Herbicide Resistant Weeds

Frankfurt, May 19th, 2014

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Talk Outline

• Global summary
• European summary
• Criteria for posting cases
• New Modules
  • Target Site Resistance
  • Non-Target Site Resistance
  • Genetics
• Survey Issues
  • Species mixtures
  • Taxonomy
  • Mapping and area estimates
International Survey of Herbicide-Resistant Weeds
20 Years Online this Month
International Survey of Herbicide-Resistant Weeds
Current Status of Survey – May 19th 2014

• 432 Unique Resistant Biotypes (Species x SOA)

• 235 Species

• 138 Dicots and 97 Grass Weeds

• over 600,000 fields/sites

• Approximately 11 new biotypes discovered per year

• WWW.WEEDSCIENCE.ORG
What is Classified as a Unique Case?

- A Unique Species X Site of Action
- *Alopecurus myosuroides* (A, B, N, K3, K1, C1, C2) = 7 Unique cases
- *Apera spica-venti* (A, B, C2) = 3 Unique cases
- Whilst multiple resistance is recorded separately, they don’t necessarily contribute to unique cases
- Otherwise every combination would contribute to a unique case, and Blackgrass would potentially account for 120 combinations
- The number of unique cases for a country may differ from the number listed in the details for a country
In Reality Almost Every Population is a Unique Case

- Each different mutation is a unique case
- Metabolic resistance – levels vary from population to population – each is a unique case
- Gene over expression – number of copies varies from population to population – each is a unique case
- Populations can be a mixture of mechanisms, each is a unique case……..etc.
Number Resistant Species for Several Herbicide Sites of Action (HRAC Codes)

Legend:
- ACCase Inhibitors (A)
- ALS Inhibitors (B)
- EPSP Synthase Inhibitors (G)
- Synthetic Auxins (O)
- PSII Inhibitors (C1, C2, C5)

Note: PSII Inhibitors Combined

Year

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Big Drivers of Resistance

- **Number of Individuals Treated**
  - Approximated by area treated x time
- **Species**
  - Some weeds are more prone to resistance than others – *Amaranthus, Lolium, Alopecurus, Echinochloa*, and *Conyza*
- **Herbicide Chemistry**
  - Number of ways weeds can evolve resistance
  - Number of species the herbicide targets
  - Area and time of use
  - These factors account for >90% impact on the outcome of resistance
<table>
<thead>
<tr>
<th>Species</th>
<th>Number of Sites of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lolium rigidum</em></td>
<td>11</td>
</tr>
<tr>
<td><em>Echinochloa crus-galli var. crus-galli</em></td>
<td>9</td>
</tr>
<tr>
<td><em>Poa annua</em></td>
<td>9</td>
</tr>
<tr>
<td><em>Alopecurus myosuroides</em></td>
<td>7</td>
</tr>
<tr>
<td><em>Eleusine indica</em></td>
<td>7</td>
</tr>
<tr>
<td><em>Amaranthus tuberculatus (=A. rudis)</em></td>
<td>6</td>
</tr>
<tr>
<td><em>Echinochloa colona</em></td>
<td>6</td>
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<td><em>Lolium perenne ssp. multiflorum</em></td>
<td>6</td>
</tr>
<tr>
<td><em>Amaranthus palmeri</em></td>
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<tr>
<td><em>Ambrosia artemisiifolia</em></td>
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<tr>
<td><em>Avena fatua</em></td>
<td>5</td>
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<tr>
<td><em>Conyza canadensis</em></td>
<td>5</td>
</tr>
<tr>
<td><em>Raphanus raphanistrum</em></td>
<td>5</td>
</tr>
<tr>
<td><em>Amaranthus retroflexus</em></td>
<td>4</td>
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<tr>
<td><em>Bromus tectorum</em></td>
<td>4</td>
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ALS inhibitor-Resistant Species by Amino Acid Substitution

<table>
<thead>
<tr>
<th>Amino Acid Substitutions</th>
<th>Number of Species</th>
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<tbody>
<tr>
<td>Trp 574 to Leu</td>
<td>27</td>
</tr>
<tr>
<td>Pro 197 to Ser</td>
<td>21</td>
</tr>
<tr>
<td>Pro 197 to Leu</td>
<td>11</td>
</tr>
<tr>
<td>Pro 197 to Thr</td>
<td>11</td>
</tr>
<tr>
<td>Pro 197 to Ala</td>
<td>9</td>
</tr>
<tr>
<td>Asp 376 to Glu</td>
<td>7</td>
</tr>
<tr>
<td>Pro 197 to GlN</td>
<td>7</td>
</tr>
<tr>
<td>Ala 122 to Thr</td>
<td>6</td>
</tr>
<tr>
<td>Pro 197 to His</td>
<td>6</td>
</tr>
<tr>
<td>Ser 653 to Asn</td>
<td>5</td>
</tr>
<tr>
<td>Ser 653 to Thr</td>
<td>5</td>
</tr>
<tr>
<td>Ala 205 to Val</td>
<td>4</td>
</tr>
<tr>
<td>Pro 197 to Arg</td>
<td>3</td>
</tr>
<tr>
<td>Ala 122 to Val</td>
<td>2</td>
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<tr>
<td>Ala 122 to Tyr</td>
<td>1</td>
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<tr>
<td>Arg 377 to His</td>
<td>1</td>
</tr>
<tr>
<td>Gly 654 to Asp</td>
<td>1</td>
</tr>
<tr>
<td>Pro 197 to Asn</td>
<td>1</td>
</tr>
<tr>
<td>Pro 197 to Ile</td>
<td>1</td>
</tr>
<tr>
<td>Ser 653 to Ile</td>
<td>1</td>
</tr>
<tr>
<td>Trp 574 to Gly</td>
<td>1</td>
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<tr>
<td>Trp 574 to Met</td>
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</table>
Number Registered Herbicides for the Major Herbicide Sites of Action

<table>
<thead>
<tr>
<th>Herbicide Sites of Action</th>
<th>Number of Registered Herbicide Actives</th>
</tr>
</thead>
<tbody>
<tr>
<td>B - ALS inhibitors</td>
<td>56</td>
</tr>
<tr>
<td>E - PPO inhibitors</td>
<td>28</td>
</tr>
<tr>
<td>K3 - Long chain fatty acid inhibitors</td>
<td>26</td>
</tr>
<tr>
<td>C1 - Photosystem II inhibitors</td>
<td>26</td>
</tr>
<tr>
<td>O - Synthetic Auxins</td>
<td>23</td>
</tr>
<tr>
<td>A - ACCase inhibitors</td>
<td>21</td>
</tr>
<tr>
<td>C2 - PSII inhibitor (Ureas and amid)</td>
<td>20</td>
</tr>
<tr>
<td>N - Lipid Inhibitors (thiocarbamates)</td>
<td>19</td>
</tr>
<tr>
<td>K1 - Microtubule inhibitors</td>
<td>16</td>
</tr>
<tr>
<td>Z - Unknown</td>
<td>14</td>
</tr>
<tr>
<td>F2 - HPPD inhibitors</td>
<td>10</td>
</tr>
<tr>
<td>F1 - Carotenoid biosynthesis inhibitors</td>
<td>7</td>
</tr>
<tr>
<td>C3 - PSII inhibitors (Nitriles)</td>
<td>6</td>
</tr>
<tr>
<td>L - Cellulose inhibitors</td>
<td>6</td>
</tr>
<tr>
<td>K2 - Mitosis inhibitors</td>
<td>3</td>
</tr>
</tbody>
</table>

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Glyphosate-Resistant “Giant” Ragweed in Roundup Ready Corn
Palmer Amaranth in Cotton

Farmers now using up to 7 herbicide applications plus hand hoeing at a cost up to $360/ha

Glyphosate 4X rate at 3 cm
Glyphosate 4X rate at 10 cm
Glyphosate 4X rate PDIR
Spread of glyphosate-resistant Kochia

Photo: Westra, CSU, 2011
Introduction Time of New Herbicide Sites of Action

- **B**: ALS inhibitors
- **H**: Glutamine synthase inhibitors
- **Z**: Cell elongation inhibitors
- **F1**: Carotenoid biosynthesis inhibitors
- **I**: DHP synthase inhibitors
- **C3**: PSII inhibitors (Nitriles)
- **K3**: Long chain fatty acid inhibitors
- **D**: PSI Electron Diverter
- **C1**: Photosystem II inhibitors
- **Z**: Unknown
- **M**: Uncouplers
- **F2**: HPPD inhibitors
- **A**: ACCase inhibitors
- **G**: EPSP synthase inhibitors
- **E**: PPO inhibitors
- **L**: Cellulose inhibitors
- **K1**: Microtubule inhibitors
- **Z**: Nucleic acid inhibitors
- **F3**: Carotenoid biosynthesis (unknown target)
- **C2**: PSII inhibitor (Ureas and amides)
- **P**: Auxin transport inhibitors
- **N**: Lipid Inhibitors (thiocarbamates)
- **K2**: Mitosis inhibitors
- **O**: Synthetic Auxins

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Species with Resistance to More than One Site of Action

Number Species with Multiple Resistance

Year


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Number of Unique Herbicide Resistant Weeds found in European Countries

72 Species
Europe's Worst Grass Herbicide-Resistant Weeds

- *Alopecurus myosuroides*
  - 11 countries, 7 SOA’s
- *Echinochloa spp.* (*E. crus-galli, E. erecta, E. oryzoides*)
  - 11 countries, 6 SOA’s
- *Lolium spp.* (*L. perenne, L. multiflorum, L. rigidum*)
  - 8 countries, 5 SOA’s
- *Apera spica-venti*
  - 7 countries, 3 SOA’s
- *Avena fatua*
  - 6 countries, 3 SOA’s
Europe's Worst Broadleaf Herbicide-Resistant Weeds

- Conyza spp. (C. canadensis, C. bonariensis, C. sumatrensis)
  - 10 countries, 5 SOA’s
- Stellaria media
  - 7 countries, 3 SOA’s
- Papaver rhoeas
  - 8 countries, 2 SOA’s
- Chenopodium album
  - 16 countries, 3 SOA’s
- Amaranthus spp. (A. retroflexus, A. hybridus, A. cruentus, A. powellii)
  - 10 countries, 3 SOA’s
Criteria for Reporting New Cases of Herbicide-Resistant Weeds

“New” vs. “Additional Cases”

“New Cases”
- First time that a species has been identified with resistance to a particular Mode of Action (MOA).
- Before a “New Case” can be added to the survey it must pass 5 criteria.

“Additional Cases”
- If the species has been shown to be resistant to the mode of action elsewhere in the world then it is considered an “Additional Case”
- Lower requirement to add additional cases, but still requires a dose response experiment that includes a susceptible control.
Five Criteria for Reporting Resistance

- **Criterion 1. Fulfillment of WSSA Definition**
  - “Resistance is the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type”

- **Criterion 2. Confirmed by Scientific Experiments**
  - Replicated trials, greenhouse dose response experiments including a susceptible control
  - May require field experiments for “low level” resistance

- **Criterion 3. Resistance Must be Heritable**
  - Can’t just dig up plants and test
  - Experiments based on seed of R and S are acceptable, genetic studies to determine inheritance are not required.
Five Criteria for Reporting Resistance cont.

• Criterion 4. Must be of practical relevance
  • The survey is intended for practical relevance rather than to document natural variations in herbicide response between weed populations.
  • If resistance is “low level” (<8 fold) then field trials may be required
  • Resistant populations must survive the highest label recommended herbicide rate under field conditions
  • "Does the herbicide provide adequate control of the weed when applied under field conditions at the highest labeled rate and the label instructions are followed?“ If the answer is no then it can be listed

• Criterion 5. A weed identified to species level
  • Must be a weed, identified to species level, and not the result of deliberate or artificial selection
Populations Naturally Vary in Response to Herbicides

R/S ratio of Pop 5/Pop 3 is approx. 3 fold.

Populations of *Avena fatua* from different regions (never exposed to herbicides)

![Bar chart showing percent control for different populations of *Avena fatua* exposed to Diclofop-methyl at different rates.]
Documentation of Target Site Resistance Cases

- ALS inhibitors – Pat Tranel & Terry Wright - Complete
- Glyphosate – Todd Gains - Complete
- ACCase inhibitors – Christophe Délye - Soon
- Photosystem II – Francios Tardif - Soon
- Microtubule inhibitors, PPO inhibitors, HPPD inhibitors
# MUTATIONS IN HERBICIDE-RESISTANT WEEDS TO ALS INHIBITORS

Amino acid substitutions that confer herbicide resistance to ALS inhibitors and that were identified in herbicide-resistant weed populations. Intentionally selected (i.e., laboratory selections) are not included in this table.


<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Subs(1)</th>
<th>Species</th>
<th>IMI (2)</th>
<th>PTB (2)</th>
<th>SCT (2)</th>
<th>SU (2)</th>
<th>TP (2)</th>
<th>Year(3)</th>
<th>Details</th>
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<tr>
<td>Ala 122</td>
<td>Thr</td>
<td>Xanthium strumarium</td>
<td>R</td>
<td>S</td>
<td>ND</td>
<td>S</td>
<td>ND</td>
<td>1995</td>
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<tr>
<td></td>
<td>Thr</td>
<td>Solanum ptycanthum</td>
<td>R</td>
<td>ND</td>
<td>ND</td>
<td>S</td>
<td>ND</td>
<td>2000</td>
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<td>Thr</td>
<td>Amaranthus retroflexus</td>
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<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<td>Details</td>
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<tr>
<td></td>
<td>Thr</td>
<td>Amaranthus powellii</td>
<td>R</td>
<td>ND</td>
<td>ND</td>
<td>S</td>
<td>ND</td>
<td>2005</td>
<td>Details</td>
</tr>
<tr>
<td></td>
<td>Thr</td>
<td>Amaranthus hybridus</td>
<td>R</td>
<td>S</td>
<td>ND</td>
<td>S</td>
<td>S</td>
<td>2006</td>
<td>Details</td>
</tr>
<tr>
<td></td>
<td>Val</td>
<td>Apera spica-venti</td>
<td>ND</td>
<td>ND</td>
<td>S</td>
<td>R</td>
<td>ND</td>
<td>2011</td>
<td>Details</td>
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<tr>
<td></td>
<td>Tyr</td>
<td>Raphanus raphanistrum</td>
<td>R</td>
<td>ND</td>
<td>ND</td>
<td>R</td>
<td>R</td>
<td>2012</td>
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<tr>
<td></td>
<td>Val</td>
<td>Echinochloa crus-galli var. crus-galli</td>
<td>R</td>
<td>S</td>
<td>ND</td>
<td>ND</td>
<td>S</td>
<td>2013</td>
<td>Details</td>
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<tr>
<td></td>
<td>Thr</td>
<td>Echinochloa crus-galli var. crus-galli</td>
<td>R</td>
<td>S</td>
<td>ND</td>
<td>ND</td>
<td>R</td>
<td>2013</td>
<td>Details</td>
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<tr>
<td>Pro 197</td>
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<td>S</td>
<td>ND</td>
<td>ND</td>
<td>R</td>
<td>R</td>
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<td>His</td>
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<td>r</td>
<td>S</td>
<td>ND</td>
<td>R</td>
<td>r</td>
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<td>Arg</td>
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<td>ND</td>
<td>ND</td>
<td>R</td>
<td>ND</td>
<td>1995</td>
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<td>ND</td>
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<td>ND</td>
<td>1995</td>
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<td>Ser</td>
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<td>ND</td>
<td>ND</td>
<td>R</td>
<td>ND</td>
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<td>ND</td>
<td>ND</td>
<td>R</td>
<td>ND</td>
<td>1995</td>
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<td>Ala</td>
<td>Brassica tournefortii</td>
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<td>ND</td>
<td>ND</td>
<td>R</td>
<td>ND</td>
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## Mutations in Herbicide-Resistant Weeds to EPSP Synthase Inhibitors

Amino acid substitutions that confer herbicide resistance to EPSP synthase inhibitors and that were identified in herbicide-resistant weed populations. Intentionally selected (i.e., laboratory selections) are not included in this table.


<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Subs(^{(1)})</th>
<th>Species</th>
<th>GLY (^{(2)})</th>
<th>Year (^{(3)})</th>
<th>Details</th>
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<td>Pro 106</td>
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<td>r</td>
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<td>Thr</td>
<td><em>Eleusine indica</em></td>
<td>r</td>
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<td></td>
<td>Thr</td>
<td><em>Lolium rigidum</em></td>
<td>r</td>
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</tr>
<tr>
<td></td>
<td>Ser</td>
<td><em>Lolium perenne ssp. multiflorum</em></td>
<td>r</td>
<td>2007</td>
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<td></td>
<td>Ala</td>
<td><em>Lolium rigidum</em></td>
<td>r</td>
<td>2007</td>
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<tr>
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<td>Ala</td>
<td><em>Lolium perenne ssp. multiflorum</em></td>
<td>r</td>
<td>2008</td>
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<td>Ser</td>
<td><em>Lolium rigidum</em></td>
<td>r</td>
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<td>Leu</td>
<td><em>Lolium rigidum</em></td>
<td>r</td>
<td>2011</td>
<td>Details</td>
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<tr>
<td></td>
<td>Thr</td>
<td><em>Digitaria insularis</em></td>
<td>r</td>
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<tr>
<td></td>
<td>Ser</td>
<td><em>Amaranthus tuberculatus (=A. rudis)</em></td>
<td>r</td>
<td>2013</td>
<td>Details</td>
</tr>
<tr>
<td></td>
<td>Ser</td>
<td><em>Echinochloa colona</em></td>
<td>r</td>
<td>2013</td>
<td>Details</td>
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</tbody>
</table>
Documentation of Non Target Site Cases

• Doug Sammons, Christophe Délye
  • Enhanced Metabolism
  • Gene Overexpression
  • Decreased Translocation
  • Sequestration
documentation of genetics for known cases

• inheritance (nuclear or cytoplasmic, number of genes, dominance when single-gene)
• fitness costs or benefits of resistance allele
• ploidy level, breeding system, chromosome number, genome size etc.
Collecting data on each resistant weed & weeds in general

- Abundance in Fields
- Seed Production
- Seed Bank Longevity
- Annual vs. Perennial
- Broadleaf vs. Grasses
- Vegetative vs. Seed Only
- Family Differences
- Seed Dispersal Mechanism
- Outcrossing vs Selfing
- Breeding System (M v D)
- Pollination System
- Genome Size
- Chromosome number
- Ploidy Level
Too Much Variability in Chromosome #, Ploidy #, and Genome Size to draw any conclusions

<table>
<thead>
<tr>
<th>List</th>
<th># Species</th>
<th>Average Chromosome #</th>
<th>Standard Deviation</th>
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<tbody>
<tr>
<td>WSSA Weed List</td>
<td>655</td>
<td>31</td>
<td>±19</td>
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<tr>
<td>Resistant Species</td>
<td>91</td>
<td>27</td>
<td>±14</td>
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</tbody>
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<table>
<thead>
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<th># Species</th>
<th>Average Ploidy #</th>
<th>Standard Deviation</th>
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</thead>
<tbody>
<tr>
<td>WSSA Weed List</td>
<td>681</td>
<td>4.4</td>
<td>±2.0</td>
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<tr>
<td>Resistant Species</td>
<td>95</td>
<td>3.1</td>
<td>±1.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>List</th>
<th># Species</th>
<th>Genome Size (Picograms 1C)</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>WSSA Weed List</td>
<td>668</td>
<td>3.6</td>
<td>±3.8</td>
</tr>
<tr>
<td>Resistant Species</td>
<td>97</td>
<td>2.5</td>
<td>±3.4</td>
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</tbody>
</table>
Species Mixtures/Hybrids - Problematic

- Some cases involve closely related species and the populations may be mixtures and or hybrids
  - *Lolium* species
  - *Echinochloa* species
  - *Amaranthus* species
  - *Conyza* species
- At present the survey only allows addition of a new case for species and not for a genus.
In January Joe did a complete audit of the taxonomy of the site.

Several species names were updated.

Issues still exist for grey areas.


**Note to Taxonomists – please pick a name and stick with it 😊.**
Estimation of Infestation Area/Mapping

• Originally the survey requested an estimation of the number of sites and the area.

• Inaccurate and rapidly out of date

• Alternative
  • Posting actual distribution and mapping studies along with the methodology used to arrive at area estimates
  • General comments from researchers about which species are most problematic in their region (without listing actual area estimates)
How Weed Scientists Estimate the Area of Herbicide-Resistant Weeds

- **Field Observations**
  - estimate problem based on observations of herbicide failures – inaccurate and not acceptable

- **Passive Testing**
  - receive samples from complaints and test – biased and not useful to estimate area of infestation

- **Active Testing**
  - Scientist travels in the test area and collects samples from heavy infestations that have a high chance of being resistant – still biased and not of a lot of use to estimate area of infestation

- **Random Field Sampling**
Random Field Sampling

• Weed scientists first define the target weed species to be tested (ie: Waterhemp and Common Ragweed), the target crops (ie: corn, soybean) and the test area (counties, region).

• Using GIS, they overlay a grid onto the test area with an appropriate amount of sample points (each intersection of the grid will be a sample location).

• They can then determine the GPS locations of each of the grid intersections and at weed seed maturity they can travel to each grid intersection (using GPS) and sample from the nearest field with the target crop.

• At each site they record the estimated densities of target species and record the percentage of fields that do not have the target species present.

• They then collect weed seed samples using appropriate sampling techniques within the field and follow a standard protocol for each sample site (eg: sampling in a W configuration over 1 hectare from more than 40 plants).

• Once samples are tested for resistance they can then calculate the percentage of fields infested with resistant weeds and multiply by the hectares of crop in the test region to get the area infested.
Please Contribute!! Email me when you…

• know of a new case that has not been posted
• have data or comments to add to an existing case
• see an error/omission/lost in translation
• have any data you want posted – maps, papers etc.
• want a new feature
• want a new graph, extracted data or summary
• have any questions regarding the survey

Acknowledgements: Thank you to all scientists that have contributed and thanks to HRAC for financial support of the survey.
Any consistent practice to control weeds year after year will result in directed evolution towards survival.

The solution is to vary weed control practices and destabilize evolution.

**Integrated Weed Management**