2016-12-562

Title: Developing a Rapid Method for Diagnosing Herbicide Resistance in Annual Bluegrass

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Objectives: Determine if agar-based rapid diagnostic tests can be used to diagnose herbicide resistance in annual bluegrass populations harvested from golf course turf.

Start Date: 2016

Project Duration: Two years

Total Funding: $57,156

Summary Text: Reports of herbicide resistance in annual bluegrass (*Poa annua* L.) are greater than any other weed species commonly found in turf. Annual bluegrass phenotypes resistant to mitotic inhibitors (e.g., prodiamine), acetolacatate synthase inhibitors (ALS; e.g., foramsulfuron, trifloxysulfuron, etc.), photosystem II inhibitors (PSII; e.g., simazine), and enolpyruvylshikimate-3-phosphate (EPSP) synthase (e.g., glyphosate) inhibitors have been identified on golf courses following continued use of the same pre- or postemergence herbicides in lieu of diversified weed management programs.

Traditional means of testing annual bluegrass for herbicide resistance can be labor intensive, costly, and time consuming. Rapid diagnostic tests have been developed to confirm herbicide resistance in weeds of agronomic cropping systems that correlate well with traditional whole plant bioassays. These tests involve transplanting weed seedlings of resistant and susceptible populations into petri plates filled with agar and discriminatory rates of herbicide. This technique has successfully been used to provide farmers confirmation of rigid ryegrass (*Lolium rigidum*) populations resistant to both ALS and acetyl co-A carboxylase inhibiting herbicides, as well as Italian ryegrass (*Lolium multiflorum*), goosegrass (*Eleusine indica*), horseweed (*Conzya canadensis*), and common waterhemp (*Amaranthus rudis*) populations resistant to glyphosate.

Research was conducted at the University of Tennessee during 2016-2017 to determine if agar-based rapid diagnostic tests could be used to confirm herbicide resistance in annual bluegrass harvested from golf course turf. Separate experiments were conducted using annual bluegrass phenotypes resistant to ALS and PSII inhibiting herbicides and glyphosate via target site mutation; an herbicide susceptible control was included in each for comparison. Single tiller plants were washed free of growing media and transplanted into autoclavable polycarbonate plant culture boxes filled with 65 mL of murashigee-skoog media amended with glyphosate (0, 6, 12, 25, 50, 100, 200, or 400 µM), trifloxysulfuron (6.25, 12.5, 25, 50, 75, 100, or 150 µM), or simazine (0, 6, 12, 25, 50, 100, 200, or 400 µM). Treatments were arranged in a completely randomized design with 50 replications and repeated in time. Mortality in agar was assessed 7 to 10 days after treatment (depending on herbicide) and compared to responses observed after treating 98 individual plants of each phenotype with glyphosate (560 g ha^{-1}), trifloxysulfuron (27.8 g ha^{-1}) , or simazine (1120 g ha^{-1}) in an enclosed spray chamber. Fisher's exact test (α =

0.05) determined that mortality in agar with 100 µM glyphosate was not significantly different than treating whole plants via traditional spray application. Similarly, mortality in agar with 12.5 µM trifloxysulfuron was not significantly different than spraying whole plants with herbicide. Mortality with all concentrations of simazine in agar was significantly different than that observed after treating resistant and susceptible phenotypes via traditional spray application. Our findings indicate that an agar-based diagnostic assay can be used to detect annual bluegrass resistance to ALS- or EPSPS-inhibiting herbicides in less than 10 days; however, additional research is needed to refine this assay for use with PSII-inhibiting herbicides.

Summary points:

- Herbicide-resistant annual bluegrass is becoming increasingly problematic on golf courses throughout the transition zone and southern United States.

- Traditional means of confirming herbicide resistance in annual bluegrass can be labor intensive, costly, and time consuming leaving superintendents with little guidance regarding proper management in-season.

- A rapid diagnostic assay in agar culture can now reliably diagnose annual bluegrass resistance (or susceptibility) to glyphosate or trifloxysulfuron in 10 days or less.

Figure 1. Herbicide-resistant annual bluegrass (*Poa annua*) following two broadcast applications of glyphosate at 1120 g ha^{-1} during winter dormancy in Rockford, TN.

Figure 2. Autoclavable polycarbonate plant culture box used to diagnose annual bluegrass (*Poa annua*) resistance to herbicides in agar culture.

Figure 3. Response of three annual bluegrass (*Poa annua*) phenotypes to 12.5 µM trifloxysulfuron in agar culture. Note that the two resistant phenotypes on the left are not affected by trifloxysulfuron while the susceptible phenotype on the right shows severe tissue discoloration 7 days after treatment.