Dynamics of Pronamide Action within *Poa annua*

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Introduction

Annual bluegrass (*Poa annua* L.) is one of the most problematic weeds in turfgrass systems. It decreases the quality of golf courses because of its light-green color, abundant seedhead production, and rapid summer decline that leaves aesthetically unpleasing brown patches in turfgrass (Yelverton 2015).

Pronamide is a mitotic inhibiting herbicide that controls annual bluegrass both pre- and post-emergence (Burt and Gerhold 1970) but may have limited efficacy when applied postemergence due to lack of root uptake (Carlson et al. 1975). Pronamide-resistant annual bluegrass was first reported on a golf course in Georgia, where the biotype survived post-emergence applications but was susceptible to pre-emergence applications (McCullough et al. 2017). Reduced absorption and translocation were reported as the non-target-site resistance (NTSR) mechanism associated with resistance.

Objectives

- Confirm pronamide resistance in three suspected pronamide-resistant annual bluegrass populations from Mississippi.
- Investigate the dynamics of pronamide at four different harvest times after foliar-only and soil-only applications within annual bluegrass.
- Sequence the α-tubulin gene from these populations to identify potential target-site mutations.

Experiment 1: Evaluation of pronamide resistance

Materials and Methods

- 5 populations [3 suspected pronamide-resistant and 2 pronamide-susceptible (S1 and S2)].
- Completely randomized design (5 replications), repeated twice in time.
- Tillers were established in native soil in greenhouse conditions.
- Pronamide was applied at 0, 0.28, 0.56, 1.12, 3.36, 6.7, and 20.2 kg ai ha^{-1} to young plants using an enclosed spray chamber.
- Visual control was assessed 4 WAT and dose response data were log transformed and fit to a sigmoidal variable slope regression model.

Results

- Pronamide resistance was confirmed in the three suspected pronamide-resistant populations (R1, R2, and R3).

Experiment 2: Evaluation of prodiamine resistance

Prodiamine resistance was assessed in the three R and two S populations.

Materials and Methods

- ***Hydroponic-assays***
  - Rapid whole-plant assay
  - 1.0 mM herbicide solution
  - Randomized complete block design (5 reps), repeated twice in time.
- ***Pre-emergence germination test***
  - 10 seeds per pot mixed sand/peat (90/10) soil
  - Completely randomized design (3 replications), conducted only once
  - Surviving plants were counted 28 days after germination.

Results

- Both tests confirmed that R1 and R2 populations were resistant to prodiamine, and R3, S1, and S2 were susceptible.

Experiment 3: Pronamide absorption and translocation

Two application methodology—foliar-only and soil-only application of pronamide— were conducted to evaluate the absorption and translocation of pronamide in the R and S annual bluegrass populations.

Materials and Methods

- Tillers from the three R and two S populations were established in pots containing potting mix under greenhouse conditions.
- Average temperature: 23 °C and photoperiod of 9 hours with LED lights.
- CRD (5 replications), repeated twice in time.
- Pronamide was applied at 1.160 kg ha^{-1} (1 × rate) in an enclosed spray chamber at 374 L ha^{-1} when plants had a height of 6.5 cm and foliar mass >0.1 g.

Foliar-Only Application of Pronamide

Before application

- Soil surface of the pots was covered with aluminum foil to prevent the herbicide from contacting the soil.

After application

- Plants were harvested 8, 24, 72, and 168 hours after treatment (HAT).
- Roots were washed and blotted dry with paper towels.
- Foliage samples were washed with 10% ethanol to remove the unabsorbed herbicide (leaf-wash samples).
- All samples (roots, foliage, leaf-wash) were stored at -80 °C until further processing.

Extraction and Quantification

- Samples were homogenized.
- Methanol (900 µL) was added as the extraction solution.
- Samples were further homogenized and centrifuged.
- HPLC system (Agilent 6470, Agilent Technologies Inc.) coupled to a mass spectrometer (Agilent 1290).
- Samples were quantitated with linear regression (Mass Hunter QQQ Analysis, Agilent).

Herbicide recovery parameters calculated

- *Absorption (%) = (roots + foliage) / (roots + foliage + leaf-wash)*
- *Basipetal translocation (%) = roots / (roots + foliage)*

Soil-Only Application of Pronamide

- Conditions were like those previously described, except for the soil type and the application method.
  - Native soil with organic matter = 0.45%.
  - Pronamide was directly applied to the soil in 20 mL of water with a syringe.
- Absorption was not measured in this experiment.
- *Acropetal translocation (%) = foliage / (roots + foliage)*

Statistical Analysis

- ANOVA (α =0.05).
- Means separation: Fisher's protected LSD test with PROC GLM procedure of SAS.
- Simple linear regression with GraphPad Prism (version 9.0).

Table 1. Absorption of pronamide by annual bluegrass plants from different populations following foliar application.

	Population				
HAT	R1	R2	R3	S1	S2
8	28 A	26 A	31 A	32 A	28 A
24	33 a	44 a	34 a	34 a	40 a
72	27 B	33 AB	24 B	34 AB	40 A
168	31 a	23 a	23 a	25 a	26 a

- Foliar absorption was similar in R and S populations, 8, 24, and 168 HAT.
- Did not exceed 44%, regardless of population and harvest time.

Table 2. Translocation of pronamide in annual bluegrass plants from different populations following foliar application (basipetal translocation).

	Population				
HAT	R1	R2	R3	S1	S2
8	11 A	5 A	4 A	4 A	12 A
24	13 a	3 b	3 b	7 ab	5 b
72	6 A	5 A	3 A	3 A	4 A
168	8 a	5 a	3 a	4 a	5 a

- Basipetal translocation was similar in R and S populations, 8, 72, and 168 HAT.
- Limited—only 3–13% of the absorbed pronamide— in all populations, regardless of harvest time.

Table 3. Translocation of pronamide in annual bluegrass plants from different populations following soil application (acropetal translocation).

	Population				
HAT	R1	R2	R3	S1	S2
8	22 B	26 AB	35 AB	25 B	39 A
24	20 b	41 a	46 a	37 a	43 a
72	32	67 A	74 A	69 A	49 B
168	24 b	41 b	69 a	72 a	69 a

- In general, acropetal translocation did not differ between R2, R3, and both S populations across harvest times.
- The R1 population translocated less soil-applied pronamide than the S populations, 24, 72, and 168 HAT.

Experiment 4: Target-site gene sequencing

Materials and Methods

- Common mutations in the target-sites of mitotic-inhibiting herbicides were sequenced for the R and S populations at Auburn University.

Results

- The mutation Thr239-Ile on the α-tubulin gene—commonly associated with resistance to dinitroaniline herbicides, including prodiamine—was discovered in the three R populations.
- Results were complicated by the discovery that the S1 population contained the same target-site mutation yet was susceptible to prodiamine.
- This mutation is associated with prodiamine resistance in R1 and R2 populations and may also be responsible for pronamide resistance in R1, R2, and R3 populations.

Discussion

- Absorption and translocation data do not strongly support a NTSR mechanism.
- This is the first report linking a target-site mutation to pronamide resistance.
- The Thr239-Ile mutation cannot be confirmed as the mechanism conferring resistance to pronamide in the 3 R populations.
- However, the Thr239-Ile mutation is the most likely mechanism of resistance.

Conclusion

- This study reports three new pronamide-resistant populations from Mississippi.
- The same Thr239-Ile mutation that leads to dinitroaniline resistance may also contribute to pronamide resistance in the three R populations.
- Target-site mutations could mask NTSR mechanisms in resistant plants.
- Both target-site- and translocation-based mechanisms may be associated with pronamide resistance in the R1 population.

Future research

- Evaluate more pronamide-resistant populations to confirm the association between the Thr239–Ile mutation and pronamide resistance.
- Research on the the α-tubulin gene expression level and where it is expressed in the plant could confirm that this target site resistance mechanism is a cause of pronamide resistance.

References

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