Insecticide Resistance: The Gene from Hell

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

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
Mechanisms of Insect Resistance to Pesticides

Increased Metabolism

Insect Resistance
Insect Resistance

- Increased Metabolism
- Target Site Modification
Insect Resistance

- Increased Metabolism
- Target Site Modification
- Reduced Penetration
Insect Resistance

- Increased Metabolism
- Target Site Modification
- Reduced Penetration
- Increased excretion
Insect Resistance

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

- Increased Metabolism
- Target Site Modification
- Change in Behavior
- Reduced Penetration
- Increased Excretion
- Sequestration
Mechanisms of Insect Resistance to Pesticides

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

Microbiome
Insect Resistance

( X )

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

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

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

Microbiome

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

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

Microbiome → Insect Resistance

?
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 cannot avoid spray
Methods of Insecticide Application
Advantages and Disadvantages of Each

**Transgenic Cotton**
- Do not have to spray
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- Insect must eat the plant to be killed

**Spray**
- Have to spray
- Need to know when to spray
- Insect can not avoid spray
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.
Mechanisms of Insect Resistance to Pesticides

- **Insect Resistance**
  - Increased Metabolism
  - Target Site Modification
  - Reduced Penetration
  - Increased Excretion
  - Sequestration
  - Microbiome

Hypothesis: Insects can become resistant to Bt and RNAi through behavioral changes.
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
Potential Mechanism of Bt Resistance by Differences in Feeding Rate

Diagram showing the insect digestive system with a focus on the peritrophic membrane in the midgut.
Potential Mechanism of Bt Resistance by Differences in Feeding Rate
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

- No. fecal pellets measure of feeding rate.
- Natural variations in feeding rates in field.

<table>
<thead>
<tr>
<th>County</th>
<th>No. fecal pellets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moore</td>
<td>10</td>
</tr>
<tr>
<td>Rockingham</td>
<td>13</td>
</tr>
<tr>
<td>Johnston</td>
<td>25</td>
</tr>
</tbody>
</table>
24-hour average fecal production per control TBW 1st instar (n = 45) fed FDT meal pads at 30°C & 20°C

**NO BT**

Can lower temperature and change feeding rate
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.

No BT

Bt in Diet

Lower temperature lowered feeding rate and increased Bt susceptibility.
Change in average percentage mortality in tobacco budworm fed on MVPII at 20°C and 30°C

Higher temp on Bt diet reduced % mortality as expected

p-value = 0.0001 (Significantly different) ; n= 64
Temp had no effect on mortality in absence of Bt
Change in average percentage mortality in tobacco budworm fed on Bt cotton at 20°C and 30°C

Higher temp on Bt cotton reduced % mortality as expected

p-value = 0.0008 (Significantly different); n=48
Change in average percentage survival in tobacco budworm fed on cotton at 20°C and 30°C

Temp had no effect on mortality on conventional cotton (no Bt)

<table>
<thead>
<tr>
<th>Temperature</th>
<th>% Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>20°C</td>
<td>93.75</td>
</tr>
<tr>
<td>30°C</td>
<td>92.9</td>
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</tbody>
</table>

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)

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

<table>
<thead>
<tr>
<th>No. fecal pellets</th>
<th>Lab Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>H. virescens</em></td>
</tr>
<tr>
<td>33.4</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Lab Strain</th>
<th>L1 mass</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. virescens</em></td>
<td>434</td>
</tr>
<tr>
<td><em>H. zea</em></td>
<td>836</td>
</tr>
</tbody>
</table>

**Hz** feed faster than **Hv** per unit neonate weight.
Variation in FDT diagnostic doses for *H. virescens* (blue) vs. *H. zea* (black & white) (n = 28-89)

Bt concentration (µg/ml)

- **Hv Hz**: Hz naturally resistant to Bt
- **Cry1AC**: Hv Hz
- **Cry1F**: Hv Hz
- **Cry1Ab**: Hv Hz

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

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

Temp effects on Bt susceptibility not result in changes in microbiome.
24-hour average fecal production (n = 56-64) of Bt susceptible (YDK) vs. resistant (YHD2) TBW L1s fed FDT meal pads

BT R strain feeds faster than S strain.

BT Resistant strain

BT Suscept. strain

TBW Lab Strain

<table>
<thead>
<tr>
<th>TBW Lab Strain</th>
<th>No. fecal pellets</th>
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</thead>
<tbody>
<tr>
<td>YDK</td>
<td>25.2</td>
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<tr>
<td>YHD2</td>
<td>34.6</td>
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</tbody>
</table>

Δ = 37.3%
Potential Mechanism of Bt Resistance by Differences in Feeding Rate

Midgut

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

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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

- 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, 08/17/2011
So what can we do about

A GENE FROM HELL

Innovation Critical
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Mosquitoes ‘developing resistance to bed nets, BBC World Service, 08/172011
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
Location of affected messages

- Up-regulated by DEET
- Down-regulated by DEET
<table>
<thead>
<tr>
<th>Sequence Name</th>
<th>Sequence Description</th>
<th>Seq. Length</th>
<th>#Hits</th>
<th>min. eValue</th>
<th>mean Similarity</th>
<th>#GOs</th>
<th>expression with DEET</th>
<th>Chromosome</th>
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<tbody>
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<td>28476829</td>
<td>ref</td>
<td>NR_001278.1</td>
<td>ncRNA_cytochrome p450 2b6-like (CYP2B7P1)</td>
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<td>ref</td>
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<td>ncRNA_pyridoxal-dependent decarboxylase domain-containing 2, pseudogene (PDXDC2P)</td>
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<td>ref</td>
<td>NR_002934.2</td>
<td>ncRNA_scavenger receptor protein family member (LOC619207)</td>
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<td>ncRNA_psoriasis susceptibility 1 candidate gene 3 (PSOR1C3)</td>
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<td>20</td>
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<td>NR_033266.1</td>
<td>ncRNA_was protein family homolog 2-like; WAS protein family 5 homolog pseudogene (WASH5P)</td>
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<td>20</td>
<td>4.20E-24</td>
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<td>gi</td>
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<td>NR_026936.1</td>
<td>ncRNA_isoform c5orf27; chromosome 5 open reading frame 27 (C5orf27)</td>
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<td>ref</td>
<td>NR_027440.1</td>
<td>ncRNA_unnamed protein product; uncharacterized LOC100272217 (LOC100272217)</td>
<td>2159</td>
<td>20</td>
<td>4.77E-25</td>
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<td>gi</td>
<td>21385260</td>
<td>ref</td>
<td>NR_024456.1</td>
<td>ncRNA_histogram demethylase uty-like; uncharacterized LOC100190986 (LOC100190986)</td>
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<td>5.62E-32</td>
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<td>NR_038080.1</td>
<td>ncRNA_chromosome 17 open reading frame 55; long intergenic non-protein coding RNA 482 (LINC00482)</td>
<td>2970</td>
<td>20</td>
<td>6.02E-147</td>
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<td>ncRNA_aquaporin adipose; aquaporin 7 pseudogene 1 (AQP7P1)</td>
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<td>11</td>
<td>1.14E-61</td>
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<td>gi</td>
<td>255306270</td>
<td>ref</td>
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<td>3</td>
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<td>ref</td>
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<td>ncRNA_endogenous retrovirus group K13, member 1 (ERVK13-1)</td>
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<td>gi</td>
<td>207113128</td>
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<td>NR_002819.2</td>
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<td>gi</td>
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<td>n/a</td>
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<td>10263</td>
<td>n/a</td>
<td>n/a</td>
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</tr>
</tbody>
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CAN WE MAKE A NATURAL REPELLENT AS EFFECTIVE AS DEET

Gold Standard is DEET

- Around for >50 years
- Broad spectrum
- Long-lasting
- Safety of Deet
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 with no restrictions
• Does not affect plastics
• Not flammable
• Licensed to Scotts
NEW INSECT RESISTANT TEXTILES

- 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