

Survey of *psbA* gene diversity and photosystem II inhibiting herbicide resistance in annual bluegrass (*Poa annua*) biotypes.

J.S. McElroy, Andrej W. Svyantek, Shu Chen, Michael Flessner, and Carol A. Mallory-Smith.
Auburn University, Auburn, AL. (2) Oregon State University, Corvallis, OR.

Abstract [No. 111-2]

Amicarbazone is a relatively new photosystem II (PS II) inhibiting herbicide introduced for use in turfgrass for selective *Poa annua* control. *Poa annua* resistance to PSII inhibiting herbicides already exists with known resistance to substituted ureas (diuron) and triazine (atrazine and simazine). Two key mutations in the *psbA* gene of *Poa annua* are known to confer resistance to PSII herbicides – Ser₂₆₄ to Gly and Val₂₁₉ to Ile. Ser₂₆₄ to Gly was previously confirmed by the authors to confer resistance to amicarbazone and triazines. Our objective was two-fold: to evaluate the effects of Ser₂₆₄ to Gly mutations on diuron response and to locate *Poa annua* populations with Val₂₁₉ to Ile mutation in order to evaluate the response of amicarbazone, diuron, and triazines to populations with this mutation. Twenty-six *Poa annua* populations have been screened with *psbA* genes partially sequenced. We have concluded that the Ser₂₆₄ to Gly mutation does not confer resistance to diuron, while it does confer resistance to amicarbazone and triazines. Secondly, while populations were identified with increased tolerance to diuron and susceptibility to triazines and amicarbazone, we have yet to elucidate a molecular mechanism of resistance as no population sequenced contained the suspected Val₂₁₉ to Ile mutation.

Problem

- Variable annual bluegrass control is often observed with amicarbazone.
- Annual bluegrass resistance to the PSII inhibiting herbicides atrazine, simazine, and amicarbazone caused by a Ser₂₆₄ to Gly mutation in the *psbA* gene has been reported previously by the authors (Perry et al., 2012)
- Annual bluegrass resistance to diuron, a PSII inhibiting herbicide, has been reported in Oregon seed fields as a result of Val₂₁₉ to Ile mutation in the *psbA* gene (Mengistu et al., 2000)

Hypothesis

Populations exhibiting reduced control with amicarbazone will contain either the Val₂₁₉ to Ile or Ser₂₆₄ to Gly mutation.

Table 2. Herbicide treatments utilized for evaluating response of annual bluegrass populations to PS II inhibiting herbicides.

Herbicide	Formulation	Product Rate	lb ai/a
Diuron	4 lb ai/gal	24 fl oz/a	1.5 lb ai/a
Diuron	4 lb ai/gal	48 fl oz/a	3.0 lb ai/a
Amicarbazone	70 % wt/wt	5 oz wt/a	0.2 lb ai/a
Amicarbazone	70 % wt/wt	10 oz wt/a	0.4 lb ai/a
Atrazine	4 lb ai/gal	24 fl oz/a	1.5 lb ai/a
Atrazine	4 lb ai/gal	48 fl oz/a	3.0 lb ai/a
Glyphosate (Roundup Pro Max)	4.5 lb ae/gal	22 fl oz/a	0.8 lb ae/gal

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Table 1. Summary of populations tested.

Population	Location	Response to PSII Inhibiting Herbicides
Auburn	Turfgrass Research Unit, Auburn, AL	Non-resistant check
Bay Point	Bay Point Golf Course, Destin, FL (Thanks to Superintendent Jamey Davis)	Non-resistant check
Bel Air	Bel Air Golf Course, Tupelo, MS (Thanks to Jim Taylor at Mississippi State for donation of all MS populations)	Resistant to atrazine, simazine
Bighorn	Bighorn Golf Club, Palm Desert, CA	Unknown
Canton	The Country Club of Canton, Canton, MS	Resistant to atrazine, simazine
DR3	Sod Fields, Oregon (Thanks to Carol Mallory-Smith)	Suspected resistance to amicarbazone
Fernwood	Fernwood Country Club, Pike, MS	Resistant to atrazine, simazine
GN1	Grand National Golf Course, Opelika, AL (Bermudagrass Green)	Unknown
GN3	Grand National Golf Course, Opelika, AL (Bentgrass Green)	Unknown
Greenville	Greenville Municipal Golf Course, Greenville, MS	Resistant to atrazine, simazine
Havnel	Unknown Miss State Population	Resistant to atrazine, simazine
Holiday	Holiday Golf Club, Panama City Beach, FL	Non-resistant check
Indianola	Indianola Country Club, Indianola, MS	Resistant to atrazine, simazine
Leflor	Leflor County Country Club, Leflor, MS	Resistant to atrazine, simazine
Meadows	Meadows Golf Course, Tupelo, MS	Resistant to atrazine, simazine
Ford	Ford Plantation, Savannah, GA (Thanks to Bert McCarty)	Non-resistant check
Purdue	Purdue University, West Lafayette, IN (Thanks to Aaron Patton)	Suspected resistance to amicarbazone
Purchase	Valley Seed Service, Fresno, CA	Non-resistant check
Redbud	Redbud Springs Golf, Kosciusko, MS	Resistant to atrazine, simazine
Rosedale	Rosedale Golf, Rosedale, MS	Resistant to atrazine, simazine
Savoy	University of Illinois Golf Courses, Savoy, IL (Thanks to Bruce Branham)	Suspected resistance to amicarbazone
TN1	Eagle Bluff Golf Course, Chattanooga, TN	Non-resistant check
UIC	University of Illinois Urbana Champagne, Champagne, IL (Thanks to Bruce Branham)	Suspected resistance to amicarbazone
Vestavia	Vestavia Country Club, Vestavia Hills, AL	Unknown
VT	Virginia Tech Golf Course, Blacksburg, VA (Thanks to Shawn Askew)	Suspected resistance to amicarbazone
Winona	Winona Country Club, Winona, MS	Resistant to atrazine, simazine

Fig 2, continued.

Pop	Diuron	Amicarbazone	Atrazine	Mutation
Auburn	Green	Green	Green	None
Savoy	Green	Green	Green	None
TN1	Green	Green	Green	None
UIC	Green	Green	Green	None
Vestavia	Green	Green	Green	None
VT	Green	Green	Green	None
GN1	Green	Green	Green	None
GN3	Green	Green	Green	None
Holiday	Green	Green	Green	None
Ford	Green	Green	Green	None
Bighorn	Yellow	Green	Green	None
DR3	Yellow	Yellow	Green	None
Havnel	Green	Yellow	Green	None
Purdue	Green	Yellow	Green	None
Purchase	Green	Yellow	Green	None
Bay Point	Green	Yellow	Green	None
Rosedale	Green	Yellow	Green	None
Winona	Green	Red	Red	Ser264 to Leu
Bel Air	Green	Red	Red	Ser264 to Leu
Canton	Green	Red	Red	Ser264 to Leu
Fernwood	Green	Red	Red	Ser264 to Leu
Greenville	Green	Red	Red	Ser264 to Leu
Indianola	Green	Red	Red	Ser264 to Leu
Leflor	Green	Red	Red	Ser264 to Leu
Meadows	Green	Red	Red	Ser264 to Leu
Redbud	Green	Red	Red	Ser264 to Leu

Materials and Methods

- Twenty-six annual bluegrass populations were collected or donated for screening. Populations with known resistance or reduced control with amicarbazone were compared to non-resistant populations.
- Populations were grown in greenhouse environment and treated with herbicides in Table 2 compared to a non-treated check. Herbicides were applied with standard spray equipment at 30 GPA and 0.25% v/v NIS.
- *psbA* genes were partially sequenced via PCR amplification and Sanger sequencing as reported in Perry et al. (2012). Sequences were aligned and compared using CLC Genomics Workbench.

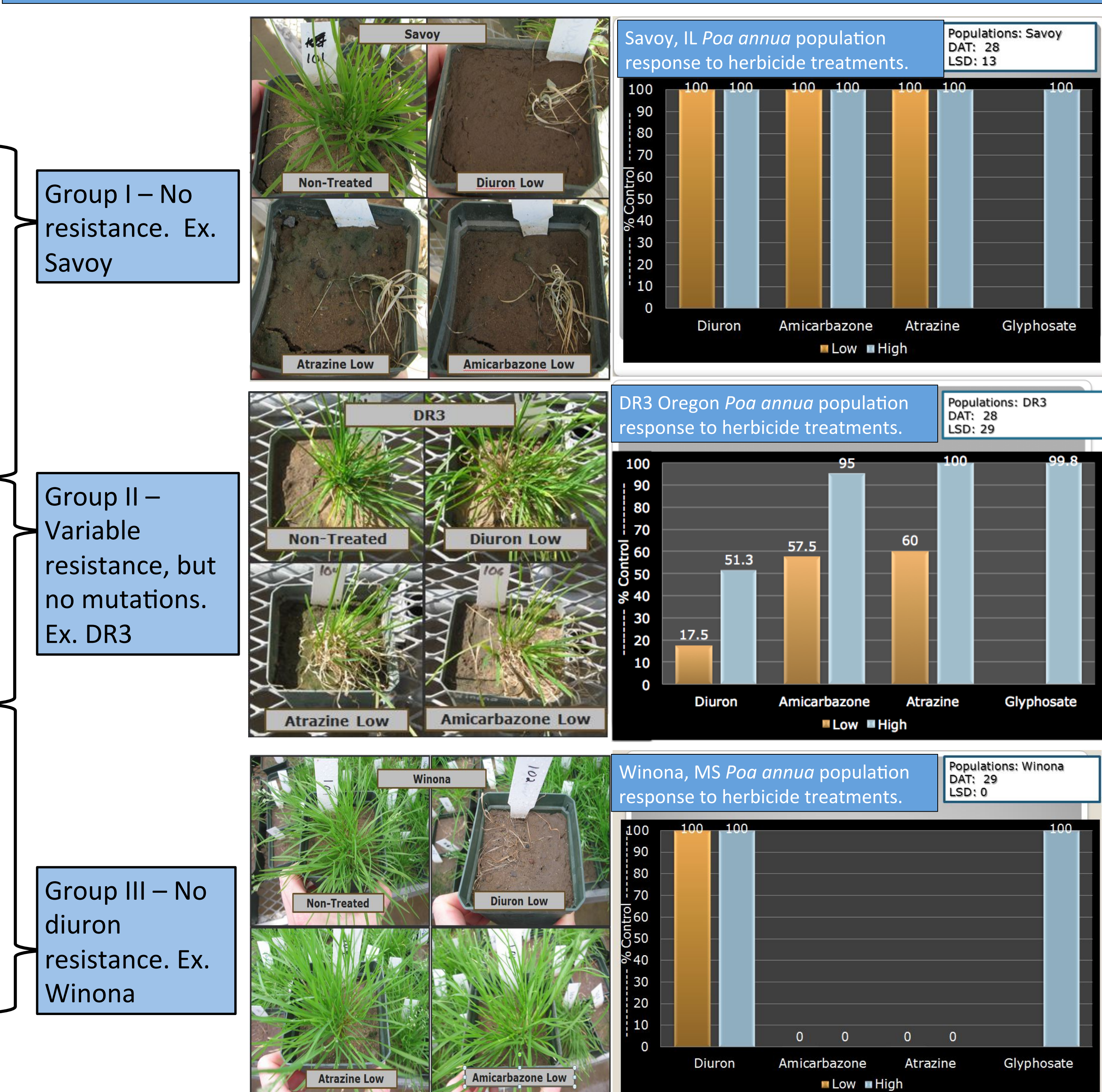
Results

- Response to PSII inhibiting herbicides could be divided into three distinct population groups. Group I – No resistance. Group II – Variable resistance. Group III – Resistance to atrazine and amicarbazone, but not diuron.
- The Val₂₁₉ to Ile mutation was not observed in any populations surveyed and thus could not describe the differences observed in Group II.
- Group III were populations with the Ser₂₆₄ to Gly mutation were resistant to atrazine and amicarbazone, but not diuron.
- Some population had suspected resistance to amicarbazone (VT, Savoy, UIC), but were found to be susceptible to all PSII herbicides.

Figure 1. Partial sequence of *psbA* gene of resistant populations containing Gly₂₆₄ and non-resistant containing Ser₂₆₄. Both contain Val₂₁₉.

Resistant – Gly ₂₆₄	(183)	MIVFOAEHNILMHPFHLGVAGVFGGSLFSAMHGSLVTSSLIRETTENES
Non-resistant – Ser ₂₆₄		MIVFOAEHNILMHPFHLGVAGVFGGSLFSAMHGSLVTSSLIRETTENES
Resistant – Gly ₂₆₄	(233)	ANEGYKFGQEBEETYNIVAHHGFGRLIFQYAGFNNSRSLHFFLAAPVVG
Non-resistant – Ser ₂₆₄		ANEGYKFGQEBEETYNIVAHHGFGRLIFQYAGFNNSRSLHFFLAAPVVG
Resistant – Gly ₂₆₄	(288)	IWFPTALGISTMAFNLMGFNFQSVVDSQGRVINTWADIINRANLGM
Non-resistant – Ser ₂₆₄		IWFPTALGISTMAFNLMGFNFQSVVDSQGRVINTWADIINRANLGM

Figure 2. Response of annual bluegrass populations to diuron, amicarbazone, and atrazine, and *psbA* mutations detected. Green denotes complete control at both rates, yellow denotes unacceptable control at low rates, and red denotes unacceptable control at high rates. An example picture and data set are presented for the herbicide grouping response.



Literature Cited

- Mengistu, L.W., G.W. Mueller-Warrant, A. Liston, and R.E. Barker. *psbA* mutation (valine₂₁₉ to isoleucine) in *Poa annua* resistant to metribuzin and diuron. *Pest Manag. Sci.* 56:209-217.
- Perry, D.H., J.S. McElroy, F. Dane, E. van Santen, and R.H. Walker. 2012. Triazine-resistant annual bluegrass (*Poa annua*) populations with Ser₂₆₄ mutation are resistant to amicarbazone. *Weed Sci.* 60:355-359.