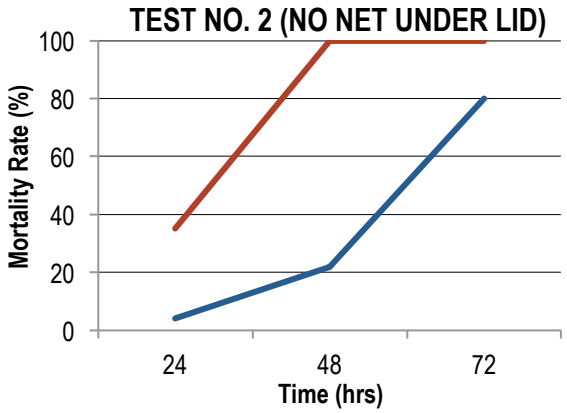
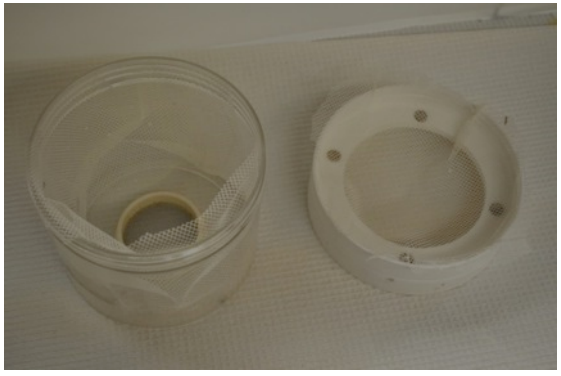
NC STATE UNIVERSITY
IMERYS TRANSFORM TO PERFORM
IVCC COMBATING INSECT BORNE DISEASE

NON INSECTICIDAL BED NETS

- NCSU mosquito efficacy studies (NCSU Agreement #130960MA, dated 05/28/2013))
  - The test consisted of 25 adult mosquitoes total (males and females) per container
  - This test system was a worst case condition where there was no host seeking behavior/net interaction (static conditions)
  - Test conducted at 27 °C and ~60% humidity
  - Physical acrylic emulsion treated bed net matrix

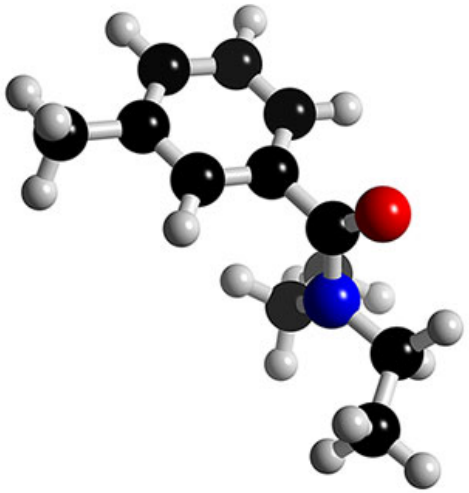






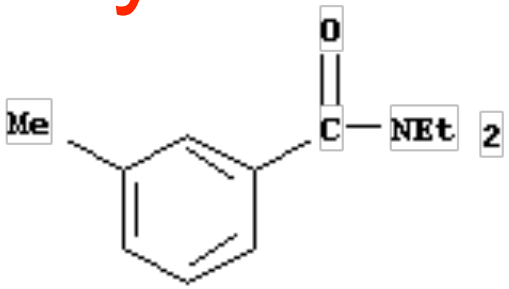
- An adult female mosquito usually lives for approximately 2 weeks
- The extrinsic incubation period of the malaria parasite is 10-21 days
  - If a mosquito does not survive longer than the extrinsic incubation period then the malaria parasites cannot be transmitted

Personal Repellents Important for the Control of Mosquitoes and Ticks



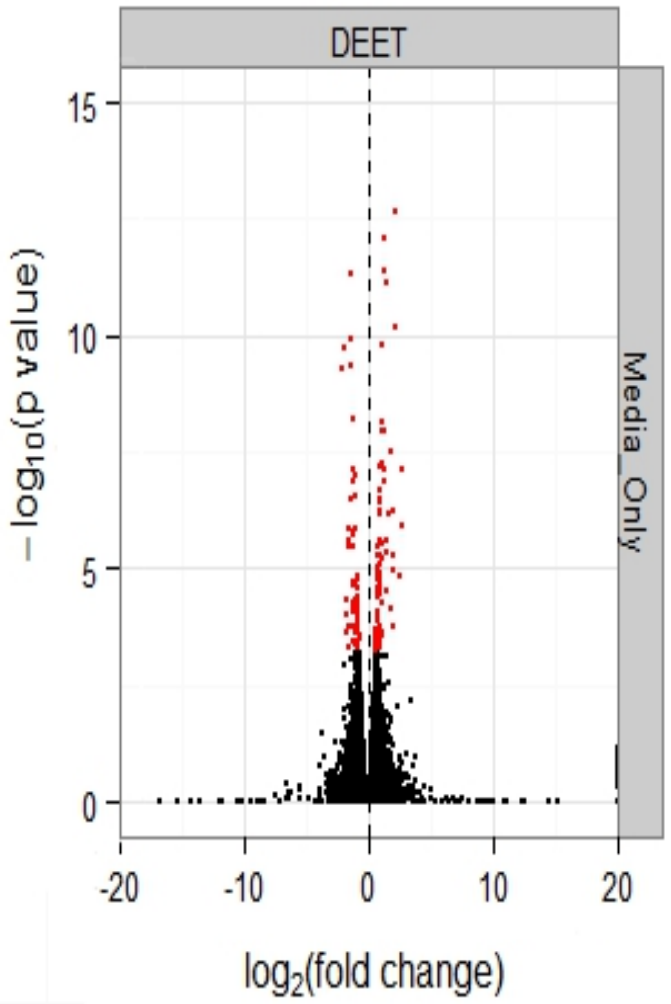
Gold Standard is DEET

- Around for >50 years
- Broad spectrum
- Long-lasting
- Safety of Deet



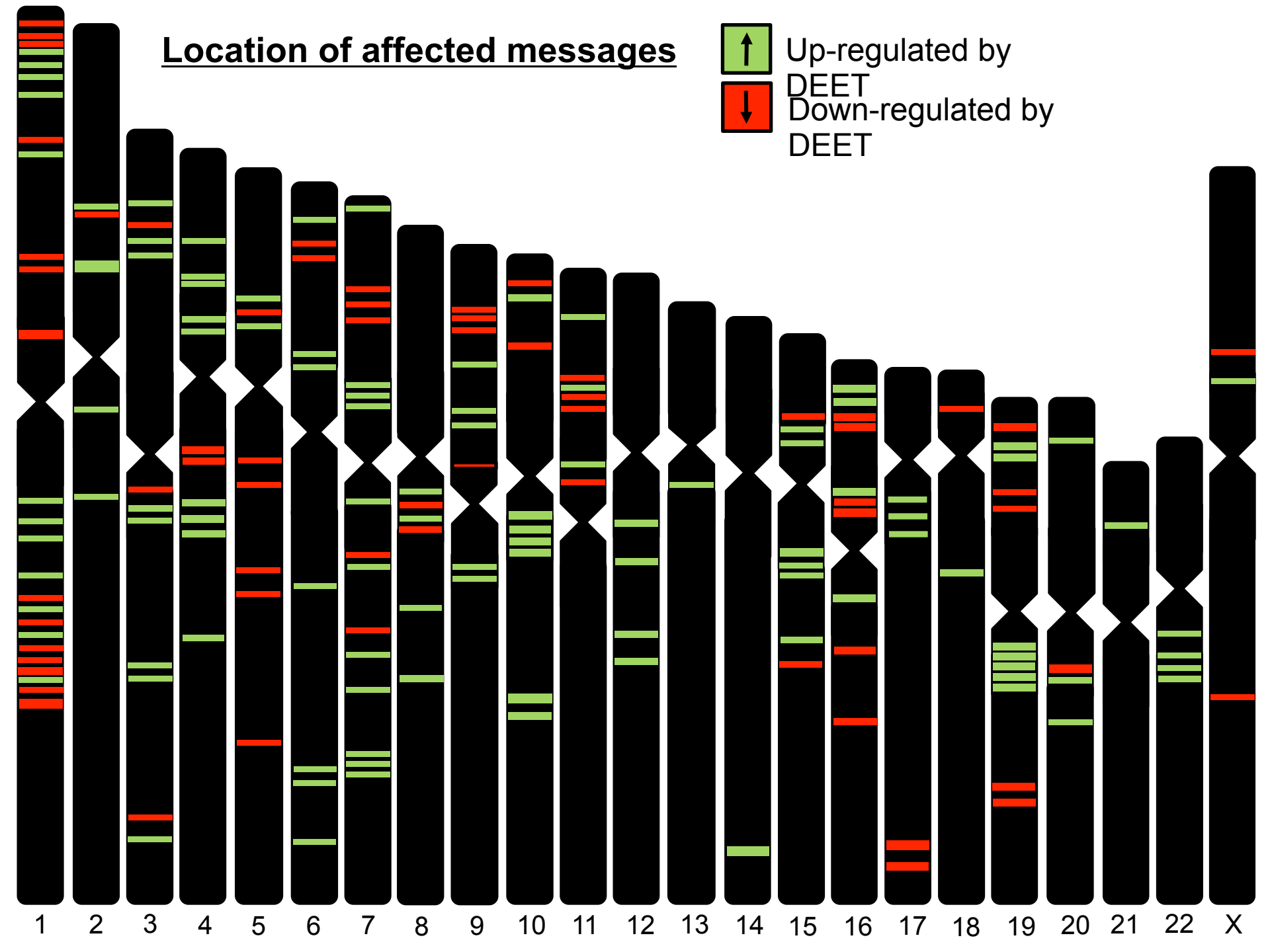
What is the impact of DEET on primary human hepatocytes after a 72 h exposure

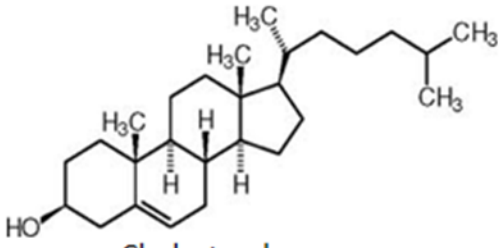
Volcano plot of comparisons between DEET and Media Only data.



172 messages up or down regulated

significant
no
yes


Location of affected messages
Up-regulated by DEET
Down-regulated by DEET
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X



Cholesterol

Down-regulated gene

Up-regulated gene

Pregnenolone → 17α-OH-Pregnenolone → Dehydroepiandrosterone

3β HSD

3β HSD

3β HSD

Progesterone → 17α-OH-Progesterone → Androstenedione

17, 20 lyase

POR

CYB5A

17β HSD

11-Deoxycorticosterone

11-Deoxycortisol

Oestrone

Testosterone

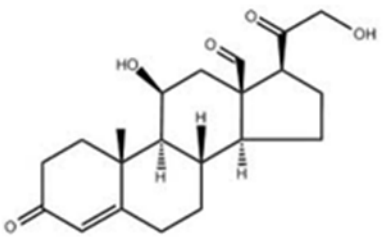
17β HSD

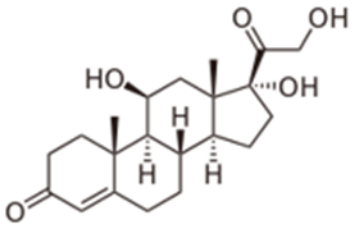
Corticosterone

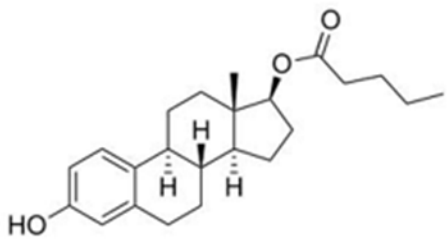
Cortisol

Oestradiol

Aldosterone

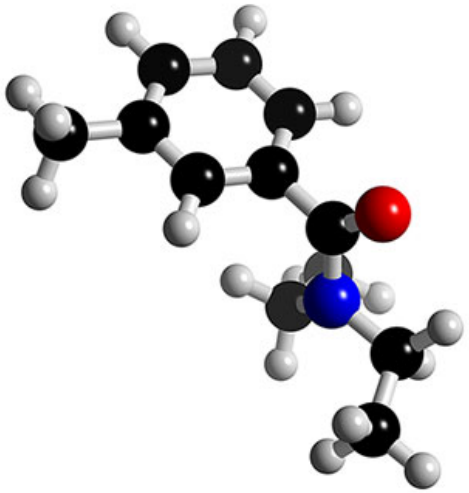






Sequence Name	Sequnece Description	Seq. Length	#Hits	min. eValue	mean Similarity	#GOs	expression with DEET	Chromosome
gi|28476829|ref|NR_001278.1|	ncRNA_cytochrome p450 2b6-like (CYP2B7P1)	3000	20	0	94.30%	10	up	chr19:41430169-41456565
gi|153791676|ref|NR_003610.1|	ncRNA_pyridoxal-dependent decarboxylase domain-containing containing 2, pseudogene (PDXDC2P)	4298	20	0	97.65%	7	down	chr16:70010201-70099851
gi|141801873|ref|NR_002934.2|	ncRNA_scavenger receptor protein family member (LOC619207)	4918	10	0	88.50%	2	down	chr10:135267431-135281953
gi|223555922|ref|NR_026816.1|	ncRNA_psoriasis susceptibility 1 candidate gene 3 (PSOR1C3)	600	20	1.19E-47	70.95%	6	down	chr6:31141511-31145676
gi|284813500|ref|NR_033266.1|	ncRNA_was protein family homolog 2-like; WAS protein family 5 homolog pseudogene (WASH5P)	1137	20	4.20E-24	93.95%	9	down	chr19:60950-70966
gi|223972624|ref|NR_026936.1|	ncRNA_isoform cra_a; chromosome 5 open reading frame 27 (C5orf27)	1931	20	2.61E-57	82.00%	14	down	chr5:95187935-95195836
gi|226053461|ref|NR_027440.1|	ncRNA_unnamed portein product; uncharacterized LOC100272217 (LOC100272217)	2159	20	4.77E-25	66.55%	4	down	chr9:133452736-133454881
gi|213385260|ref|NR_024456.1|	ncRNA_histone demethylase uty-like; uncharacterized LOC100190986 (LOC100190986)	2453	20	5.62E-32	81.10%	16	down	chr16:21443344-21445776
gi|327412331|ref|NR_038080.1|	ncRNA_chromosome 17 open reading frame 55; long intergenic non-protein coding RNA 482 (LINC00482)	2970	20	6.02E-147	77.00%	9	down	chr17:79276623-79283048
gi|219555684|ref|NR_002817.2|	ncRNA_aquaporin adipose; aquaporin 7 pseudogene 1 (AQP7P1)	3180	20	2.34E-51	92.25%	15	down	chr9:67270214-67289492
gi|302699227|ref|NR_036530.1|	ncRNA_unnamed protein product; uncharacterized LOC100289230 (LOC100289230)	1876	19	5.31E-25	73.68%	0	down	chr5:98264837-98266713
gi|345842504|ref|NR_027455.3|	ncRNA_flj44451 fis; uncharacterized LOC100131434 (LOC100131434)	2348	13	5.26E-96	91.69%	0	down	chrX:148609131-148621312
gi|341932552|ref|NR_040662.1|	ncRNA_hla complex isoform cra_a; HLA complex P5 (non-protein coding)(HCP5)	2547	11	1.14E-61	70.73%	1	up	chr6:31430956-31433586
gi|255306270|ref|NR_028272.1|	ncRNA_nuclear paraspeckle assembly transcript 1 (non-protein coding)(NEAT1)	3756	5	9.82E-43	97.80%	0	down	chr11:65190268-65194003
gi|378548194|ref|NR_046377.1|	ncRNA_isoform cra_a; hCG1813624 (LOC728040)	763	3	6.49E-39	83.67%	2	down	chr4:74374519-74394250
gi|338797704|ref|NR_040023.1|	ncRNA_endogenous retrovirus group K13, member 1 (ERVK13-1)	8336	n/a	n/a	n/a	n/a	down	chr16:2708389-2723440
gi|207113128|ref|NR_002819.2|	ncRNA_metastasis associated lung adenocarcinoma transcript 1 (non-protein coding)(MALAT1)	8708	n/a	n/a	n/a	n/a	down	chr11:65265232-65273939
gi|342307077|ref|NR_039981.2|	ncRNA_uncharacterized LOC100216546 (LOC100216546)	9419	n/a	n/a	n/a	n/a	down	chr7:104622193-104631612
gi|211938434|ref|NR_024368.1|	ncRNA_uncharacterized LOC402483 (FLI45340)	10263	n/a	n/a	n/a	n/a	down	chr7:128281294-128301052

CAN WE MAKE A NATURAL REPELLENT AS EFFECTIVE AS DEET



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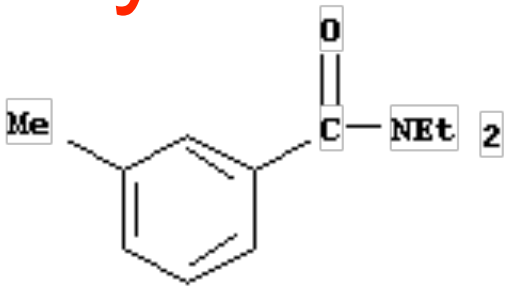
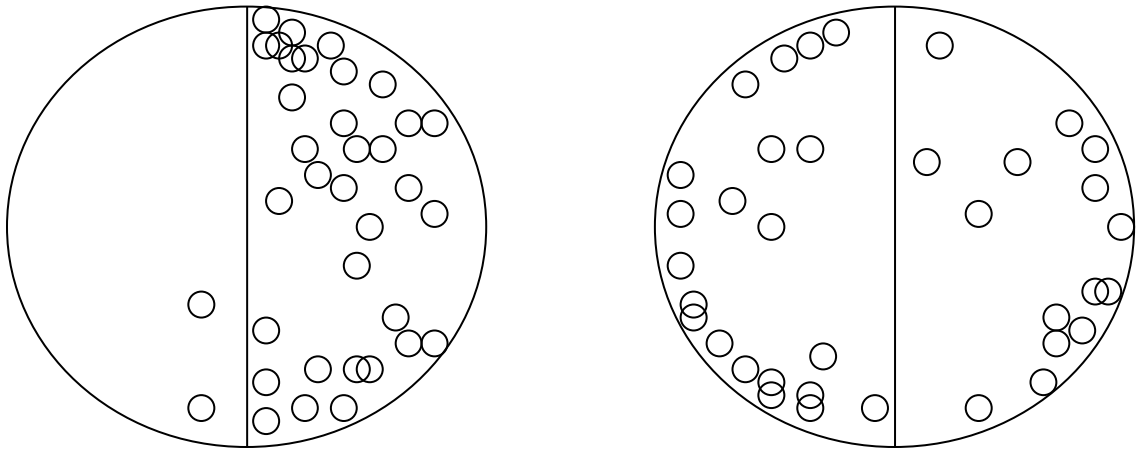


NEW

Insect repellent, BioUD

Two-choice Test on Filter Paper (3.5 h after treatment with repellent) *Abylomma americanum*



Based on actives, undecanone was 13-fold more active than DEET for *A. americanum*, *D. variabilis* and *I. scapularis*

Advantages of BioUD

- More effective than deet (mosquitoes/ ticks)
- Natural compound
- EPA registered for humans
  restrictions
- Does not affect plastics
- Not flammable
- Licensed to Scotts

NEW INSECT RESISTANT TEXTILES NEEDED

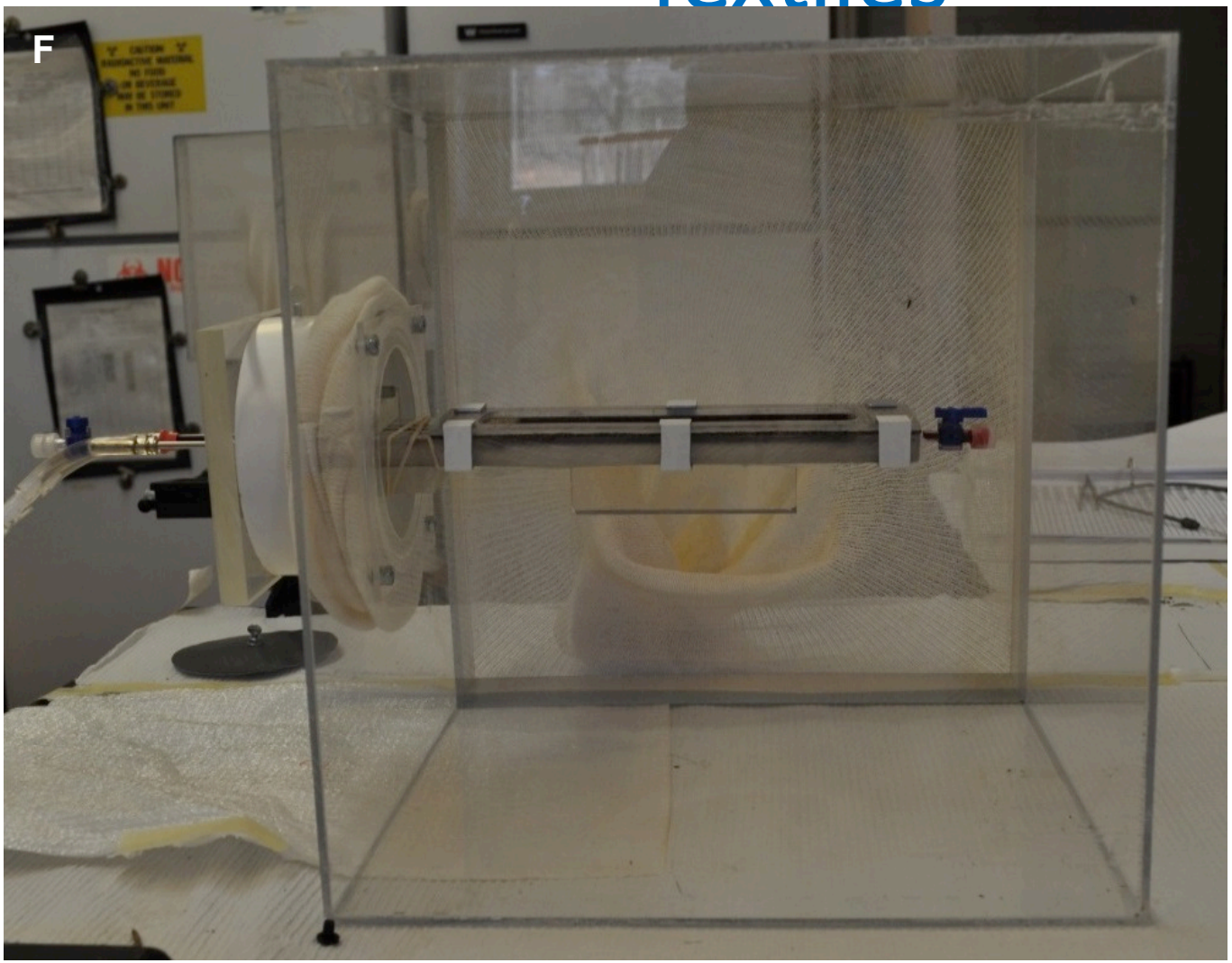
- Vector-borne diseases remain a significant threat in military operations resulting in reductions in manpower, lost duty days and decreased combat effectiveness.

- Current garments for protection from mosquito bites use cloth treated with the insecticide, permethrin. Effectiveness of this technology is on the decline because of insect resistance to permethrin.

- Also, there are potential health risks to insecticide exposure and public aversion to chemicals.

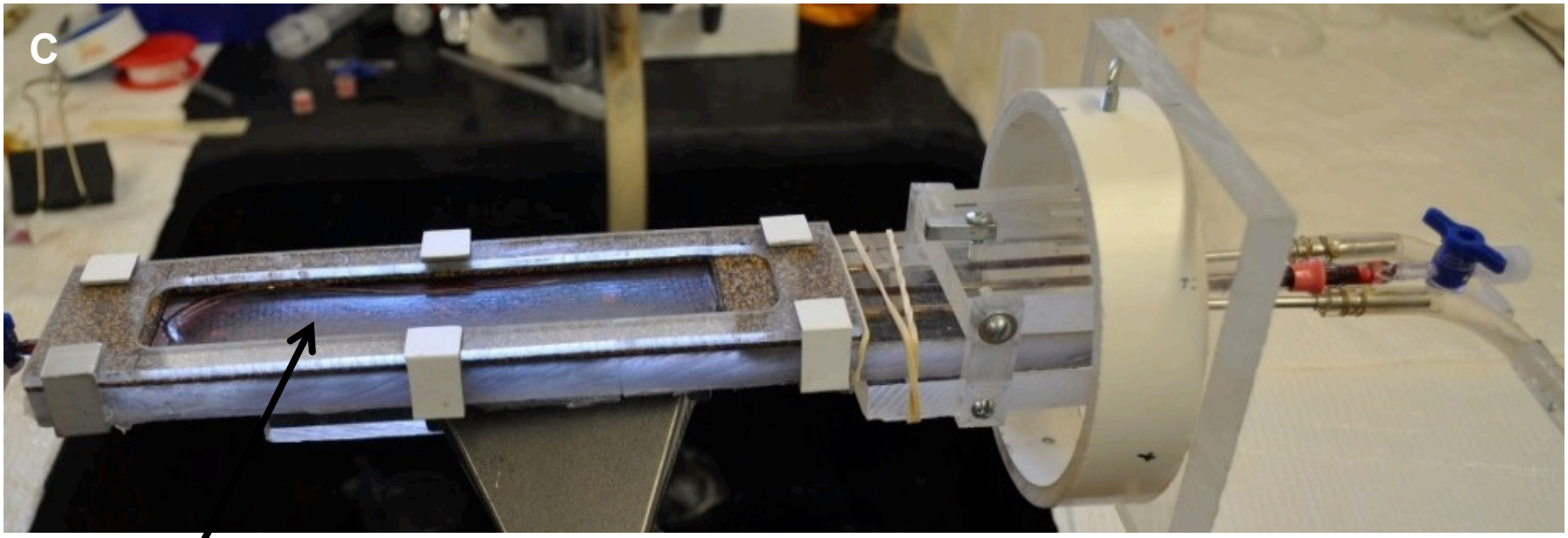
- Significant other uses for insect resistant textiles for malaria control and protection of general public (including infants and children).

New *In vitro* Bioassay System for Mosquito Biting through Textiles



25 mosquitoes/cage
(less than 10 days old)

New *In vitro* Bioassay System for Mosquito Biting through Textiles

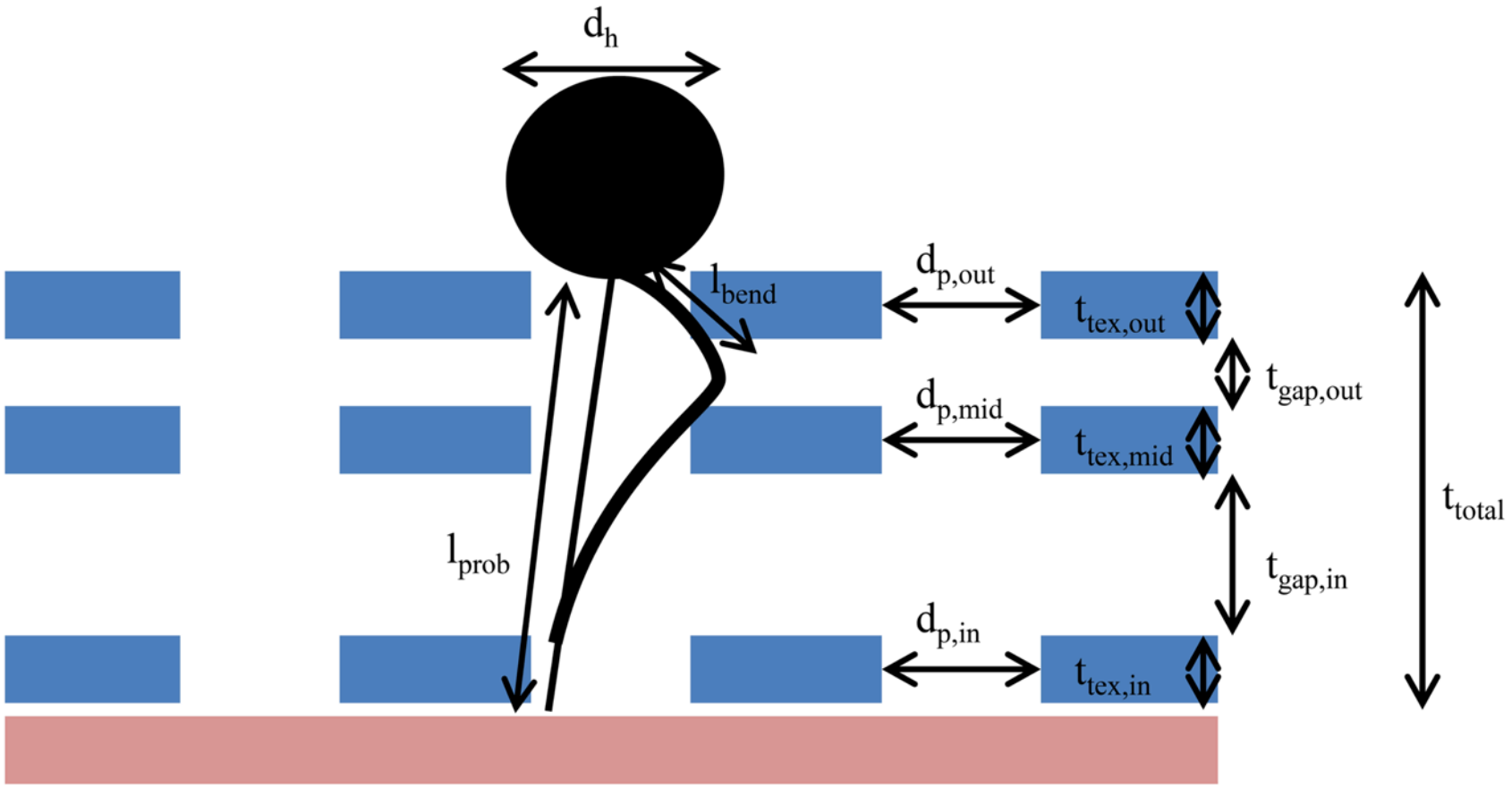


Surface temperature
88 degrees C

Rationale for Cloth

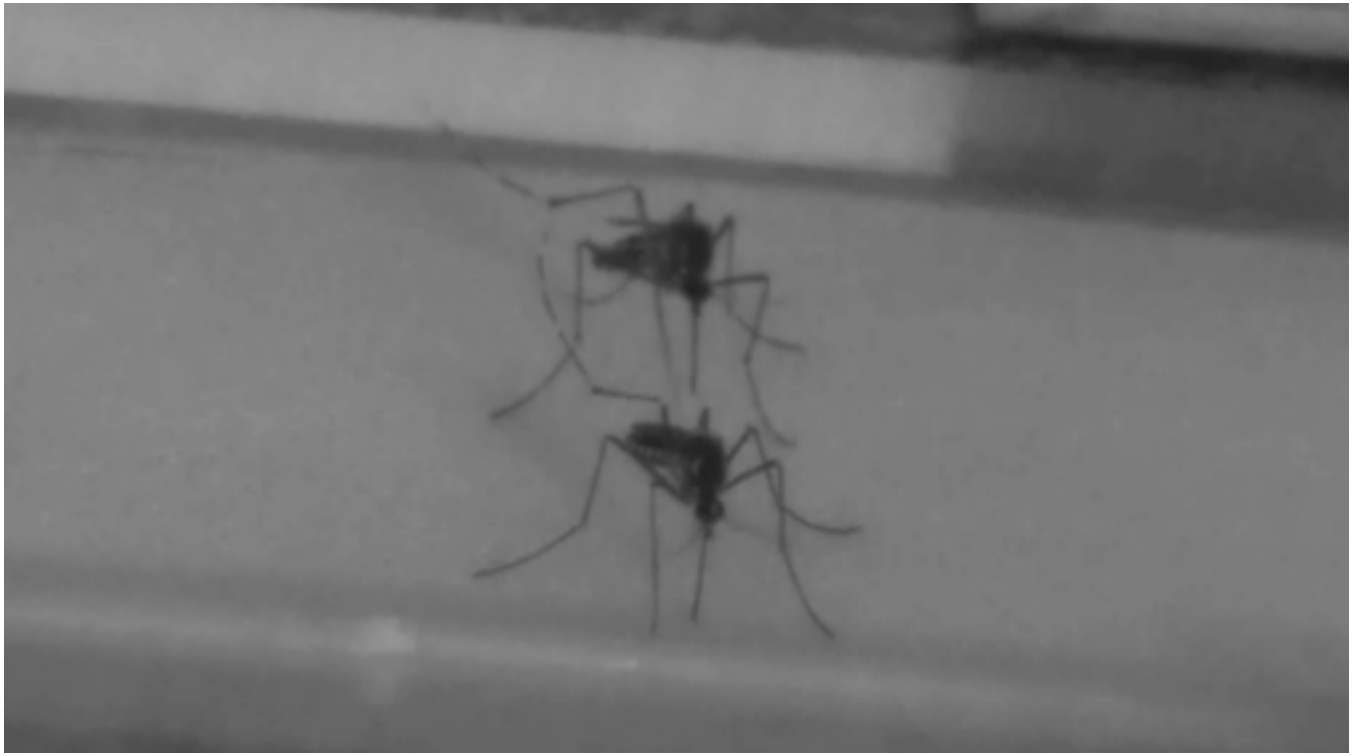
Factors affecting biting resistance

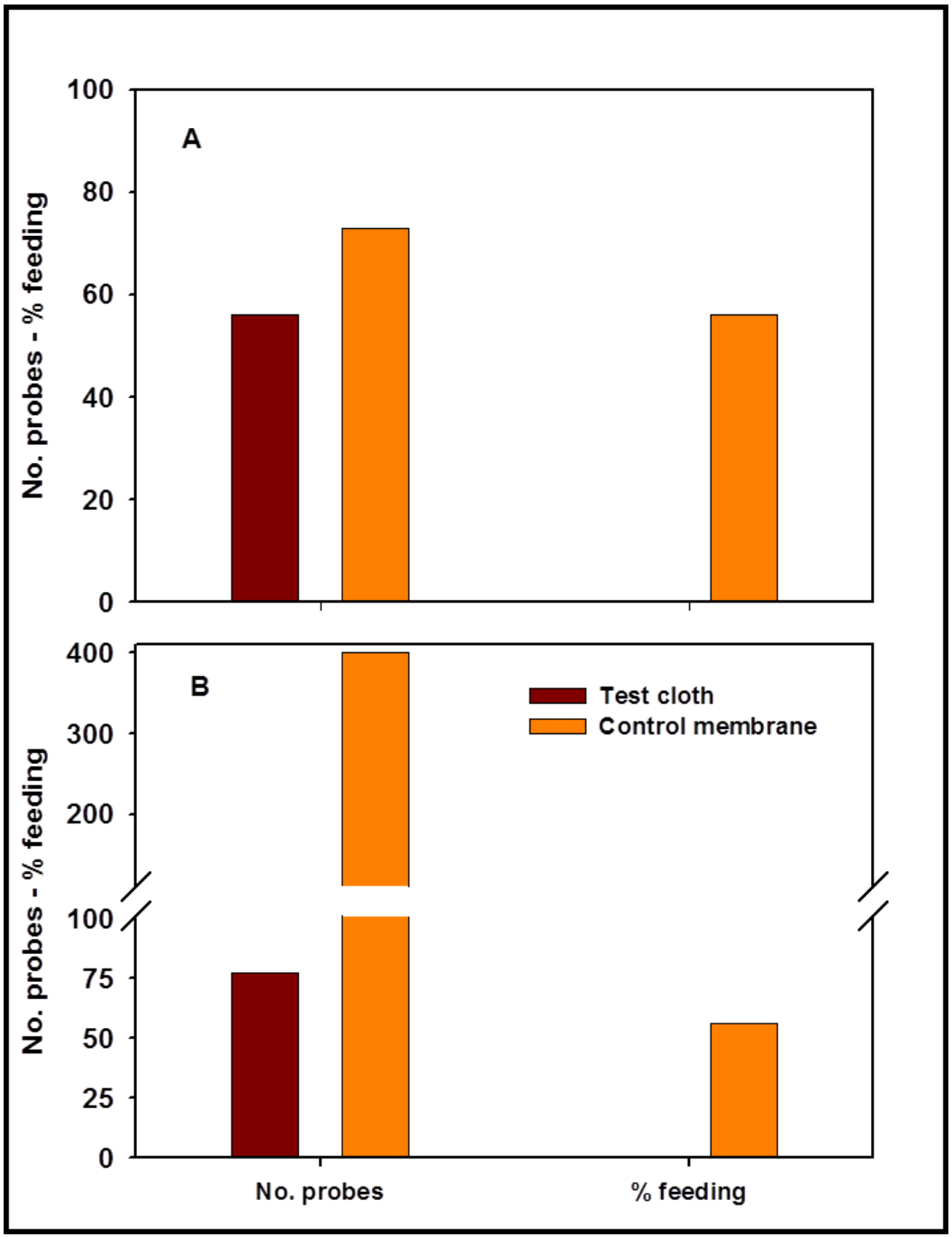
across 3 layers



Bite Resistance Cloth
Open Cell NCSU-0735

Bite Resistance Cloth
Close Cell NCSU-0501





Bite Resistance Cloth CloseCell NCSU-0501

Aedes aegypti

Anopheles gambiae

Summary

- Insecticide resistance is not going away
- There could be “genes from hell” out there
- Need to think “out of the box” for new control methods
- Synetheic chemistry and molecular biology may not be the whole solution

Questions