Reprinted with permission from: Leafy Spurge Symposium. Riverton, WY. July 9-10, 1986. pp. 21-23.

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

Cotyldedon chlorophyll assay for measuring injury to leafy spurge

WILLARD L. KOUKKARI and DAVID D. BIESBOER

University of Minnesota, St. Paul, MN

Introduction

Recently, we initiated a project to examine the biology and physiology of leafy spurge, with a major focus on competitive species and allelochemicals. The chemicals when isolated from from the organism, will need to be assayed for allelopathic properties. Several assays are currently being examined. Some of the assays involve species other than leafy spurge, e.g., water cress. However, there are definite advantages for developing an assay that directly incorporates the species being studied. A biological assay which appears to be versatile in measuring the effects of various agents, including those of a chemical nature, is the cotyledon chlorophyll stress bioassay (Koukkari *et al*. 1984). This bioassay, which can be traced to earlier herbicide studies (Koukkari and Johnson 1979) has been used with several species to study the effects of chemical, mechanical, biological and thermal agents, as well as temporal changes in plant sensitivity during the course of a single day (Koukkari *et al*. 1984). Adapting this bioassay, perhaps with some modifications, to examine the effects of chemical stress to leafy spurge may provide a more direct method for the testing of allelochemicals.

Materials and methods

Seeds of leafy spurge, *Euphorbia esula* L., were either planted directly in soil (Experiment A) or first treated with 0.2% $KN0_3$ and then sown in soil contained in 200 cc plastic cups. Plants were maintained in controlled environment chambers under conditions similar to those described elsewhere (Koukkari 1980, Koukkari *et al*. 1984).

When the plants were ca. 27 days old (Experiment A), or 28, 33 and 38 days old (Experiment B), they were subjected to mechanical injury. This was accomplished by removing all but the top two leaves and the cotyledons from each plant.

Eleven day old plants (Experiment C) were inoculated with bacteria[1] by puncturing the center of each leaf with a dissecting needle containing *Psuedomonas syringae* pv. *tagetis*.

[1] Courtesy B. W. Kennedy, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Chlorophyll from the cotyledons was extracted with 95% ethanol, absorbance read at 654 nm, total chlorophyll calculated (Wintermans and DeMots 1965), and the values expressed as micrograms per milligram dry weight.

Results and discussion

Before testing the effects of chemical agents, it was necessary to confirm the basic premise that in leafy spurge, as in other species, the levels of chlorophyll in cotyledons of injured plants should be higher than those of uninjured plants. Leafy spurge was not an exception to what had been observed in other species. The levels of chlorophyll in the cotyledons of injured plants were more than twice the levels found in the uninjured plants (Table 1A).

The level of cotyledon chlorophyll may remain relatively high for at least 20 days after mechanical injury (Table 1B). In contrast, the uninjured plants have practically no detectable chlorophyll in the cotyledons.

Strain caused by bacterial stress may be quantified also by the cotyledon chlorophyll assay. The levels of cotyledon chlorophyll were approximately twice as high in the diseased plants (Table 1C).

Table 1. Total chlorophyll levels of leafy spurge, *Euphorbia esula*, L., on the 16th (A) 10th, 15th, 20th (B), and 10th day (C) following mechanical injury (A,B) or bacterial inoculation (C). All plants maintained on a 24 hour regimen of 16 hours of light followed by 8 hours of darkness at ca. 23 to 24° C. Each value represents the mean ug total chlorophyll per mg dry weight ± the standard error.

A. Mechanical (n = 4* and 8)**

Injury	Cotyledon Chlorophyll
None*	5.9 ± 1.7
Leaves Excised**	14.1 ± 2.1

B. Mechanical (n = 7)

Days After	Injury	
Injury	None	Mechanical
10	8.2 ± 2.9	14.8 ± 2.3
15	2.1 ± 1.1	10.2 ± 1.3
20	0.0 ± 0.0	10.2 ± 2.5

C. Bacterial (n = 2)

Inoculation	Cotyledon Chlorophyll
None	15.8 ± 7.7
Water Only	20.6 ± 0.6
Pseudomonas syringae pv. *tagetis*	35.3 ± 0.5

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