Reprinted with permission from: Communication Through Research: Proceedings of Great Plains Agricultural Council Leafy Spurge Task Force Symposium. July 26-28, 1993. pp. 27-29.

Published by: Great Plains Agricultural Council Leafy Spurge Task Force.

## Organogenesis in leafy spurge

D. G. DAVIS

USDA-ARS Biosciences Research Laboratory, State University Station, Fargo, North Dakota 58105-5674

The process of organogenesis in leafy spurge (*Euphorbia esula* L.) continues to be the major research project in this laboratory, since it is believed that the long range control of leafy spurge will require a combination of biological and chemical methods. The main target sites for chemical control are likely to be the tissues and cells that regenerate into shoot or root buds. The most effective herbicides (picloram, 2,4-D and dicamba) are thought to have physiological effects similar to auxin under certain conditions. The action of 2,4-D, picloram, and the natural auxin, indoleacetic acid (IAA) and its analog indole-butyric acid (IBA), have been determined in the model system that has been in use in this laboratory for several years. This model system consists of aseptic etiolated hypocotyl segments grown on agar media containing the nutrient formula of Gamborg *et al.* (1968), with 2% sucrose as the carbon source.

Both IAA and IBA increase root formation, but 2,4-D does not, unless the tissue is transferred to media free of 2,4-D after 2 to 5 days of treatment (Davis and Olson 1993). Picloram strongly inhibits organ formation, so that mostly callus tissues are formed. In contrast to IAA or 2,4-D, picloram at low concentrations did not increase root formation above the level of the control, even when the hypocotyl segments were transferred to basal medium without growth regulators. The inhibition of root formation by a specific inhibitor of ornithine decarboxylase, an enzyme involved in the biosynthesis of putrescine (the immediate precursor to the polyamines; Slocum and Flores 1991) was reversed by IAA and IBA, but not by 2,4-D. The possible reversal of the action of this inhibitor by picloram was not evaluated, since no stimulation of root formation by picloram at any concentration has been observed.

For the past two years the research emphasis has been on the action of the diamine putrescine, the polyamines spermidine and spermine, and the effects of inhibitors of the biosynthesis of these compounds (Davis and Olson 1992). Canavanine and canaline (analogs of the putrescine precursors, arginine and omithine, respectively) were tested for their effects on the formation of roots and shoots on the hypocotyl segments. Inhibition of both root and shoot formation occurred with canavanine (10  $\mu$ M or greater) and canaline (25  $\mu$ M or greater). The inhibition by canavanine was not reversed by simultaneous treatment with arginine. Comparable experiments on the possible reversal of canaline inhibition by ornithine are underway.

The effects of a chemical (designated fraction F-117), extracted from Sunnhemp (*Crotalaria juncia* L.), that has shown activity against photosynthetic tissues of leafy spurge, causing a bleaching of the tissues (Leather 1993), has also been tested in the leafy spurge hypocotyl system. In darkness, this chemical inhibits root formation in etiolated leafy spurge hypocotyls only slightly at the highest concentration tested (0.5 mM).

Agar-solidified media is used in the model hypocotyl system. Efforts to improve the system are being tested continuously. A system whereby liquid nutrient medium replaces the agar containing medium is presently being developed to avoid the presence of low concentrations of additional nutrients or contaminants contained in the agar. Growth of both roots and shoots is better in the liquid cultures if the cultures are shaken continuously, with the result that the organs become entangled and are difficult to visualize unless they are physically separated.

Special tissue culture dishes are used in these experiments and they must be opened to facilitate organ counts, thereby exposing the tissues to possible contamination. One type of dish has a small opening to the atmosphere, so that some gas exchange with the external air is possible. Ethylene, ethane, ethanol, methyl jasmonate or other volatile components generated by the tissues and that may influence organ formation, can escape to the outside of the vessels. This reduces, but does not eliminate, the possible complication of their involvement in organogenesis. On the other hand, airborne contaminants may move into the tissue culture dish through the opening. Handling of these dishes takes considerably longer times for routine counts of organs than the petri dishes, so the simplicity and ease of counting organs in petri dishes is lost.

Agar is presently used to support the hypocotyls in the petri dishes, to allow oxygen to reach the tissues, and for efficient visualization of the roots and shoots without requiring opening of the dishes (which risks contamination and introducing inequalities of the environmental conditions between experiments). The possibility of replacing agar with Gelrite<sup>®</sup> (Scott Laboratories, Troy, MI) was investigated. Gelrite is transparent and gives a better appearing support than agar, but the possibility of the inadvertent release of micronutrients from the Gelrite may not be greatly different from that of agar. In one experiment, root production was more prolific on Gelrite, but these results were not substantiated in two other experiments. Therefore, the use of Gelrite as an agar replacement did not appear to be advantageous.

One cm lengths of hypocotyl segments for routine experiments were selected early in the development of the model system, based on the results of experiments comparing the regenerative capacities of hypocotyl segments of 2 to 15 mm in length. Hypocotyl segments 5 mm or less in length produced few or no organs in the absence of exogenous growth regulators. This limits the response that can be observed to only those chemicals that stimulate organ formation or growth, since inhibition of either organ will not be observed simply because almost no organs are formed in control tissues of that size. However, hypocotyl segments as short as 2 mm can be used to study the effects of exogenous chemicals on auxin-induced root formation, and/or cytokinin-induced shoot formation, nearly independent of the formation of the other organ.

## Literature cited

- Davis, D. G., and P. A. Olson. 1992. Uptake and translocation of polyamines and inhibitors in hypocotyl segments of leafy spurge. Proceedings, Leafy Spurge Symposium of the Great Plains Agricultural Council, Publication No. 144, pp. 2-3 to 2-9.
- Davis, D. G. and P. A. Olson. 1993. Organogenesis in leafy spurge (*Euphorbia esula* L.). In Vitro Cellular & Developmental Biology-Plant. Accepted for inclusion in July 1993 issue.
- Gamborg, O. L., R. A. Miller, and K. Ojima. 1968. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. 50:148-151.
- Leather, G. R. and L. E. Forrence. 1993. Phytotoxicity of an allelochemical from Sunn Hemp (*Crotalaria juncea* L.) Weed Science Society of America, Denver, CO. Abstract 168, p. 56.
- Slocum, R. D. and H. E. Flores, eds. 1991. Biochemistry and Physiology of Polyamines in Plants. CRC Press, Boca Raton, Florida, USA. 264 pp.