Developing robust identification assays for *Amaranthus palmeri* in seed mixtures

UMWISC/NAISMA
Oct. 15th, 2018

Speaker:
Anthony Brusa
Amaranthus palmeri

Common names:
- Palmer Amaranth
- Pigweed
- Carelessweed

Native to SW US and NW Mexico, and recently introduced to MN

Closely related to waterhemp

Image © Rebekah D. Wallace
Why do we care?

Extremely prolific (250,000 seeds from one individual)

Image © Lisa Behnken
Why do we care?

Extremely prolific (250,000 seeds from one individual)

Fast growing (2-3 inches per day)
Why do we care?

Extremely prolific (250,000 seeds from one individual)

Fast growing (2-3 inches per day)

Yield losses been up to 91% in corn and 79% in soybean (MN Department of Agriculture)
Palmer Amaranth in MN

First introduced through prairie seed mixtures

First confirmed sighting in MN was September 2016

Early stage of invasion is the best time for control efforts
Project Goals

1) Design of genetic markers for the identification of *A. palmeri* vs. related species

2) Development of a testing protocol for implementation by the MN Department of Agriculture
How do we identify *A. palmeri*?

We can differentiate species based on species-specific SNPs (Single Nucleotide Polymorphism)

<table>
<thead>
<tr>
<th>Species</th>
<th>Sequence</th>
<th>Length</th>
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</thead>
<tbody>
<tr>
<td><em>Amaranthus palmeri</em></td>
<td>TCTCCCATGCCTCGCCGCTTCTGGATGCTGCTTAAAGGGAGCCCAGGGGTCTCGAGCTGCT</td>
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<td><em>Amaranthus spinosus</em></td>
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<tr>
<td><em>Amaranthus powellii</em></td>
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<tr>
<td><em>Amaranthus retroflexus</em></td>
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</tr>
</tbody>
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Preliminary work by Colorado team
How do we identify *A. palmeri*?

We can differentiate species based on species-specific SNPs (Single Nucleotide Polymorphism).

The state of this SNP can be used to call species.
How do we identify the state of an SNP?

Need a method that is
- Reliable
- Easy to read
- Binary (We only care if the SNP is Palmer or not)
KASP

Kompetative Allele Specific PCR
- Florescence based genotyping for a single allele
- Works for both SNPs and indels
- Developed by LGC

KASP used 3 primers
- 2 forward (allele specific), 1 reverse (universal)
- Forward primers end with SNP
KASP Marker Design

Allele A

Allele B
KASP Marker Design

Allele A

Allele B
KASP Marker Design

Allele A

Allele B
KASP Marker Design

Allele A

Allele B

Plus a reverse primer downstream
KASP runs on qPCR machine
Obtaining Plant Tissue
Material supply from GRIN
## Sampling Coverage

<table>
<thead>
<tr>
<th>Species</th>
<th>Populations</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. <em>palmeri</em></td>
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<td>A. <em>spinosis</em></td>
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<td>A. <em>albus</em></td>
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<tr>
<td>A. <em>blitoides</em></td>
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<tr>
<td>A. <em>arenicola</em></td>
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<tr>
<td>A. <em>hybridus</em></td>
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<tr>
<td>A. <em>powelii</em></td>
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</tr>
<tr>
<td>A. <em>retroflexus</em></td>
<td>5</td>
</tr>
</tbody>
</table>

Each population is 48 individuals, 24 validation and 24 sequencing.
Sampling Coverage
DNA extraction
Sequencing

Extracted DNA sent to University of MN Genomics Center (UMGC) for Genotyping by Sequencing (GBS)

Chris Saski (Clemson) has agreed to share his *A. palmeri* assembly, which will help map GBS reads

Results will be processed through the TASSEL GBSv2 pipeline (Buckler Lab, Cornell)
Validating Genetic Markers

We have 1 existing primer (double SNP) from our team at CSU

We will use the new GBS data to develop and test 2 more markers
We get something that looks like this, but we need to determine what group an individual belongs to.
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Computers are really bad at doing things that are “easy” for humans.
Hierarchical Clustering

Each sample begins as its own cluster

Cluster Dendrogram
Hierarchical Clustering

Nearby clusters are fused, reducing total number by 1

Cluster Dendrogram
Hierarchical Clustering

Process repeats, producing a few large clusters...

Cluster Dendrogram
Hierarchical Clustering

...until all samples are in a single group.
Hierarchical Clustering

Cutoff is based on # of clusters we want to find.
Hierarchical Clustering

Cutoff is based on # of clusters we want to find.
Hierarchical Clustering Output
Cluster Assignment for 8 Plates
HCLUST Performance

Tested 700 individuals

3 errors total
- 2 erroneous negative calls
- 1 erroneous ID
  - A single *A. arenicola* individual was IDed as Palmer

Found 2 populations that suppliers misidentified!
Good News: WI2011 is not Palmer
Bad news: AREN6 is Palmer
Marker calls backed up by morphology
Further Development

So we have a working KASP marker, but that was based on a relatively small number of populations

Plan to continue work in 2 Directions
- Validate against more populations
- Develop more markers
Final Goal: Bulk Seed Testing

Preliminary work by Colorado team
Contributors

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- Anthony Brusa
- Jim Anderson
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- David Marks
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Kansas State University
- Kevin Dorn

Clemson University
- Eric Patterson
Funding Acknowledgement

Funding provided by the Minnesota Invasive Terrestrial Plants and Pests Center through the Minnesota Environment and Natural Resources Trust Fund.
Questions