

ASSAY COMPARISON FOR MEASURING SHIKIMATE ACCUMULATION IN GLYPHOSATE-TREATED PLANT SPECIES. Keith Kretzmer, Mason Hughes, Laylonda Maines and Doug Sammons, Monsanto Company, St Louis, MO 63146

Glyphosate inhibits 5-enolpyruvyl-shikimate-3-phosphate (EPSP) synthase in the shikimic acid pathway of susceptible plant species, resulting in the accumulation of shikimate. A limited survey was undertaken in order to measure shikimate levels in the leaves of both weed and crop plant species, either treated or untreated with glyphosate. Identification and quantification of shikimate was done by HPLC-MS and by HPLC-uv. Extracts from glyphosate treated leaves were also assayed spectrophotometrically and quantification of shikimate was compared between these assays.

The results showed that, as expected, shikimate accumulated in leaves of plants treated with glyphosate. The level of accumulation varied, depending on the plant species, from 230 ug/gFW in cotton, to 7200 ug/gFW in both soybean and alfalfa. In many species, quinic acid also accumulated in treated leaves, and in certain species, to levels greater than shikimate. Further, in many species, there were significant levels of either shikimate or quinic acid, or both shikimate and quinic acid, in untreated leaves.

Finally, it was shown that velvetleaf accumulated shikimate and dehydroshikimate at approximately equal levels after glyphosate treatment (808 and 883 ug/gFW, respectively). Dehydroshikimate was not detected in any other species tested.

The shikimate values measured spectrophotometrically agreed reasonably well with those measured by HPLC for all plant sample extracts tested. It is shown that the spectrophotometric method also detects quinic acid, although the periodate oxidation of quinic acid is much slower than that of shikimate. It is proposed, then, that it may be possible to measure both shikimate and quinate in a single extract well simply by measuring absorbance at 2 timepoints: shikimate would be measured immediately after the addition of sodium hydroxide/sodium sulfite and quinate would be measured after an incubation time of 60 to 90 min.

Since plants treated with glyphosate may accumulate both shikimate and quinic acid, and in some cases more quinic acid than shikimate, a more sensitive analytical method such as HPLC-MS and HPLC-uv may be necessary for measuring a plant's response to glyphosate. In addition, there are likely several plant species which have significant levels of naturally occurring shikimate and quinic acid, so shikimate measurement alone may not necessarily be directly correlated to glyphosate treatment.