

# Non-Target Site Resistance Mechanisms in Annual Bluegrass (*Poa annua*)

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## Introduction

- Herbicide resistance can be divided into two groups, target site resistance (TSR) and non-target site resistance (NTSR) (Gaines et al., 2020)
- NTSR is characterized by reduced absorption, and/or translocation of an herbicide, sequestration in a plant part or increased metabolism of an herbicide (Rigon et al., 2020)
- A population of annual bluegrass in Alabama resistant to PS-II inhibiting herbicides demonstrated reduced absorption, translocation, and metabolism to an application of atrazine (SyvanteK et al., 2016)
- TSR and NTSR mechanisms can combine at the individual level to produce populations with higher resistance levels (Gaines et al., 2020)
- Multiple annual bluegrass populations from five states in the southeastern United States were evaluated

## Objective

- Determine if select annual bluegrass (*Poa annua* L.) populations exhibit non-target site resistance mechanisms.

## Materials and Methods

- Location
  - Raleigh, North Carolina
- Evaluated herbicide and rates
  - Simazine = 2.24 kg ai ha<sup>-1</sup>
- Evaluated populations
  - All populations underwent a comprehensive dose response study to confirm resistance
  - Six suspected resistant and one susceptible population were evaluated
- Radiolabeled technical material
  - Radioactive solutions for each treatment consisted of a 1:1 v/v mixture of HPLC-grade water and methanol, with a non-ionic surfactant NIS (0.25% v/v)
  - 5 kBq radioactivity of <sup>14</sup>C-simazine
- Harvest timings
  - 0, 4, 12, 24, 96 and 192 hours after treatment (HAT)
- Evaluated plant parts
  - Treated leaf, treated leaf wash, other leaves, crown, and roots

### Trial Initiation

- Prior to herbicide application, the third youngest leaf was covered with tinfoil.
- Herbicide applications were made using a CO<sub>2</sub>-pressurized sprayer equipped with an 8002 EVS nozzle calibrated to deliver 187 L ha<sup>-1</sup>.
- A 1 µl droplet of radioactive herbicide solution was placed on the adaxial surface of the covered third youngest fully expanded leaf.

### Laboratory Methods

- Treated leaves were rinsed in a scintillation vial containing 20 ml 50:50 methanol: deionized water with the addition of a NIS (0.25% v/v) to remove any unabsorbed herbicide.
- All plant parts were dried for 48 hours at 60° C, weighed, and combusted with a biological oxidizer

### Experimental Design and Data analysis

- Randomized block design with a factorial treatment arrangement
- Four replicates
- Two experimental runs

### Statistical Analyses

- Data subjected to ANOVA ( $\alpha \leq 0.05$ )
- Means were separated using Fisher's Protected LSD ( $\alpha = 0.05$ )



Figure 2a. <sup>14</sup>C-simazine solution  
 Figure 2b. Liquid Scintillation Analyzer  
 Figure 2c. OX-500 Biological Material Oxidizer

## Results

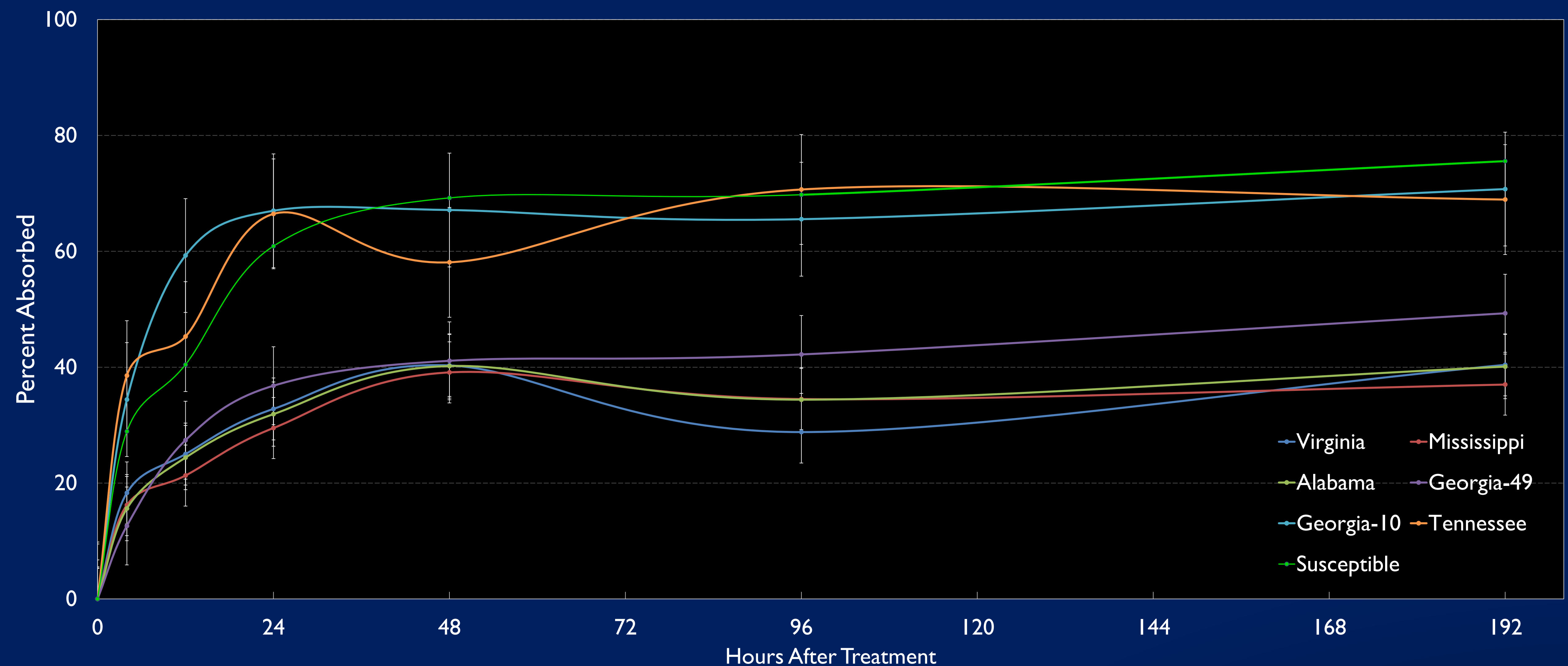


Figure 1. Two-way interaction of hours after treatment (HAT) and population on <sup>14</sup>C-simazine absorption in annual bluegrass

Table 1. Population-by-plant part interaction on <sup>14</sup>C-simazine translocation in annual bluegrass.<sup>a</sup>

|                                    | Alabama                     | Georgia-49 | Virginia | Mississippi | Georgia-10 | Tennessee | Susceptible |
|------------------------------------|-----------------------------|------------|----------|-------------|------------|-----------|-------------|
| <b>Plant Part</b>                  | % of recovered <sup>b</sup> |            |          |             |            |           |             |
| Wash                               | 60.7                        | 56.5       | 58.2     | 65.1        | 33.6       | 38.0      | 38.3        |
| Treated Leaf                       | 37.0                        | 41.5       | 38.9     | 34.1        | 64.2       | 59.7      | 60.7        |
| Shoot                              | 2.0                         | 1.8        | 2.8      | 0.7         | 1.3        | 1.7       | 0.7         |
| Crown                              | 0.2                         | 0.04       | 0.1      | 0.06        | 0.4        | 0.4       | 0.1         |
| Root                               | 0.1                         | 0.04       | 0.02     | 0.02        | 0.3        | 0.2       | 0.2         |
| LSD <sub>(0.05)</sub> <sup>c</sup> | 3.2                         |            |          |             |            |           |             |

<sup>a</sup>Data pooled over two experimental runs and six harvest timings.  
<sup>b</sup>% of recovered = (total radioactivity in plant part) ÷ (total radioactivity recovered in plant) × 100  
<sup>c</sup>Fishers Protected LSD conducted at P = 0.05

## Discussion

- Analysis of variance indicated a significant two-way interaction between population and HAT for <sup>14</sup>C-simazine absorption
- Georgia-49, Alabama, Georgia-10, and Mississippi populations absorbed 13% to 19.1% less <sup>14</sup>C-simazine compared to the susceptible population at 12 HAT (Figure 1)
- This trend continues to 192 HAT where the Georgia-49, Alabama, Georgia-10, and Mississippi populations absorbed 26.2% to 38.5% less <sup>14</sup>C-simazine compared to the susceptible population
- Significant differences were not observed in <sup>14</sup>C-simazine absorption for the Virginia and Tennessee populations when compared to the susceptible population
- These data suggest reduced absorption does not contribute to resistance in the Virginia and Tennessee populations
- Findings in this study are similar to SyvanteK et al., (2016) in which an annual bluegrass population resistant to atrazine absorbed 50% less than a susceptible population
- Analysis of variance indicated a significant two-way interaction between population and plant part for <sup>14</sup>C-simazine translocation
- Translocation data show 19.2 to 26.6 % more <sup>14</sup>C-simazine in the treated leaf of the susceptible population when compared to the Alabama, Georgia-49, Virginia and Mississippi populations (Table 1)

## Conclusions

- Reduced absorption may contribute to resistance in the Georgia-49, Alabama, Georgia-10, and Mississippi populations
- Translocation data depict no statistical differences between populations in regards to movement of <sup>14</sup>C-simazine out of the treated leaf

## Future Research

- Conduct metabolism research to determine if resistant populations exhibit increased metabolism of simazine
- Conduct absorption, translocation studies on additional annual bluegrass populations

## Literature Cited

- Gaines TA, Patterson EL, Neve P (2019) Molecular mechanisms of adaptive evolution revealed by global selection for glyphosate resistance. *New Phytol* 223(4):1770–1775.
- Gaines TA, Duke SO, Morran S, Rigon CAG, Tranel PJ, Küpper A, Dayan FE. Mechanisms of evolved herbicide resistance. *J Biol Chem*. 2020 Jul 24;295(30):10307–10330.
- Mendes KF, Silveira RF, Tornisiello, MHI VL (2017). Procedures for Detection of Resistant Weeds Using <sup>14</sup>C- Herbicide Absorption, Translocation, and Metabolism. In (Ed.), *Herbicide Resistance in Weeds and Crops*. IntechOpen.
- Rigon, Carlos & Gaines, Todd & Kuepper, Anita & Dayan, Franck. (2020). Metabolism-Based Herbicide Resistance, the Major Threat Among the Non-Target Site Resistance Mechanisms. *Outlooks on Pest Management*. 31. 162-168. 10.1564/v31\_aug\_04.
- SyvanteK AVV, Aldahir P, Chen S, Flessner ML, McCullough PE, Sidhu SS, McElroy JS (2016) Target and non-target resistance mechanisms induce annual bluegrass (*Poa annua*) resistance to atrazine, amicarbazone, and diuron. *Weed Technol* 30:773–782