Reprinted with permission from: Leafy Spurge Symposium. Riverton, WY. July 9-10, 1986. pp. 18-20.

Published by: Great Plains Agricultural Council: Leafy Spurge Symposium.

The effect of glyphosate on bound auxins in leafy spurge

DAVID D. BIESBOER and JAMES H. WESTWOOD

Department of Botany, 220 Biological Sciences Center, University of Minnesota, St. Paul, MN 55108

Introduction

Last year, we reported on the effects of the herbicide glyphosate on the amount of free indole-3-acetic acid (IAA) in various organs of leafy spurge. This year, we are reporting on the effect of glyphosate on auxin conjugates in leafy spurge. The primary intent of this research was to either support or refute speculation that the phytotoxicity of glyphosate is due to glyphosate-induced reductions in levels of IAA in the plant.

Conjugation of IAA

The majority of conjugated IAA in plants has been found to be either ester or amide conjugates (Cohen 1982). Ester conjugates appear to be the major form of bound IAA in seeds of cereal grains while amide conjugates predominate in legumes (Bandurski and Schulze, 1977). Ester conjugates include indole-3-acetyl myoinositol, IAA-myoinositol-arabinose, and IAA-glucose (Cohen and Bandurski, 1982). Approximately one-half of the ester conjugates are in the form of high molecules weight cellulosic glucans that contain one IAA residue for every 7 to 50 glucose units (Cohen and Bandurski, 1982). Endogenous ester conjugates have rarely been isolated from plant tissues except for seeds but the presence of IAA-myoinsitol has been confirmed in corn shoots and reported to represent about 19% of the ester conjugates in them (Chisnell, 1984).

Amide conjugates have been identified less frequently in plants. IAA-acetylaspartate is the only known endogenous amide conjugate and has been reported to comprise one-half of the total IAA in soybean (Cohen, 1982). Other amides conjugates have been detected in plants after the application of exogenous IAA to them. These include IAA-alanine, IAA-glutamic acid, IAA-glycine, IAA-lysine, and IAA-valine (Sembdner *et al*., 1980).

The conjugates are thought to serve several functions in plants such as the storage of IAA, protection of IAA against oxidation, transport of IAA, and hormonal homeostasis (Cohen and Bandurski, 1982).

Methods and materials

Plant material was greenhouse-grown leafy spurge, started from seed, and 4 months old. Plants were divided into 3 groups: glyphosate treated, decapitated plants, and controls. Details of the experimental treatment are the same as that given in last years report (Westwood and Biesboer, 1985). For conjugate analysis, the aqueous fractions from ether partitioning (Westwood and Biesboer, 1986) were combined and frozen until used. Ester conjugates were those compounds that could be partitioned in acidic ether after hydrolysis with IN NaOH at 23º C for 1 hour. Amide conjugates were compounds that could be partitioned in acidic ether after hydrolysis with 7N NaOH at 100º C for 3 hours. The aqueous phases were divided into two equal parts and one-half was subjected to mild hydrolysis while the other half underwent strong hydrolysis. Solid NaOH was used to adjust normalities, and ddH_2O was added as necessary to maintain a constant volume in the 7N hydrolysis. At the end of the hydrolysis period, both samples were acidified to pH3 using H_3PO_4 and partitioned 4 times against 10 ml aliquots of Et_2O. The ether phases were saved after each extraction and pooled before being brought to a pH greater than 8 using NH_4OH. The ether was evaporated under a stream of nitrogen gas leaving a small aqueous sample containing the freed IAA. Losses of conjugated IAA during the initial extraction and partitioning the efficiency of the strong hydrolysis were measured using known amounts of ^{14}C-aspartate in appropriate controls. Isolation and detection of IAA was done by reversed phase HPLC, ion-pair chomatography, and sequential fluorescent (excitation = 254nm; emission=340nm) and electrochemical (potential=+0.8 V) detection as previously reported (Westwood and Biesboer, 1985).

Results and discussion

Glyphosate treatment did not appear to affect the concentrations of conjugates in the roots, shoots, or leaves of leafy spurge. Two way analysis of variance did not reveal significant differences between control, decapitated, or glyphosate treated plants at the 90% confidence level. Amounts of conjugates did not increase or decrease during the time course of the experiment (Westwood and Biesboer, 1986). It is our opinion that the decrease in free IAA in leafy spurge caused by glyphosate treatment noted in last years report, could not be accounted for by increased conjugation of IAA.

In summary, it is worthwhile to compare the relative concentrations of different forms of IAA in the various organs of leafy spurge (control plants; Table 1). The crown region of the plant had the highest concentration of free IAA followed by shoots and roots containing 41.0, 32.5, and 25.6% of the free IAA respectively. The crowns may be expected to have the highest level of free IAA because of the large number of adventitious buds that will develop into adventitious shoots. Secondly, the differences in levels of conjugated IAA were less pronounced except that the concentrations of IAA-ester conjugates were higher in the shoot and roots than the crown region of the plant. It has been demonstrated that IAA conjugates can act as a storage form of 1AA (Hangarter and Good, 1981) and raises the possibility that the aerial portions of the plant may store 1AA and hydrolyze it when buds in the stem begin to grow. Concentrations of endogenous free and conjugated IAA in vegetative tissues exist in the ratio of about one-third free IAA and two-

thirds conjugated IAA. Finally, of the conjugates, esters and amides occur in approximately equal amounts.

Table 1. Relative distribution of the different forms of IAA in untreated 4-month-old leafy spurge plants.

Form of IAA	Percent of total IAA in individual plant parts			
	Shoot	Crown	Root	Average
Free	32.5	41.0	25.6	33.7
Ester conjugates	43.4	26.6	47.2	38.1
Amide conjugates	24.1	32.4	27.2	28.2

References

Bandurski, R. S. and A. Schulze. 1977. Concentration of indole-3-acetic acid and its derivatives in plants. Plant Physiol. 60: 211-213.

Chisnell, J. R. 1984. Myo-inositol esters of indole-3-acetic acid are endogenous, components of *Zea mays* L. shoot tissue. Plant Physiol. 74: 278-283.

Cohen, J. D. 1982. Identification and quantitative analysis of indole-3-acetyl-Laspartate from seeds of *Glycine max.* L. Plant Physiol. 70: 749-753.

Cohen J. D. and R. S. Bandursid. 1982. Chemistry and physiology of the bound auxins. Ann. Rev. Plant Physiol. 33: 403-430.

Hangarter, R.P. and N.E. Good. 1981. Evidence that IAA conjugates are slow-release sources of free IAA in plant tissues. Plant Physiol. 68: 1424-1427.

Sembdner, G., D. Gross, H. W. Liebisch, and G. Schneider. 1980. Biosynthesis and metabolism of plant hormones. *In* Encyclopedia of Plant Physiology, ed. by J. MacMillan, Springer-Verlag, Berlin. Vol. 9-. 281-390.

Westwood, J. H. and D. Biesboer. 1985. The influence of glyphosate on endogenous levels of free IAA and phenolic compounds in leafy spurge. Leafy Spurge Symposium Proceedings, pp. 5-13.

Westwood, J.H. and D. Biesboer. 1986. The influence of glyphosate on endogenous levels of free and conjugated IAA and phenolic compounds in leafy spurge (*Euphorbia esula* L.). M.S. Thesis. University of Minnesota. pp 1-57.