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Progress report: Translatable mRNA's in crown and root buds of leafy spurge

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Abstract:

Leafy spurge is a problematical weed because of the manner in which it perenniates. The crown and roots of the plant produce a large number of dormant shoot buds throughout the growing season. These buds are under correlative inhibition and when the top of the plant is killed by herbicides or mowing, these buds are released from inhibition and grow rapidly. Eradication of the plant is difficult because translocation of herbicides to both the root and crown buds is often incomplete allowing the plant to re-grow soon after treatment. Factors that control dormancy and development of these buds is certainly the most under-researched area of leafy spurge biology and may hold the key to controlling leafy spurge. It is our broad objective to begin studying the molecular biology of root and crown bud development in leafy spurge.

What evidence suggests that control of root bud and crown bud formation may be crucial for the eradication of leafy spurge? In an enlightening article, Watson (1985) presented a model to show how a leafy spurge population might decline under various methods of control. Seed production might appear to be a weak link in the reproduction of leafy spurge. However, the simulations of Watson show that even if 100% of leafy spurge seed production is controlled for 15 years, the population will not decline. This simulation also holds true for 80% and 90% control of above-ground shoots with 10 to 20% control of seed and root/crown bud production. Satisfactory control can only result, according to this model, by controlling 95-99% of the top growth of a population and 40 to 80% of root and crown bud formation. Top growth is easily eliminated with herbicide treatment, however, we know little about the control of, or growth and development of the root and crown buds.

As stated above, it is our broad objective to begin studying the molecular biology of root and crown bud development in leafy spurge. Our specific objectives are to:

- 1) Determine whether changes in gene expression, as determined by alterations in the levels of translatable mRNA's, accompany release of root and crown buds from dormancy. If we identify mRNA translational products whose abundances significantly increase or decrease shortly after release of the buds from dormancy, then our next objective is to:
- 2) Isolate several cDNA clones that are complementary to the developmentally regulated mRNA's.

In order to determine whether changes in gene expression occur following release of buds from dormancy, we have begun to isolate RNA from inhibited root and crown buds, from rapidly developing root and crown buds released from correlative inhibition by removal of shoots from growing plants, and from crown buds that have been actively growing for a significant period of time. The use of growing buds as one of the controls should allow us to screen out those RNA's that function primarily in the growth process, e.g. components of the translational and transcriptional machinery, "garden variety" cell wall components, components of the photosynthetic apparatus, etc. RNA preparations will be translated *in vitro* in the presence of a labeled amino acid, and the translation products displayed on a two-dimensional polyacrylamide gel.

Single dimension gels of total protein fractions isolated from the various buds at different stages of development have indicated that certain proteins are specific to the different buds. However, isolations of mRNA from the various buds have been problematical. Modified PAS/TNS phenol extractions of buds (Gantt and Key, 1983) do not seem to be able to remove enough contaminating proteins to be able to effectively translate isolated mRNA in our wheat germ translation system. Methods using guanidium salts are better (MacDonald *et al.*, 1987) but still have not yielded RNA of sufficient quality to allow for effective translations. Work is in progress to improve our methods for the isolation of mRNA from buds of leafy spurge.

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