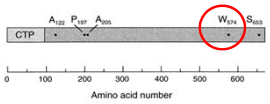
Patterns in the Inheritance of Resistance to ALS-Inhibiting Herbicides in Giant Ragweed Infers Link with Self-Incompatibility

Benjamin C Westrich, Subramanian Sankaranarayanan, Sharon A Kessler, Bryan G Young

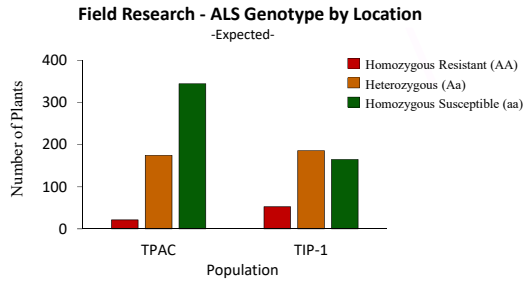
Distribution of ALS-R Giant Ragweed

- Surveys have identified giant ragweed biotypes resistant to ALS-inhibitors (ALS-R) in 13 of 15 states across the Midwest[1]
  - ALS-R giant ragweed found in 60% of populations tested[2]
- Resistance is conferred by a dominant point mutation in ALS (W574L)



[1]Regnier et al. (2016)
[2]Harre (2017)

Where are the Homozygotes Mutants?



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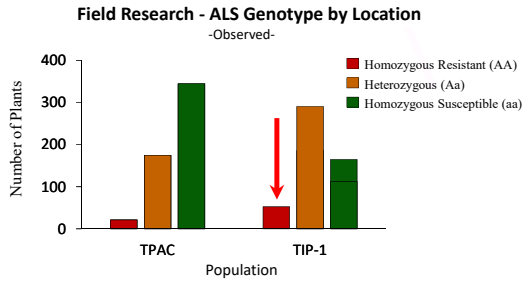
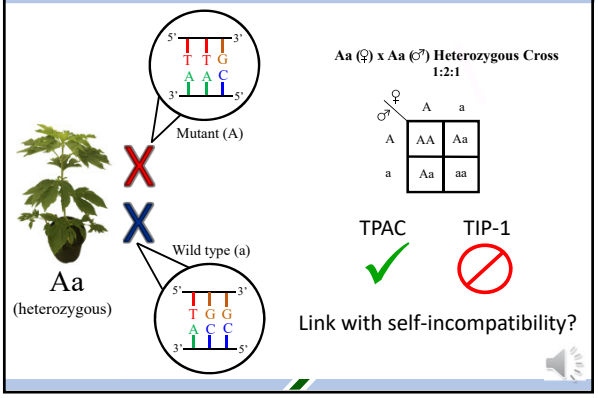


Mendelian inheritance[1], no fitness penalty[1,2]

[1]Patzoldt and Tranel (2002)
[2]Marion et al. (2017)

Outcrossing of Heterozygous Plants



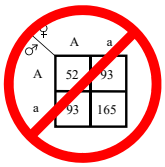
Link with self-incompatibility?

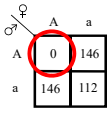
Self-Incompatibility

- Self-incompatibility (SI) mechanisms are utilized by ~60% of flowering plants to maintain genetic diversity[1]
- Pollination fails when both pollen and pistil share the same SI allele(s)
- Pollination between incompatible gametes is halted between pollen-style adhesion and ovule fertilization
  - Partial or "leaky" SI mechanisms can occur as a result of complex dominance interactions
- *Senecio squalidus* (Asteraceae) serves as a model plant for study of sporophytic SI[2]
  - **No studies examining SI in giant ragweed**

[1]Nettancourt 2001)
[2]Hiscock (2000)

Hypothesis





The lack of homozygous-resistant giant ragweed plants at TIP-1 may be the result of a self-incompatibility response when both gametes possess the ALS-R mutation

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Objectives

1. Confirm presence of an SI mechanism
2. Validate field experiments in controlled cross
   a) Absence of homozygous mutant progeny
3. Confirm that resistance is transferred through both pollen and ovules
   a) Reciprocal cross to investigate segregation distortion
4. Determine whether homozygous mutants are produced in a cross between populations

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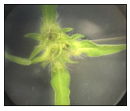
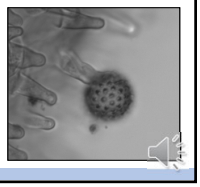
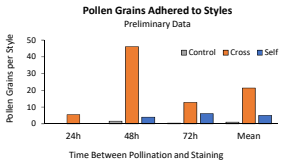
Methods: Observing Pollination

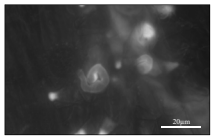
- Giant ragweed plants grown in greenhouse for 2 months
  - Growth chamber (12h photoperiod) to initiate flowering
- Self- and cross-pollinated branches created by emasculation and covering
- Pistils were hand-pollinated then collected and stained for microscopy
  - Fixed in 3:1 EtOH:Acetic Acid for 12 hours
  - Softened with 1 M NaOH for 3h
  - Stained with aniline blue for 24h
  - Dissected styles from ovules
  - Observed with Nikon Eclipse Ti2
  - Counted attached pollen grains, observed pollen tubes

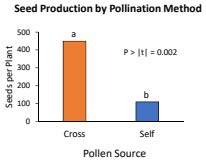


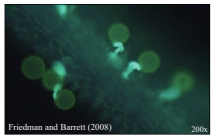


Predominate SI Mechanism - Pollen Rejection









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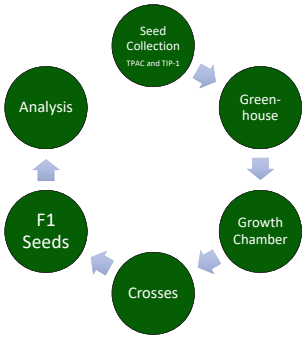
Methods: Generating F1



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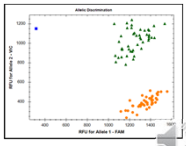
Methods: Analyzing F1

- DNA was primarily extracted directly from F1 seeds (n=100)
- Subset of F1 populations were planted in the greenhouse
  - Leaf DNA extracted
  - Sprayed with cloransulam (42 g ai ha^{-1})
    - Visual assessment (alive/dead) and biomass collection after 28 days
- Seed and plant genotypes determined by TaqMan® SNP genotyping assay[1]
- PROC FREQ in SAS 9.4 for Chi-squared goodness-of-fit test



[1]Harre et al. (2017)

Crosses Conducted with TIP-1 Plants

Cross	Notes	Research Question
aa x self	Susceptible self-pollination	Control
Aa x self	Heterozygous self-pollination	Control
♀Aa x Aa♂	Heterozygous female, Heterozygous male	Are AA progeny produced?
♀Aa x aa♂	Reciprocal (heterozygous female, susceptible male)	Is A transmitted through ovules?
♀aa x Aa♂	Reciprocal (susceptible female, heterozygous male)	Is A transmitted through pollen?
Aa x Aa*	Interpopulation cross	Are these trends affected by increased genetic variation?

*Plant from TPAC population

Medelian Inheritance

Aa ♀ x Aa♂ Heterozygous Cross
1:2:1

♂ \ ♀	A	a
A	AA	Aa
a	Aa	aa

Aa♀ x aa♂ Reciprocal Cross
0:1:1

♂ \ ♀	A	a
a	Aa	aa
a	Aa	aa

aa♀ x Aa♂ Reciprocal Cross
0:1:1

♂ \ ♀	a	a
A	Aa	Aa
a	aa	aa

Results: Genotype of F1 Populations

Cross	Plant ID	Genotypic ratio of F1 AA:Aa:aa Expected[a]	Actual	P-value χ^2	Conclusions
aa x self	31	0:0:38	0:0:38	na	
Aa x self	21	25:50:25	1:72:27	****	
Aa x self	3	8:17:8	1:32:0	****	
♀Aa x Aa♂	20	25:50:25	0:98:2	****	
♀Aa x aa♂	15	0:50:50	0:99:1	****	
♀aa x Aa♂	33	0:50:50	0:89:11	****	
Aa x Aa[b]	13	25:50:25	0:70:30	****	
Aa[b] x Aa	45	25:50:25	0:60:40	****	

**** $P < 0.0001$
[a]P value for $\chi 2$ in "Expected" column
[b]Plant was from TPAC population

Discussion

- Data show reduced pollen retention, seed set in self-pollinated plants
  - Pollen rejection is the predominant SI mechanism in giant ragweed
- Confirmed field observations
  - **Homozygous mutant progeny were not produced in heterozygous cross**
  - **Reciprocal crosses lacked homozygous susceptible progeny**
- Linked inheritance of ALS and SI genes could explain why heterozygosity of ALS is favored
  - Similar linkages proposed, yet to be investigated[1,2]

[1]Scarabel et al. 2015
[2]Ntoanidou et al. 2017

Implications

- Crossing susceptible and heterozygous plants strongly favors resistant progeny
  - Facilitates rapid spread of resistance
  - Mutant (resistant) pollen is more compatible with wild type pistils than wild type pollen (and vice versa)
- Identification of SI locus and behavior in new species

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Future Research

- Elucidation of a novel link between a herbicide target gene and SI gene(s)
- Additional Crosses
  - Test this hypothesis more directly
  - Examine other populations for similar inheritance patterns

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