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Membrane perception of 2,4-D as the initial interaction leading to phytotoxicity in leafy spurge

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The shoots of leafy spurge are controlled satisfactorily by auxinic herbicides such as 2,4-D, but crown and root bud regrowth is not readily controlled. Leaf absorption and translocation of 2,4-D to the roots occur but the herbicide is lost very rapidly from the root tissues. To improve crown and root bud control, 2,4-D absorption by leafy spurge shoots and translocation to roots must be increased concomitantly in leafy spurge. The mechanism of influx and efflux of 2,4-D from leafy spurge cells is unknown at present. The involvement of either passive and/or active mechanisms in the influx and efflux of 2,4-D may determine if the loss of translocated 2,4-D from root tissues can be regulated or modulated. The objectives of our research were to increase foliar absorption of 2,4-D and translocation to roots of leafy spurge and to investigate the mechanism of loss or efflux of the herbicide from leafy spurge cells.

Three ¹⁴C-labeled and unlabeled, variably hindered esters of 2,4-D (methyl, *sec*-butyl, and *t*-butyl) were synthesized for our studies. Unlabeled 2,4-D and its three esters were sprayed separately on 6-week-old leafy spurge plants at 0.04kg/ha acid equivalent per compound. The 2,4-D acid was most phytotoxic with the methyl and *sec*-butyl esters slightly less phytotoxic and *t*-butyl ester showing very little herbicidal activity.

Excised leaf sections (0.5 cm) were used to determine uptake, metabolism and accumulation of 2,4-D in leafy spurge. Maximum uptake of all compounds were complete within 4 hours with loss from tissues occurring with all compounds except *t*-butyl ester over the next 20-hour period. Only 6% of applied 2,4-D was absorbed within 4 hours whereas about 25% of the three esters were absorbed over the same period. Total uptake of the compounds in leaf tissues after 4 hours expressed as nmol/g f.w. (in parenthesis) was: 2,4-D (64), three esters (ave. of 162). Rapid hydrolysis of the methyl and *sec*-butyl esters to 2,4-D occurred in leaf tissues with only limited hydrolysis of the *t*-butyl ester. At 4 hours after treatment, the accumulation of 2,4-D in nmol/g f.w. of leaf tissue treated with 2,4-D and its esters were: (a) 2,4-D (48); (b) methyl ester (157); (c) *sec*-butyl ester (133); and (d) *t*-butyl ester (29). Very little metabolism of 2,4-D other than the hydrolysis of its esters occurred over a 24-hour period when severe herbicidal response to the herbicide was observed in whole plants.

The results described above indicated that 2,4-D acid is the herbicidal molecule. In whole plants, the least absorbed form (2,4-D) causing very little accumulation of the active molecule in leaf tissues is most phytotoxic to leafy spurge. The effects on the plasma membrane potential in root cortex cells from the zone of elongation indicated that leafy spurge cells appear to recognize or perceive the acid form as the biologically active molecule and not the esters of 2,4-D. An interaction between the plasmalemma and the 2,4-D acid during influx of the herbicide may be the physiologically significant process in the herbicidal action of 2,4-D.