The Road Ahead for Resistance Diagnostics

1 Oct 2015, Dr. Todd Gaines
Outline

• Herbicide Resistance Diagnostics Currently
  1. Biological assays – Greenhouse
  2. Biological assays – Rapid tests
  3. Biochemical assays
  4. Genetic assays

• The Road Ahead
  1. Genomics and weed biology
Option 1: Greenhouse Resistance Assays

*Amaranthus tuberculatus* and Glyphosate

**Susceptible**

<table>
<thead>
<tr>
<th>11.53</th>
<th>5.76</th>
<th>2.88</th>
<th>1.45</th>
<th>1.08</th>
<th>0.72</th>
<th>0.54</th>
<th>0.36</th>
<th>0.18</th>
<th>0.09</th>
<th>0.04</th>
<th>0.02</th>
</tr>
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</table>

**Resistant**

| 11.53 | 5.76 | 2.88 | 1.45 | 1.08 | 0.72 | 0.54 | 0.36 | 0.18 | 0.09 | 0.04 | 0.02 |

Cost: $$-$$$$

Time: months

Data Points: $10^{2-3}$

*L. Lorentz, Bayer CropScience*
Option 2: Rapid Assays

Cost: $

Time: weeks

Data Points: $10^{1-3}$

Kaundun et al, 2011, Weed Res. 51: 284-293
Option 3: Biochemical Assays

Shikimate accumulation assay for glyphosate resistance
(Shaner et al., 2005, Weed Sci., 53:769-774)

Cost: $-$
Time: days
Data Points: $10^{1-2}$

Glyphosate-resistant *Amaranthus palmeri*,
Gaines et al, 2010, PNAS, 1029-1034

Glyphosate-resistant *Ambrosia trifida*,
Van Horn et al, CSU, unpublished
Option 3: Biochemical Assays

- Measure enzyme activity

| $K_{\text{cat}}$ [µM P1, µg\(^{-1}\) protein] |
|-----------------|-----------------|
| 0               | Susceptible     |
| 10              | Resistant       |
| 20              |                 |
| 30              |                 |
| 40              |                 |
| 50              |                 |

Increased EPSPS activity in glyphosate-resistant *Amaranthus tuberculatus*, Lorentz et al., 2014, JAFC, 8134-8142

Target-site EPSPS mutations reduce glyphosate inhibition in glyphosate-resistant *Eleusine indica*, Yu et al., 2015, Plant Physiol, 167:1440-1447

Cost: $$
Time: days
Data Points: 10\(^{1-2}\)
Option 3: Biochemical Assays

• Metabolite detection

  Cost: $$$-$$$$
  Time: days-month
  Data Points: $10^{1-2}$

• Can increase throughput in 96-well plates
  – BCS WRCC

Resistant

Susceptible

Option 4: Genetic Assays

- Central dogma of molecular biology
Option 4: Sequence One Gene

- Target-site mutations
  - Amplify using PCR, sequence, align

Glyphosate-resistant *Ambrosia trifida*, Van Horn et al, CSU, unpublished
Option 4: Genotype one nucleotide

- Target-site mutations - dCAPS
  - Amplify using PCR, use restriction digest to cleave sequence, run gel, identify mutations

Cost: $
Time: hours
Data Points: 10^{1-2}

dCAPS markers to identify EPSPS mutations at Thr102 in glyphosate-resistant *Eleusine indica*, Yu et al, 2015, Plant Physiol, online ahead of print
Option 4: Genotype one nucleotide, Advanced

- Pyrosequencing
  - E.g., ACCase and ALS mutations by Bayer CropScience
  - High throughput

Cost: $
Time: hours
Data Points: $10^{2-3}$

- KASP markers
  - Two primers, genotype one nucleotide
  - <$0.20 per data point
  - Very high throughput

BCS, WRCC

LGC Genomics
Option 4: Genetic Assays

• Quantitative PCR
  – measure variation in gene copy number
  – Transcriptional marker for gene expression

Glyphosate-resistant
*Kochia scoparia*

R (above) and S (below) genomic DNA Ct curves for EPSPS and ALS
Option 4: Gene Copy Number

Wiersma et al., 2015, Planta, 241:463-474

Cost: $$
Time: hours
Data Points: $10^{1-2}$
Distribution of *EPSPS* Copy Number in *K. scoparia*

![Graph showing the distribution of EPSPS copy number in samples from Canada and the United States. The graph includes a formula for calculating copy number: \(2^{\Delta CT} = -(CT_{EPSPS} - CT_{ALS})\).]
EPSPS Copy Number in K. scoparia from Beta vulgaris fields

Gaines & Kniss, 2015, unpublished
Option 4: Transcriptional Marker

\[ R^2 = 0.854 \]

Cost: $$$-$$$$  
Time: days  
Data Points: $10^{1-2}$

Wiersma et al., 2015, Planta, 241:463-474
Option 4: Transcriptional Marker

**Lolium rigidum**

RNA-Seq analysis of rye-grass transcriptomic response to an herbicide inhibiting acetolactate-synthase identifies transcripts linked to non-target-site-based resistance

Arnaud Duhoux · Sébastien Carrère · Jérôme Gouzy · Ludovic Bonin · Christophe Delye

2015

Increased expression of two CytP450, one GT, and one GST

Red: resistant to pyroxsulam AND iodosulfuron/mesosulfuron

Green: susceptible to both

Diagnose as Resistant
Option 3/4: Antibody for protein

Wiersma et al., 2015, Planta, 241:463-474

<table>
<thead>
<tr>
<th>A. palmeri</th>
<th>K. scoparia</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>Susceptible</td>
</tr>
<tr>
<td>R</td>
<td>Resistant</td>
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</tbody>
</table>

55 kDa  
43 kDa  
34 kDa

EPSPS

Protein marker

Candidate for antibody strip test

Cost: $  
Time: minutes  
Data Points: $10^{1-2}$
The Road Ahead

Genomics and weed biology
Genomics to Improve Diagnostics

• Genotype by Sequencing (GBS)
  – Sequence partial subset of genome
  – Obtain 10,000+ single nucleotide polymorphisms (SNPs)
  – SNPs are genetic markers
  – Next-generation sequencing

Cost: $$
Time: weeks
Data Points: $10^{6-8}$
Applications of GBS

- Population genomics
- Use SNPs for relatedness
- Test hypotheses

GWAS: Genome-wide association study
- Developed in crops, humans
- Panels of lines/varieties with known relatedness
- Statistical models
- Associate variant (SNP) with desired trait
- Bioinformatics

Single origin of R
Multiple origins of R
GWAS – Water Uptake in *Phaseolus vulgaris*

<table>
<thead>
<tr>
<th>Chr</th>
<th>Position</th>
<th>p value</th>
<th>$R^2$ (%)</th>
<th>Allele</th>
<th>Effect</th>
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<td>1.83E-07</td>
<td>14.6</td>
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<td>3.7</td>
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</table>

Replace water uptake with herbicide resistance – we can map the locus and have SNP markers


Gaines – BCS Global IWM 2015
GWAS for Resistance Diagnostics

• Must have assembled reference genome

• Must have multiple collections with variation in phenotype

• Must have some knowledge of population genetic structure

How to achieve it?

– Continue phenotyping diverse collections
  • Metabolic resistance
  • Gene copy number variation

– GBS resources
  • Assemble reference genomes
  • Population genomics analysis

Cost: $$$$  
Time: years  
Data Points: $10^{6-8}$
How do we get there?

- Metabolic resistance
- Major species:
  - *Amaranthus palmeri*
  - *Amaranthus tuberculatus*
  - *Lolium rigidum*
  - *Alopecurus myosuroides*
- Possible diagnostic
  - Group I: metabolic type I
  - Group II: metabolic type II
  - Group III: Target-site and metabolic, type I or II

![Venn diagram showing groups and SNPs]

- SNPs 1-20
- SNPs 21-40
- Target-site SNPs

Cost: $$-$$$ 
Time: weeks 
Data Points: $10^{6-8}$
Summary

• Apply powerful genomics tools to diagnose resistance
• Develop SNPs to diagnose the multiple mechanisms present in weed populations
  – Biology, bioinformatics
• Sequencing gets faster and cheaper every year
  – “Disposable lab on a chip”
  – Custom-designed arrays with panel of diagnostic SNPs
Thank you for listening!