The Hunt for Herbicide Tolerant Rice for California

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Rice Weed Control in California
Challenging, complex, expensive, weed resistance, and regulated
Weed control is the primary production issue for California rice growers

- Resistant weeds are a problem for California!
- Weedy red rice has not been a weed problem for California but it looks like that is changing too.
- Herbicide tolerant varieties have been successfully developed and commercialized in maize, soybeans, sorghum, canola, and rice. These include transgenic traits (GM) and non-transgenic traits (Clearfield® rice).
- GM rice “is out of the question” for California and most other places currently.
- The BASF Clearfield® technology has not been made available in California and a second non-transgenic system, Provisia™ (ACCase inhibitor based) is in development with “no plans to come to California.” RiceTec, Inc.-Adama just announced the development of similar herbicide resistant rice technologies.

Reasons given why not California:

“Insufficient value capture”, continuous rice cropping, water seeding, drift on non-target crops, limited acreage, regionally limited varieties, and regulatory obstacles.
RES Efforts 2009-13

The RES Breeding Program has been screening herbicides for resistant mutants since 2009. Challenges include:

✓ Getting a simple and effective screening method
✓ Not having escapes, especially in the field
✓ Identifying an effective registerable herbicide for weed control
✓ Herbicides where a successful mutant can be recovered. (really have not seen success anywhere with glyphosate or glufosinate unless there are transgenic)
✓ Generation of enough material to find a rare event

Our initial effort has involved field and greenhouse screening of registered California rice herbicides, Granite® (penoxulam) and Prowl® (pedimethalin). The idea was to try to identify mutants that would provide a very high level of tolerance to an existing registered California herbicide (avoiding the regulatory/registration obstacles & costs).

• Greenhouse screening of $X_2$ populations with a high rate of Granite was not successful although mutants for plant height and type were recovered, and the project was discontinued.
• Using Prowl initially identified some putative resistant mutants in germination tests, but testing in the greenhouse and field has not seen success.
A plant breeding assistant was hired to support the project in 2015.

Induced mutant populations of were generated using chemical mutagens and radiation and advance in the greenhouse and field for screening 2014-6.

Greenhouse and field screening of material was conducted year round involving different herbicides.

We advanced, confirmed, and evaluated any putative herbicide resistant mutants.
2014-16 field screening involved about 20 seeded acres (8 ha) with different herbicides that were very effective on weeds and rice. Dozens of putative tolerant mutants were recovered and advance to confirm resistance.

Yes.... This truly was a spray and pray!
In July of 2014, 29 putative resistant mutant $M_3$ seedlings were recovered from a greenhouse screening with oxyfluorfen (Goal 2XL®)
Oxyfluorfen Mutants Spray Chamber Test of seed from putative mutants.

- 1-9 were not killed (all in the same tray from the same screen) but other selections had no survivors.
- 1-9 were weakened and some died in the lines (segregating?).
- Retested a few seeds again and confirmed resistance in 1-9.

Nov-Dec. 2014
Figure 1

Oxyfluorfen Seedling Rate Study
M-206 and M-206 Mutant Lines 1 to 9

Avg. Seedling Height (mm)

Oxyfluorfen (Goal 2XL) pt./acre

Days

M-206  1  2  3  4  5  6  7  8  9

7 10 14 7 10 14 7 10 14 7 10 14 7 10 14
• Advanced the survivors 1-9 from spray chamber test in winter greenhouse.
• Tolerance trait inheritance crosses made and tolerance segregation noted in greenhouse F₂ population, F₃ progeny test and other F₂ in winter plantings.
• Evaluated/purified lines (sterility or other changes noted and segregation).
• Crossing program, DNA mapping, and target gene sequencing initiated.
• Seed increase for 2016 field studies made in RES and winter Hawaii nurseries.
• 2015 Field application timing & rate studies and weed control studies initiated.
2015 Glenn County Field Test - CCFS

Water seeded 9 lines and M-206 as spokes in the ring

M-206 Control

Goal Line Stand!

GoalTender®
2pt/acre preplant
Mutant Lines

No crop competition

Goaltender @ 2 pt/A
Pre-flood
Glenn County

06/25/2015
**Additional 2015 Discoveries**

1. Two additional resistant mutants were recovered in greenhouse screen (same lot and event).

2. There are M-206 survivors and recovery at Glenn ring test.

3. In a pre-flush application of oxyfluorfen the drill seeded mutant lines and M-206 all emerged (not shown).
Genetics and Breeding

Oxyfluorfen is a Protoporphyrinogen Oxidase-Inhibiting (PROTOX) herbicide.

- This is the same Herbicide Resistance Action Committee Group as Shark® (carfentrazone) but a different chemical family.
- The PPO gene has been identified in the literature as the site that provides resistance for PROTOX herbicides.
- Drs. Andaya and Sanchez completed a project to sequence the PPO gene in the mutant selections and M-206.

Leaf tissues were collected from G1-9 and M206. DNA Extraction and Purification were done thru Qiagen Plant Maxi Prep (Column purification). DNA sent to Arizona Genome Institute (AGI). AGI sequenced the 4.5 kb region of Chromosome 1 containing the candidate gene, PPO. AGI designed sequencing primers based on Nipponbare sequence.

Sequence comparison between mutants and M-206 will reveal if the mutation is indeed in this candidate gene.
No differences in base sequence of the PPO gene for the lines 14G1-9 and M-206

The mutation causing the oxyfluorfen tolerance is somewhere else, new? Mapping studies in progress indicate the mutation is on chromosome 5.
Inherited as a single recessive gene and we named it **ROXY**

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<th>Phenotype</th>
<th>Observed</th>
<th>Theoretical</th>
<th>$X^2$ for 3:1 inheritance</th>
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<tbody>
<tr>
<td>Susceptible</td>
<td>136</td>
<td>139</td>
<td>=0.25</td>
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<tr>
<td>Resistant</td>
<td>50</td>
<td>47</td>
<td>0.50&lt;P&lt;0.70</td>
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**14G4/M-206 F₂ plants**

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<th>$X^2$ for 3:1 inheritance</th>
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<tbody>
<tr>
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<td>124</td>
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<tr>
<td>Resistant</td>
<td>49</td>
<td>41</td>
<td>0.20&lt;P&lt;0.10</td>
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**A-202/14G7 F₂ plants**

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<tbody>
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<td>Resistant</td>
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<td>143</td>
<td>0.99&lt;P&lt;0.95</td>
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Segregating A-202/14G7 F₃ lines a cross to susceptible variety
F$_2$ and Selected F$_3$ Populations 2016

- Tolerant 15G4 lines 5’ rows (regular seeding rate, seed increase)
- F$_2$ populations segregating for tolerance
- Tolerant F$_3$ bulk populations selected in Hawaii
- Tolerant G lines (low seeding rate)

Drill 40’ plots (very low seeding rate)
Applied GT 2pt./a and flushed 2x
All emerged and applied Clincher®. Applied GT 2pt./a on emerged plants

Susceptible Parents

1. Trait is inherited from crosses.
2. Tolerant plants were selected from treated winter nursery.
3. Post emergence spray of GT effective for selecting for tolerant plants.
4. Complete weed control in open water areas through July.
2016 replicated large plot tests confirmed the resistance of the Roxy mutant lines to GoatTender (GT) compared to M-206.

Water seeded plots with GT applied pre-flood @ 2pt./acre
Breeders are selecting improved lines from crosses with the ROXY mutation. M-206 backcross lines are available for further yield and weeds testing in 2017. Seed from the mutant lines will be further evaluated as well. Efforts are continuing to secure a registrant and labeling for use on water seeded rice in California. A US patent for the ROXY mutant allele was filed 9/2016.