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Accumulation of Shikimate in Corn and Soybean Exposed to Various Rates of Glyphosate

W. Brien Henry, USDA-ARS, Central Great Plains Research Station, 40335 County Road GG, Akron, CO 80723-0400; **Clifford H. Koger**, USDA-ARS, J. Whitten Delta States Research Center, 141 Experiment Station Road, Stoneville, MS 38776-0345; and **Dale L. Shaner**, USDA-ARS, Northern Plains Area, Water Management Resource Unit, Fort Collins, CO 80526-8119

Corresponding author: W. Brien Henry. Brien.Henry@ars.usda.gov

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Abstract

Glyphosate is used extensively throughout the world as a burndown and in-crop herbicide. A spectrophotometric assay was evaluated as an inexpensive and rapid procedure to measure shikimate accumulation in conventional corn and soybean following glyphosate application. Shikimate levels peaked between 4 and 7 days after application in both corn and soybean. It is useful to understand the behavior of shikimate in plants so that management decisions can be made regarding conventional crops' exposure to glyphosate. Further refinement of this assay may lead to an assay that can be used to monitor glyphosate drift or detect glyphosate resistant weeds.

Introduction

Glyphosate is a non-selective herbicide that controls many troublesome annual and perennial weeds (2,6,8,15,16). Its broad-spectrum efficacy and price has resulted in extensive glyphosate use for burn-down prior to planting and also in-season on glyphosate-resistant (GR) crops. In the United States in 2004, GR-varieties were planted on 60% and 80% of the cotton and soybean acreage, respectively (18). As the number of glyphosate-treated acres increases, so too does the likelihood of drift onto neighboring susceptible crops.

Glyphosate is difficult to detect and quantify in plants; however, shikimate, a precursor in the amino acid pathway inhibited by glyphosate, accumulates in excess of endogenous levels after treatment with the herbicide (5,9,17). Shikimate is easily extracted from plants and has been used as a surrogate for determining if plants have been exposed to glyphosate (1,3,4,13,17).

There are increasing reports of glyphosate drift onto sensitive crops, particularly in the Southern U.S. (D. Muzzi, Delta Farm Press, 16 April 2004; V. Boyd, Rice Farming, February 2004; D. Muzzi, Delta Farm Press, 26 April 2002). Often the damage is not seen until 5 to 7 days after glyphosate exposure. However, crops may show stress unrelated to glyphosate drift, and it is important to determine if injury is due to glyphosate or some other factor. Measuring shikimate levels in plants can be a method for determining if injury is due to glyphosate. However, such measurements need to be rapid and inexpensive to be useful.

There are two methods for measuring shikimate: an HPLC assay and a spectrophotometric assay (1,3). The two methods were used to measure the effects of glyphosate on shikimate levels in cotton (13), and the reported

difference between the two procedures was small. The HPLC shikimate assay is labor intensive and costly with respect to both reagents and equipment (1,9), whereas the spectrophotometric assay is relatively inexpensive and rapid (3).

The spectrophotometric assay promises to be a simple lab assay that could be used to measure shikimate levels in a large numbers of samples, which could occur in case of a drift incident. However, there is little information on what levels of glyphosate injury can be detected based on shikimate levels or how long after glyphosate exposure shikimate levels remain high enough to be measured. The objective of this research was to determine how shikimate levels in corn and soybean change over time after being treated with different concentrations of glyphosate.

Site Description and Experimental Protocol

Field studies were conducted at Stoneville, MS; Akron, CO; and Ft. Collins, CO in 2003 and 2004.

2003. Conventional and GR corn and soybean were planted in 10-x-15-ft plots in a randomized complete block design with four replications of each treatment at each location. To minimize the potential for drift between plots, all plots were separated from adjoining plots by a minimum of 10 ft. The recommended glyphosate rate was 0.75 lb a.e./acre and the rates used throughout this study were based upon this 1x application rate. Glyphosate (Roundup WeatherMAX formulation) was applied at 0.00, 0.047, 0.188, and 0.375 lb a.e./acre (a 0, 1/16, 1/4, and 1/2 times the recommended rate, respectively) in 20 gal/acre spray volume, to 6- to 7-leaf corn and to 2nd to 3rd trifoliolate soybean.

A cork borer was used to excise leaf discs (12 per leaf) for a total leaf area of approximately 0.22 inch². Corn leaf discs were extracted from the youngest emerging leaf on both sides (six discs from each side) of the midrib at a point equidistant between the tip and base of the leaf. Soybean leaf discs were extracted from the youngest, fully expanded trifoliolate on both sides of the midrib (two discs on each side) from each of the three trifoliolate leaflets. Samples were collected at 1, 4, 7, 14, and 21 days after glyphosate application (DAA). Samples were frozen and stored in 1-oz vials prior to analysis.

2004. Conventional corn and soybean were planted in 10-x-30-ft plots in a randomized complete block design with four replications at each location. All plots were again separated from adjoining plots by a minimum of 10 ft. Glyphosate (Roundup Custom formulation) was applied at 0.023, 0.047, 0.094, 0.188, and 0.375 lb a.e./acre (1/32, 1/16, 1/8, 1/4, and 1/2 times the recommended rate, respectively), with Activator 90 surfactant at 0.25% v/v, at 20 gal/acre, to 8- to 9-leaf corn and 4- to 5-leaf soybean. Three leaf-discs were excised from six different plants resulting in 18 leaf-discs (0.33 inch²) for each sample. Corn leaf discs were sampled in a similar manner as in 2003, except three discs were excised from the youngest leaf coming from the whorl from six different plants. Soybean samples were collected from the newest fully expanded trifoliolate leaf, with one leaf-disc excised from each trifoliolate leaflet of six different leaves. Samples were collected 1, 4, 7, and 14 days after application. Samples were frozen and stored in 1-oz vials prior to analysis.

Laboratory protocol. The following solutions were used in the assay: 0.25 M HCl; 0.25% periodic acid/0.25% meta-periodate; 0.6 M NaOH/0.22 M Na₂SO₃. Shikimate standards were developed by adding known amounts of shikimate to vials containing leaf discs not exposed to glyphosate so that shikimate levels could be reported as µg of shikimate per ml of HCl solution (for example, add 12.5 mg shikimate to 12.5 ml 0.25 M HCl). The 1 mg/ml shikimate solution was then diluted 1 to 10 in 0.25 M HCl, to give a 0.1mg/ml shikimate solution.

One ml of 0.25 M HCl was placed into each vial and the vial was shaken vigorously to ensure all leaf-discs were submerged in acid. Samples were incubated at room temperature for 1.5 h, at which point the discs were a uniform grey-green color. A 100 µL aliquot of the 0.25% periodate/0.25% meta-periodate was as added to each well of a 96-well microtiter plate. A 25 µL aliquot of the 0.25 M HCl extract was pipetted into each of two wells so that two replicate

extract samples were assayed per vial. The plate was incubated at room temperature for 1 h. The reaction was stopped by adding 100 μ L of the 0.6 M NaOH/0.22 M Na₂SO₃ solution into each well. The optical density (OD) from each well was measured at 380 nm using a spectrophotometer. Data were analyzed by an analysis of variance test of fixed effects with differences presented as Least Squares Means.

Shikimate Response to Glyphosate in Corn and Soybean

The soybean and corn injury response to glyphosate was comparable across locations (Figs. 1 and 2). Rates below 0.188 lb of glyphosate generated both injury and measurable shikimate accumulation but were inconsistent with respect to crop and location (*data not shown*). Shikimate accumulation in response to glyphosate treatment was determined by comparing shikimate levels in treated samples to the endogenous shikimate levels in an untreated control. Excluding the 0.188 lb rate on soybean and corn at Akron, the shikimate assay was able to detect the 0.375 and 0.188 lb rates of glyphosate across locations. Glyphosate injury varied slightly across locations. In both years, injury levels at Akron were slightly lower than at the other two locations because of larger crops at time of application (2003) and multiple irrigation/rainfall events following application (2004) allowing the plants to recover from the injury symptoms. The diminished injury response at Akron also translated into lower shikimate accumulation. Shikimate in GR crops did not accumulate beyond levels present in the untreated control plants, regardless of rate or time after application.

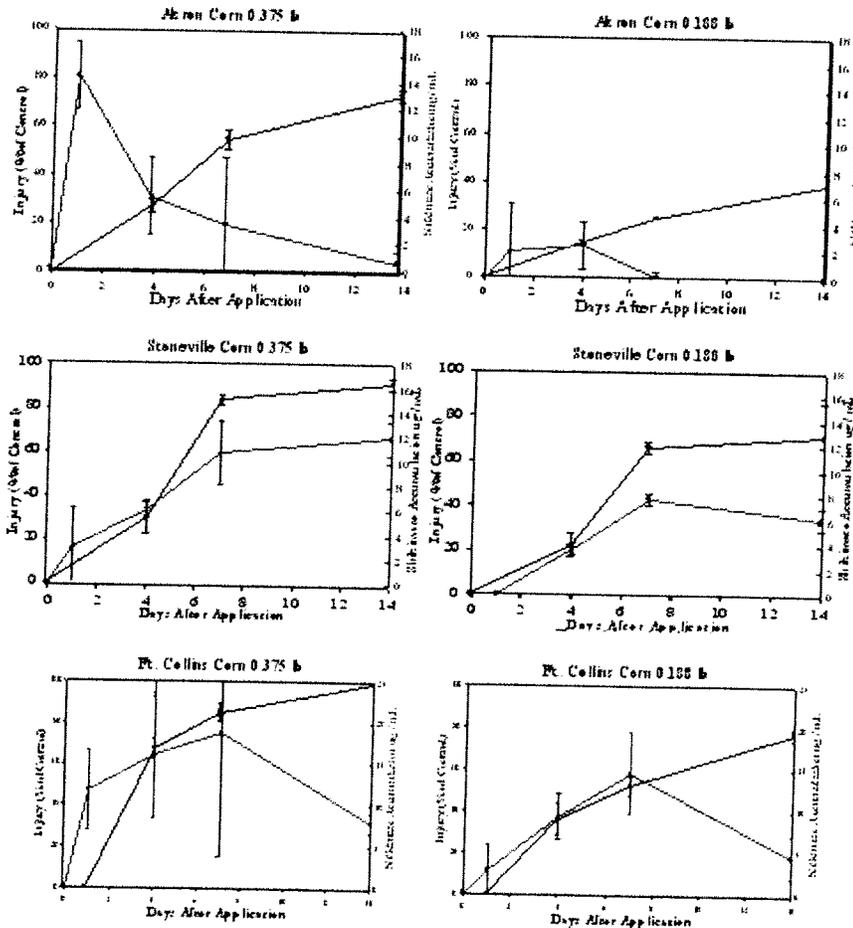


Fig. 1. Shikimate accumulation with respect to glyphosate injury in corn. Injury ratings and shikimate accumulation were measured at 1, 4, 7, and 14 days after application.

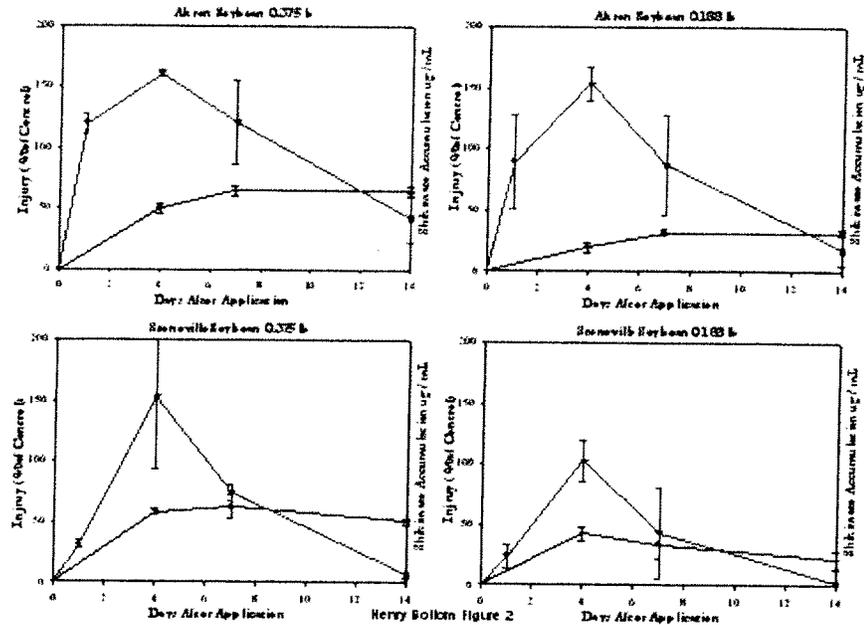


Fig. 2. Shikimate accumulation with respect to glyphosate injury in soybean. Injury ratings and shikimate accumulation were measured at 1, 4, 7, and 14 days after application.

Conclusions

There are two key factors from these experimental data that should be considered when sampling plants for shikimate analysis to assess glyphosate injury: first, in these trials, shikimate accumulation typically peaked between 4 and 7 DAA and then declined (Figs. 1 and 2); second, visual injury symptoms in crops developed more slowly than shikimate accumulation. Glyphosate injury was typically visible between 7 to 10 days, several days after the peak concentrations of shikimate were present in the upper leaves of the plants that were sampled. This means that in order for a producer to use this assay to detect drift, leaf tissue samples need to be gathered at the first sign of crop injury to detect shikimate and to confirm glyphosate exposure. The producer will also have to gather samples from untreated plants to use as a control. These samples should come from the same crop in a nearby location to which glyphosate has not been applied or from a GR crop. With respect to sampling, it is also important to note the potential limitations of this assay for drift detection. Although the assay was able to detect elevated shikimate levels following glyphosate application as low as the 0.023 lb rate in soybean at 4 DAA, only the 0.375 and 0.188 lb rates resulted in consistently detectable increases across years and locations. This means that a crop manager would need to sample from the edge of a field that would have received the highest exposure to glyphosate. In summary, the benefits of this assay include its speed, relative low cost, and its ability to be performed with a basic spectrophotometer, which is a fairly common and relatively inexpensive piece of laboratory equipment. A limitation of this assay is its inability to consistently detect shikimate accumulation at rates below 0.188 lb glyphosate which are possible in a drift situation. However, it is potentially a fast and inexpensive way for a crop manager to confirm that injury along a field border was caused by glyphosate. If implementing this test to detect glyphosate injury, crop managers must remember the following key points: (i) Shikimate accumulation in response to glyphosate exposure is transitory in nature (Figs. 1 and 2) and samples should be collected as soon as glyphosate injury is suspected; (ii) in addition to collecting injured plants, non-injured plants should also be collected for comparison; and (iii) plant samples should be stored in airtight plastic bags in a freezer until analysis can be conducted.

In addition to drift, glyphosate resistance is a growing problem particularly if glyphosate is applied multiple times within a short time frame (7,12,14). With further development of sampling techniques and understanding of the shikimate

response in glyphosate-susceptible plants, this assay may provide a useful technique to detect weeds resistant or tolerant to glyphosate.

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