MONTANA STATE UNIVERSITY

Starch utilization, root bud correlative inhibition, and endogenous indole-3-acetic acid levels in leafy spurge (Euphorbia esula L.)
by Scott Jay Nissen

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Crop and Soil Science
Montana State University
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Abstract:
Leafy spurge (Euphorbia esula L.) is a rapidly spreading perennial rangeland weed which continues to persist and spread despite increased efforts at biological and chemical control. The persistence of leafy spurge can be traced directly to the plant's root carbohydrate reserves and its effective means of vegetative reproduction. Research was initiated to examine aspects of these two important survival mechanisms .

Utilization of leaf, stem, root and latex starch was monitored in leafy spurge plants during a 52 day light starvation period. Leaf, stem and root starch levels decreased rapidly in light starved plants; however, detectable levels of starch were present even after 52 days without light. Latex starch levels did not change significantly. Amylase activity was present in the latex; however, latex starch granules were found to be resistant to enzymatic hydrolysis. Results indicated that latex starch granules do not function as a source of utilizable carbohydrate.

Root buds were found to be quiescent during most of the growing season due to correlative inhibition rather than innate dormancy. Innate dormancy occurred when plants were in full flower; however, elongation could be stimulated by chilling intact plants for 8 days at 4 C. Exogenous applications of indole-3-acetic acid and napthalene-acetic acid at concentrations of 10^{-3} M and 10^{-5} M respectively, completely inhibited elongation of excised root buds. Significant increases in root bud elongation were produced by 1 mM 2,3,5-tri-iodobenzoic acid applied to stem and root tissue. These data provide evidence for the involvement of IAA in correlative control of root bud growth.

Primary root and root bud endogenous IAA levels were determined at three phenologic stages: vegetative, full flower and post flower. Free IAA levels were highest in root bud of full flowering plants which were found in previous studies to have a diminished capacity to elongate. Levels of conjugated IAA increased during phenologic development. Primary root free IAA levels did not appear related to lowered root bud elongation during full flower.

STARCH UTILIZATION, ROOT BUD CORRELATIVE INHIBITION,

AND ENDOGENOUS INDOLE-3-ACETIC ACID LEVELS IN

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Scott Jay Nissen

This thesis has been read by each member of the thesis committee and been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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Date

Chairperson, Graduate Committee

Approved for Major Department

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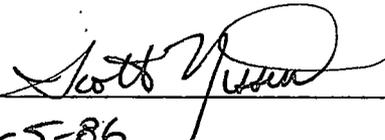Head, Major Department

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TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Leafy spurge (Euphorbia esula L.) is a rapidly spreading perennial rangeland weed which continues to persist and spread despite increased efforts at biological and chemical control. The persistence of leafy spurge can be traced directly to the plant's root carbohydrate reserves and its effective means of vegetative reproduction. Research was initiated to examine aspects of these two important survival mechanisms.

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Root buds were found to be quiescent during most of the growing season due to correlative inhibition rather than innate dormancy. Innate dormancy occurred when plants were in full flower; however, elongation could be stimulated by chilling intact plants for 8 days at 4 C. Exogenous applications of indole-3-acetic acid and napthalene-acetic acid at concentrations of 10^{-3} M and 10^{-5} M respectively, completely inhibited elongation of excised root buds. Significant increases in root bud elongation were produced by 1 mM 2,3,5-tri-iodobenzoic acid applied to stem and root tissue. These data provide evidence for the involvement of IAA in correlative control of root bud growth.

Primary root and root bud endogenous IAA levels were determined at three phenologic stages: vegetative, full flower and post flower. Free IAA levels were highest in root bud of full flowering plants which were found in previous studies to have a diminished capacity to elongate. Levels of conjugated IAA increased during phenologic development. Primary root free IAA levels did not appear related to lowered root bud elongation during full flower.

CHAPTER 1

INTRODUCTION

Leafy spurge (<u>Euphorbia</u> <u>esula</u> L.) is a rapidly spreading perennial rangeland weed which infests one million hectares in the Northern United States and Prairie Provinces of Canada, including 250,000 hectares in Montana (Noble et al., 1979). Current chemical control measures are costly and require reapplication every 2 to 3 years. Despite state and regional efforts to increase public awareness and coordinated research efforts this weed continues to infest more Montana rangeland each year.

Leafy spurge has several characteristics which are common to other difficult to control perennial weeds. First, an extensive root system with tremendous stored starch reserves and ability to spread laterally at significant rates (Selleck et al., 1964). Secondly, an effective form of vegetative reproduction by means of underground buds which produce new shoots. The combination of these characteristics make leafy spurge a tenacious competitor capable of reducing rangeland carrying capacity by as much as 75% (Alley et al., 1984).

Starch Storage

Long-term survival of leafy spurge is a result of root-stored starch reserves which can be slowly utilized over extended periods. This characteristic allows leafy spurge to survive for extended periods

when top growth has been removed by mowing, grazing or chemical treatment. Arny (1932) previously investigated seasonal root carbohydrate levels in leafy spurge and found the pattern of starch usage was similar to other perennial plants. Root starch decreased rapidly in the spring and reached lowest levels at flowering, followed by rapid and continued starch accumulation until the growing season ended. Very little research has been published on carbohydrate utilization in leafy spurge since Arny's initial efforts over 50 years ago. Since root reserves of available carbohydrate are critical to the plant's survival, it was felt that several unanswered questions existed in this area. The role of latex starch as a source of available carbohydrate is addressed in Chapter 2.

Vegetative Reproduction

A major factor contributing to the persistence of leafy spurge is its ability to produce new shoots from root buds (Coupland and Alex, 1955; McIntyre, 1972, 1979; Raju et al., 1964). When the plant is left undisturbed, root buds remain quiescent or "dormant." Root buds will produce new shoots if the current season's top growth is removed. In some instances where non-residual herbicides, shallow tillage, or grazing have been used, shoot densities have increased after treatment (Selleck et al., 1964).

The process by which leafy spurge controls the growth of root buds remains in question. Internal competition for nutrients and water have been suggested by McIntyre (1972, 1979), while others suggest that root buds are under correlative inhibition by the main stem(s) (Budd, 1973).

Determining the mechanism by which leafy spurge controls root bud growth has considerable significance for the eventual control of this plant. Chapter 3 describes a series of experiments designed to examine this mechanism in leafy spurge.

Endogenous IAA Levels

Correlative inhibition refers to a process by which one plant part controls the growth and development of another plant organ some distance away (Goodwin et al., 1978). The current theory of correlative inhibition suggests that IAA, working directly or through the production of ethylene, is a major element in the control of one meristemic region over another. The most widely studied system is the control of the shoot apex on axillary bud growth. Recent studies have shown that IAA translocating basipetally from the shoot apex stimulates the production of ethylene at the internode (Blake et al., 1983). High ethylene levels are responsible for the growth inhibition of axillary buds (Blake et al., 1983; Yang, 1980; Zimmerman et al., 1977).

Although indirect evidence suggests that IAA is involved in the control of leafy spurge root bud growth (Budd, 1973), no one has attempted to demonstrate a direct link by determining endogenous IAA levels. IAA is present in plant tissue in nanogram amounts, is susceptible to photoxidation and enzymatic hydrolysis, and is sometimes difficult to separate from substances showing similar chemical and physical properties. For these reasons quantitative determination of endogenous IAA levels can be a difficult task. Chapter 4 involves the

determination of endogenous IAA levels in root and root buds of leafy

spurge by high performance liquid chromatography (HPLC).

CHAPTER 2

STARCH UTILIZATION IN LEAFY SPURGE (Euphorbia esula L.)
DURING LIGHT STARVATION STRESS

Introduction

Euphorbia esula L. is a herbaceous perennial belonging to the
family, Euphorbiaceae. Specialized cells called laticifers are
characteristic of the genus Euphorbia. Laticifers contain a milky
substance known as latex, which is exuded readily from above ground
plant parts when they are broken or damaged. A wide array of secondary
and at least one primary metabolite are found in the milky latex. These
secondary metabolites include: rubber, tetracyclic triterpenoids,
glycerides, waxes, flavonoids, and alkaloids (Nielsen et al., 1977).
Starch, the primary metabolite, is found in plastids of distinct
morphology.

The function of many of these secondary compounds is not clearly
understood. It has been suggested that laticifers serve as storage
systems for toxic metabolic by-products or as protection from insects
(Bonner & Galston, 1947). The role of latex starch as reserve carbo-
hydrate has been suggested (Sperlich, 1939), but has been carefully
examined for only two species, Euphorbia heterophylla and Euphorbia
myrsinites (Biesboer & Mahlberg, 1978). Latex starch did not appear to
serve as a utilizable starch reserve in these two Euphorbia species.
The present study was undertaken to determine if the "weedy nature" of

E. esula is due in part to the ability to utilize latex starch during prolonged stress periods.

Methods and Materials

Plant Material

E. esula L. plants were cloned by root cuttings from a single plant and propagated in cone-tainers or in polyvinyl chloride (PVC) pipe (0.1 m diameter by 1 m long) filled with 50:50 mixture of greenhouse potting soil and sand. Plants were grown under greenhouse conditions, watered daily, and fertilized once each week with commercial liquid fertilizer (Pete's Professional, 20-20-20). Cone-tainer grown plants used in the light starvation study were pruned of all top growth, transferred to a growth chamber, and allowed to regrow for 4 weeks. The growth chamber was set at a constant temperature of 25°C and 16 h photoperiod with incandescent and fluorescent lights providing a photo flux density of 150 $\mu E \cdot m^{-2} \cdot s$. Amylase activity was determined from latex samples taken from 1- to 2-year old E. esula growing in PVC pipe under greenhouse conditions. Each experiment was repeated at least twice with similar results.

Light Starvation Study

Forty plants were selected for the light starvation study, based on visual uniformity of top growth. Plants were then transferred to a growth chamber without lights set at a constant temperature of 25°C. Three plants were removed from the dark growth chamber at 3 to 4 day

intervals over a period of 52 days. Latex samples were taken by making a small incision in the stem of each plant with a disposable eye surgery scalpel and collecting exuded latex into 10 μl capillary tubes. The plants were then divided into three parts: leaves, stem, and primary roots, immediately frozen with solid CO_2, and lyophilized. Latex samples were also frozen immediately with solid CO_2 and stored at -40°C until the end of the light starvation period.

Starch Assay

A modification of the procedure described by Outlaw and Manchester (1979) was used for starch determinations. Starch was solubilized and reducing sugars were destroyed by heating tissue and latex samples to 100°C for 12 h in 200 μl of 100 mM ethanolic 0.2 N KOH. Enzymatic hydrolysis of starch polymers by amyloglucosidase (E.C. 3.2.1.3) proceded for 12 h at 55°C. Free glucose was determined using a coupled enzyme assay. The glucose reagent consisted of 0.5 U/ml glucose-6-P dehydrogenase (E.C. 1.1.1.49), 0.8 U/ml hexokinase (E.C. 2.7.1.1), 3.6 mM $MgCl_2$, 1 mM ATP, 0.1 mM NADP, 3.6 mM DTT in 360 mM Tris-HCl buffer (pH 8.5). All enzymes and cofactors were purchased from Sigma (St. Louis, MO).

Light and Scanning Electron Microscopy

Latex samples taken 0, 21, and 52 d after beginning the light starvation period were examined using both scanning electron (SEM) and light microscopy. Latex starch granule morphology and surface topography were determined using SEM. Size measurements were made on

a larger sample (n=50), using a light microscopy equipped with a stage micrometer. Sample preparation for SEM consisted of fixing latex samples in 4% glutaraldehyde for 1 h, followed by washing with 100 mM K-phosphate buffer (pH 5.9) (Biesboer & Mahlberg, 1978) and dehydrating in ethanol. Starch grains were sedimented between steps with low speed centrifugation. Fixed and dehydrated starch grains were transferred to microscope cover slips and sputter coated with gold. Latex samples viewed with the light microscope were placed directly on glass slides and mixed with a drop of I-KI to stain starch grains. Length and width of starch granules were measured and statistically compared.

Latex Amylase Activity

Latex proteins were isolated, using the method of Biesboer and Mahlberg (1982). Amylase activity was measured by adding 50 μg of isolated latex protein to 3 ml of 100 mM K-phosphate buffer (pH 5.9) containing 1 mM Ca-acetate and soluble corn amylopectin (2 mg/ml). The enzymatic reaction was allowed to progress for 10 min at 25°C and was stopped by placing the reaction vial in a boiling water bath for 3 min. Aliquots of 100 μl were taken from the reaction vial and placed in 0.9 ml 100 mM K-phosphate buffer (pH 6.0) containing 2 units (U) of α-glucosidase (E.C. 3.2.1.20). Maltose units cleaved from the corn starch by amylase activity were reduce to glucose by α-glucosidase. Glucose was then measured using the coupled enzyme assay described in the starch assay.

Enzymatic Hydrolysis of Latex Starch

Fresh latex samples (200 µl) were collected from greenhouse grown plants to determine susceptibility of raw latex starch to enzymatic hydrolysis. Latex samples were washed several times with 80% (v/v) ethanol to remove soluble sugars and various non-carbohydrate latex components. Latex starch granules were sedimented with low speed centrifugation and finally resuspended in 2 ml of 100 mM K-phosphate buffer (pH 5.9), containing 1 mM Ca-acetate (amylases) or 2 ml of 100 mM Na acetate (pH 4.5) (amyloglucosidase). One U of latex amylase, Bacillus subtilis α-amylase or amyloglucosidase from Aspergillus niger, was added to approximately equal amounts of raw latex starch and incubated at the appropriate temperature for 10 min. Enzymatic activity was terminated by heating the samples to 100°C for 3 min. The presence of maltose or glucose was measured by the same procedure described for determining amylase activity.

Results

Leaf, stem, and primary root starch levels decreased by 67, 83, and 80%, respectively during the 52 d light starvation period (Figure 1). Leaf starch levels reached a steady state after 6 d. After 20 d of total darkness complete leaf abscission occurred, even though measurable starch was present (Figure 1). Stem and primary root starch decreased more slowly, but still maintained measurable starch levels.

In contrast to tissue starch levels, no significant change in latex starch was measured. The initial level of starch in the latex
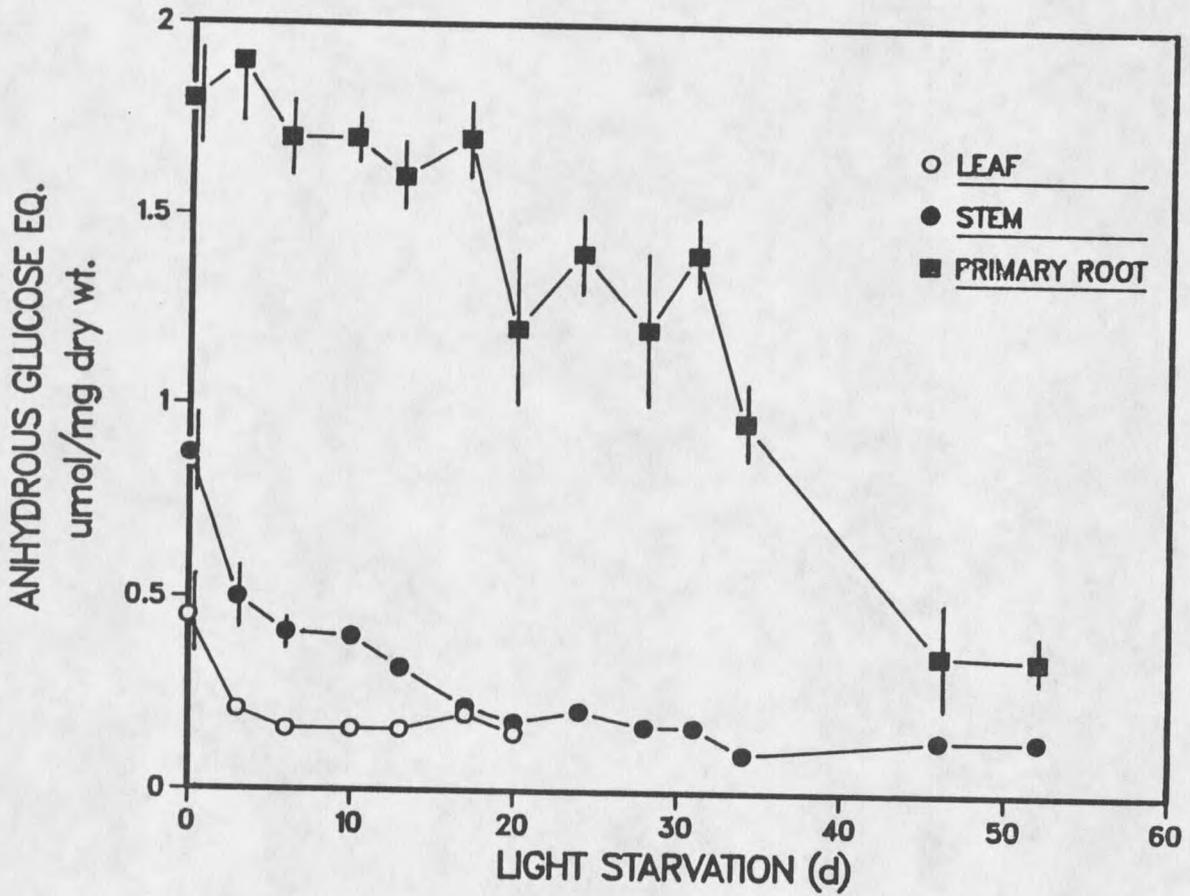
Figure 1. Starch utilization by E. esula during 52 d light starvation
           period.

was 92 ± 12 nmol anhydrous glucose equivalents/10 μl and after 52 d of darkness, starch in the latex measured 95 ± 10 nmol anhydrous glucose equivalents/μl (Figure 2).

SEM indicated that E. esula latex starch granules were basically rod shaped with blunt ends or a slight tendency toward the osteoid morphology described by Mahlberg (1975) for the latex starch grains of E. milli. The general morphology was not visibly different for starch grains from normal and light starved plants (Figure 3). Examination of surface topography showed a smooth surface even after 52 days of light starvation (Figure 3). Latex starch granules measured by light microscopy at 0, 21, and 52 d of light starvation ranged in length and width from 29.9 ± (0.9) to 31.1 ± (1.0) μm and 4.1 ± (0.07) to 4.4 ± (0.09) μm, respectively. No statistically significant changes in latex starch grain length or width were observed during light starvation period.

Amylase activity was present in latex of E. esula at approximately 6.6 ± 0.5 U/mg protein (n=6). One unit (U) of activity is defined as the amount of enzyme required to hydrolyze 1 umol of maltose from soluble starch at pH 5.9 and a temperature of 25°C.

No hydrolysis of raw latex starch was detected by latex amylase, B. subtilis α-amylase, or amyloglucosidase from A. niger.

Discussion

Measurable starch was present in all parts of E. esula after 52 days in total darkness. This indicates that some starch component comprising 20 to 30% of the total storage carbohydrate was not being utilized as a source of metabolic energy during this period. Latex
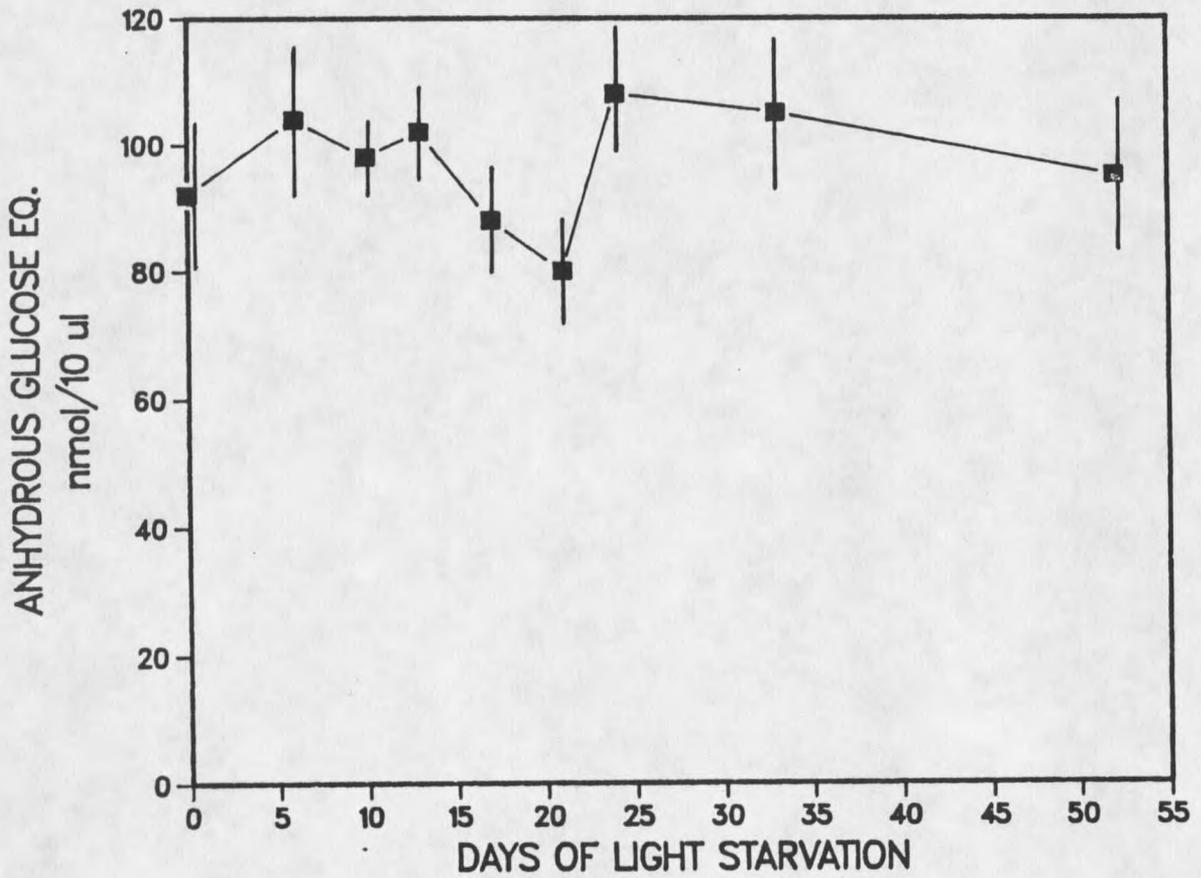
Figure 2. Latex starch levels measured over 52 day light starvation period. Each value represents the mean of triplicate samples.
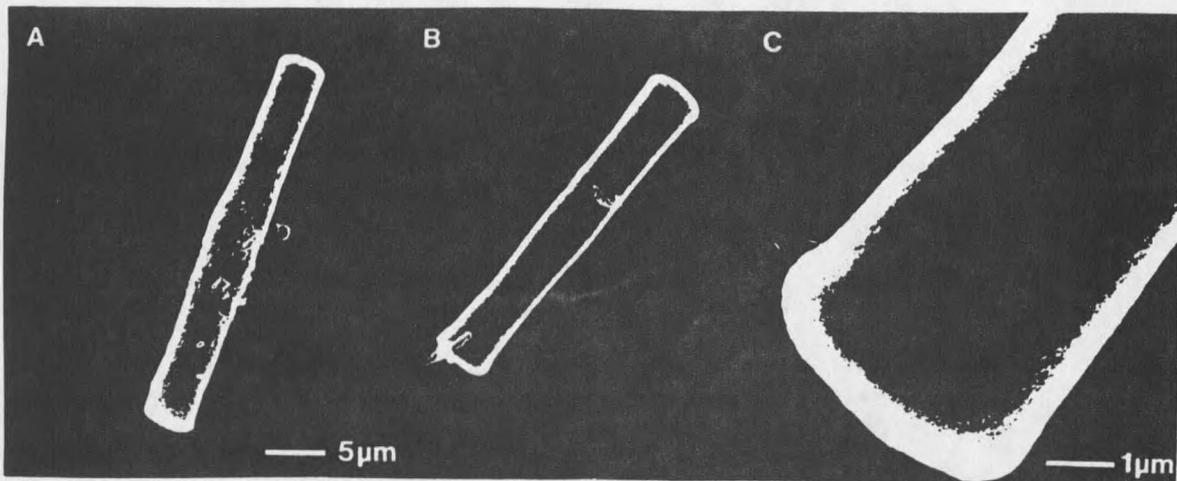
Figure 3. Scanning electron micrographs of latex starch grains from
(a) plant grown under normal photoperiod (16 h), (b) grown
without light for 52 days, and (c) high magnification SEM of
latex starch grain from plants grown without lights for 52
days.

starch levels did not change significantly during the light starvation experiment, indicating that the source of residual starch found in the leaves, stems, and primary roots originates from latex starch granules. These data, in addition to studies of E. heterophylla and E. myrsinites (Biesboer & Mahlberg, 1978), indicate that a high priority is placed on formation and maintenance of starch grains in the latex of these Euphorbia spp., even under severe stress conditions. E. heterophylla and E. myrsinites maintained constant latex starch levels during a 48 day light starvation study and diverted over 40% of their embryonic starch reserves for production of latex starch granules when seeds were germinated and grown in total darkness (Biesboer & Mahlberg, 1978).

SEM has been used previously to study the activity of amylases on starch granules (Gallant et al., 1973). Enzymatic activity can be easily visualized as a pitted and highly irregular starch grain surface. The smooth surface of all E. esula starch grains examined indicates that no enzymatic hydrolysis occurred in normal or light starved plants. Light microscope studies verified the results of SEM studies. The consistency of length and width measurements, even after 52 days without light, provides further evidence of the non-utilizable nature of latex starch grains of E. esula.

Lack of enzymatic hydrolysis, even in the presence of active latex amylases, could indicate that latex starch is resistant to hydrolysis because of an unusual composition. The actual composition of E. esula latex starch granules was not determined; however, based on the blue-black color produced by iodine staining, it would appear that the amylose/amylopectin ratio is not unusual. Amylose content of latex

starch granules from E. marginata, E. tirucalli, and E. heterophylla was determined and found to range from 18 to 20%, which is considered typical for most non-waxy starches (Biesboer & Mahlberg, 1981).

Walker and Hope (1962) previously found that resistance to enzymatic hydrolysis is not necessarily related to starch composition. They studied the action of salivary, B. subtilis, and A. oryzae α-amylases on potato, waxy-maize, and protozoal starch. Potato starch was found to be highly resistant and could not be degraded by any amylases tested. The lack of enzymatic degradation of E. esula latex starch granules by latex amylases, B. subtilis α-amylase, and A. niger amyloglucosidase is not necessarily an indication of unusual starch composition.

Results discussed in this paper do not provide evidence of the function of these non-utilizable starch grains. It has been suggested that they stem the flow of latex from non-articulated laticifers when plant parts are damaged (Biesboer & Mahlberg, 1981). What remains to be determined is why a constitutive system, which encumbers 20 to 40% of measured plant starch, does not have a more readily apparent function.

CHAPTER 3

CORRELATIVE INHIBITION AND DORMANCY
IN ROOT BUDS OF LEAFY SPURGE (Euphorbia esula L.)

Introduction

Leafy spurge was introduced into the United States from Europe in soil used as ship ballast and in contaminated grain brought from Russia by Mennonite immigrants (Dunn, 1985). This perennial, herbaceous weed has since spread to infest 26 states and six Canadian Provinces. The North American infestation was estimated at 1.1 million hectares in 1979 (Noble et al., 1979). Leafy spurge is well suited to semi-arid rangeland in Montana, Wyoming, North and South Dakota. In these states leafy spurge is considered the most serious range weed problem, while in other states it finds suitable habitat along road sides and in waste areas.

Leafy spurge persists despite all efforts at cultural, biological and chemical control. One characteristic which contributes to its persistence is an extensive root system which possesses numerous adventitious buds. New shoots can be produced by both crown and root buds. Crown buds develop at the soil surface each fall. The following spring these buds rapidly elongate to produce that season's shoot growth. Root buds are located along the entire length of the root system, although the majority occur in the upper 30 cm of the soil profile (Coupland & Alex, 1955). These buds do not grow unless the

current season's top growth is removed by tillage, grazing, or chemical treatments. A significant increase in shoots/m^2 can result from root bud growth after shallow tillage or chemical treatments which do not have a soil residue (Selleck et al., 1962). The term "bud dormancy" (Dosland, 1969) has been used to describe the developmental arrest of these root buds. However, the distinction should be made between dormancy within the bud (innate dormancy) (Sanders, 1978) and dormancy enforced by the main shoot (correlative inhibition) (Goodwin et al., 1978).

The physiological processes involved in developmental arrest of root buds are not well understood. Research has shown that root bud growth in certain perennial plants is influenced by phytohormones, chilling temperatures, plant nutrient status and internal water relations (Kefford & Caso, 1972; McIntyre, 1972; McIntyre, 1979; Raju, 1964). Shoot axillary bud inhibition is more clearly defined. Ethylene production induced by indole-3-acetic acid (IAA) appears to be responsible for correlative inhibition of axillary buds (Blake et al., 1983; Zimmerman et al., 1977). Ethylene levels at the leaf axis have been shown to drop dramatically after removal of the shoot apex, and complete release of axillary buds has been demonstrated by direct application of ethylene synthesis inhibitors (Blake et al., 1983; Yang, 1980; Zimmerman et al. 1977).

The ability of leafy spurge to produce new shoots from root buds has long been recognized as a major factor contributing to this plant's persistence (Budd, 1973; Coupland & Alex, 1955; McIntyre, 1972, 1979; Raju et al., 1964). Developing a more complete understanding of the

mechanisms involved in control of root bud growth may provide insights to aid in control of this plant and other perennial weeds. The objectives of this study were to determine: a) if innate dormancy or correlative inhibition occurred in root buds at various leafy spurge growth stages; b) the effects of chilling temperatures on root bud elongation; and c) the influence of exogenously applied auxins (IAA, NAA) on root bud growth.

Materials and Methods

Plant Material

Leafy spurge plants used in these experiments were propagated by root cuttings from a single plant. Root cuttings were grown in cone shaped pots 4 cm in diameter at the top, tapering to a diameter of 1 cm over a 21 cm length (155 cm^3 volume). Pots were filled with a 50:50 (v/v) mixture of greenhouse potting soil (58% sand, 18% silt, 24% clay, and OM 2.8%) and sand. Pots were placed in a growth chamber set at a constant temperature of 25 C with photosynthetic flux density of 150 $\mu E \cdot m^{-2} \cdot s^{-1}$ and 16 hours photoperiod. After 3 months root cuttings had developed into plants with 35 cm of top growth and root systems which filled the entire volume of rooting media. These plants were transplanted to large tubes 0.1 m diameter by 1 m length made of polyvinyl chloride (PVC) and filled with the same mixture of potting soil and sand. Plants were grown under greenhouse conditions at a constant temperature of 25 ± 3 C. Natural light was supplemented during winter months with banks of incandescent and fluorescent lights providing a

photosynthetic flux density of 200 $\mu E \cdot m^{-2} \cdot s^{-1}$ and extending the photo-period to 12 h.

Root Bud Growth Potential

Initial studies were conducted to determine the relationship of bud size and location to shoot growth potential. Greenhouse grown plants were removed from PVC tubes and root systems washed free of soil with tap water. Root buds were selected at random from 1 m long roots starting 10 cm below the crown. Initial bud size, distance of the selected bud from the crown, and diameter of the root at the selected bud were determined. After measurement, the root was cut 1 cm above and 1 cm below the measured bud. Root pieces were then placed horizontally in 32 x 24 x 9 (L x W x H) cm germination boxes filled to a depth of 1 cm with sterile sand. The sand had previously been saturated with distilled water. Root segments were pushed into the sand so that cut ends of roots were in contact with moist sand. A total of 15 to 25 root sections, each containing a single root bud, were placed in each germination box and incubated in the dark at 20 C for 15 day. Distilled water was added every 3 days to maintain sand in saturated condition. Root bud elongation was measured at the end of the 15-day period and compared by regression analysis to initial bud size, distance from the crown, and root diameter. The growth response of 891 root buds collected from 35 different plants was determined.

Plant Growth Stage

The occurrence of correlative inhibition and innate dormancy during the yearly growth cycle of leafy spurge was determined during the 1984 and 1985 growing seasons. On April 2, 1984 and April 5, 1985 one-year-old plants grown in 1 m PVC tubes were moved from the greenhouse to the Post Agronomy Farm, Bozeman, MT. The top growth was removed and the PVC tubes placed in holes in the ground just larger than the diameter of the tubes. The holes were deep enough so that the top of the tubes were even with the soil surface. Root buds were collected on May 15, June 2, June 14, June 25, August 23, 1984 and May 1, May 20, June 5, June 18, August 15, 1985. These sampling dates correspond to five growth stages: 1) spring vegetative growth, 2) bract formation, 3) early flowering, 4) full flower, and 5) late summer regrowth, respectively. Two plants at each growth stage were removed from the PVC tubes and roots washed free of soil with tap water. Root buds were removed from the parent root system by cutting the root 1 cm above and 1 cm below the selected bud. The initial root bud length was determined immediately following removal from the parent root system. Root segments were incubated in sterile sand as previously described. Root bud elongation was measured after 15 days and the initial length subtracted. Treatments consisted of 20 to 25 root buds and results from the two years were combined for statistical analysis. Mean elongation at each growth stage was compared by Newman-Kauls sequential studentized range test.

Chilling Temperatures

Experiments were conducted to determine the effect of chilling temperatures on root bud growth. This experiment was conducted in the same manner as the one previously described. Paired plants were selected on approximately the same sampling dates as before, representing the same five growth stages. Root buds were collected immediately from one plant, while the other plant, still in the PVC tube, was placed in a cold room at 4 C for 8 days. Root buds were collected as before, following the 8 day chilling treatment. Elongation was measured every third day over a 15 day period and the cumulative growth was compared by analysis of variance. Treatments consisted of 15 to 20 root buds. The experiment was conducted for two growing seasons and the data combined.

Exogenous Auxins

The influence of exogenous IAA and NAA on root bud growth was examined utilizing 2-cm root sections containing a single root bud. IAA concentrations of 0, 10^{-3}, 10^{-5}, 10^{-7}, 10^{-9}, and 10^{-11} M and NAA concentrations of 0, 10^{-3}, 10^{-5}, 10^{-7}, 10^{-9}, 10^{-11}, and 10^{-13} M in 25 mM 2(N-morpholino)ethane sulfonic acid (MES) buffer (pH 6.1) were used. Plastic test tubes were filled with 1.5 ml of solution and sealed with three layers of parafilm. A small hole was made in the parafilm and the proximal end of the root section inserted 0.5 cm into the buffered auxin solution. Root sections with attached test tubes were placed in germination boxes filled with sterile sand and incubated

for 9 days under conditions previously described. Ten root buds were used per treatment and the experiment was repeated four times. Root bud growth at each auxin level was expressed as a percent of the control and data from four experiments combined. Treatment means were compared by LSD.

TIBA Experiments

TIBA, an IAA translocation inhibitor (19), was used to further investigate the role of IAA in correlative inhibition of leafy spurge root buds. Whole plants growing in cone-shaped pots under growth chamber conditions were used (see Plant Materials section). Plants were first removed from pots and roots washed free of soil with tap water. The number of root buds longer than 3 mm was determined and expressed as a percent of the total number of root buds present. Plants were then repotted in sterile sand. TIBA was applied continuously to stem tissue which had been abraded with 600 grit silicon, carbide powder. A small plastic vial was placed around the abraded stem tissue, sealed at the bottom and sides with petroleum jelly, and then filled with water or a 1 mM TIBA solution. The top of each vial was sealed around the plant stem with paraffin. Root treatment consisted of watering with 10 ml of 1 mM TIBA daily for five consecutive days. A third treatment combined stem and root application. Plants were maintained under growth chamber conditions previously described. Ten days after the first treatment application plants were removed from pots and root bud length was remeasured. The number of root buds longer than 3 mm was determined and expressed as a percent of total

root buds present. The difference between starting and ending values was expressed as a percent increase. The percent increase in root buds longer than 3 mm for control and TIBA-treated plants was compared by Newman-Kauls sequential studentized range test. The number of root buds per plant ranged from 15 to 40, and 5 plants were used per treatment. The experiment was repeated three times with the same qualitative but somewhat different quantitative results. The results of a typical experiment are shown.

Results and Discussion

Root Bud Growth Potential

There was no significant relationship between root bud growth and initial bud size, distance of the bud from the crown, and root diameter at the root bud. Table 1 shows the average of 35 simple, correlation coefficients determined between measured parameters. No significant p values were found between root bud growth and other variables. The potential of each root bud to produce a new shoot appeared to be unrelated to size or location on the root system. These results are consistent with those of other investigators working with leafy spurge root buds (Raju et al., 1964) and shoot bud production on root systems of rush skeletonweed (Chondrilla juncea L.) (Kefford & Caso, 1972).

Growth Stage

Root buds taken from plants in the first three growth stages elongated rapidly after removal from the root system (Table 2). Root

Table 1. Average correlation coefficient (r) from 35 regressions comparing the growth response of leafy spurge root buds to size and positional differences.

	Growth[a]	Size[b]	Distance[c]
Size[b]	0.31	----	----
Distance[c]	-0.22	-0.31	----
Diameter[d]	0.07	0.11	-0.48

[a] Growth refers to the elongation of root buds excised from leafy spurge roots system and allowed to grow for 15 days.

[b] Size refers to the initial length of the root buds before removal from the intact root system.

[c] Distance is a distance of the bud from the crown.

[d] Diameter is the root diameter at the root bud.

Table 2. Average elongation of leafy spurge root buds on 2 cm root sections 15 days after removal from plants at five growth stages.[a]

Plant growth stage	Root bud growth[b]
	(mm)
Vegetative	44.6 b
Bract formation	48.6 b
Early flowering	51.7 b
Full flowering	11.5 a
Late summer regrowth	65.6 c

[a] Combination of data from 1984 and 1985 growing season.

[b] Means followed by the same letter are not significantly different at P=0.05 according to Newman-Kauls sequential studentized range.

buds appeared to have no innate dormancy during these periods and were quiescent because of correlative inhibition by the main shoot. When buds were taken from plants that were fully flowering root bud elongation was considerably less, indicating the presence of innate dormancy. Once late summer regrowth occurred, the period of innate dormancy appeared to be over. A wide variety of perennial plants show the same seasonal patterns in adventitious shoot production (Cuthbertson, 1972; Dore, 1953; Eliasson, 1971a; Marston & Village, 1972; Raju et al., 1964; Schier, 1973; Sterrett et al., 1968). It has been suggested that the reduction in root bud elongation associated with flowering in some plants is related to increased IAA levels in root tissue, a direct result of IAA translocated from the shoot (Eliasson, 1971b; Schier, 1973; Sterrett et al., 1968).

Early attempts were successful in demonstrating a significant increase in root extractable IAA associated with flowering in European aspen (Populus tremula L.) (Eliasson, 1971a) and Quaking aspen (Populus tremuloides L.) (Schier, 1973). If high IAA levels during flowering imposed an innate dormancy, increased root bud elongation in the fall might be the result of significantly lower root IAA levels. The growth of numerous axillary buds in late summer and fall (Lym & Messersmith, 1983) would indicate that apical dominance is reduced because of lowered IAA translocation from shoot apex. Marston (1972) has demonstrated that in raspberries, plant propagation can be successful during summer dormancy period if the shoot meristem and axillary buds are removed 3 weeks prior to removal of root cutting. This treatment would appear to reduce endogenous IAA levels in the root. McIntyre (1972,

1979) has suggested the main factor contributing to growth inhibition of leafy spurge root buds is competition between root buds and above ground shoot for available resources such as water and nitrogen. However, the period of innate dormancy associated with full flowering indicates that root bud inhibition is controlled by endogenous factors during part of the plant's growth cycle.

Chilling Temperatures

Many plants require a period of chilling temperatures before vegetative buds can resume growth in spring (Nooden & Weber, 1978). Preliminary experiments indicated that root buds which failed to elongate after release of correlative inhibition could be induced to grow if plants were first chilled at 4 C for 8 days. At four of five growth stages examined there was no significant response to chilling treatments. Root bud elongation of chilled and non-chilled plants at the vegetative, bract, early flower, and late summer regrowth stages have been averaged together and results are shown in Figure 4a. Root bud elongation was significantly increased by chilling plants which were in full flower (Figure 4b). Chilling temperatures apparently reduced innate dormancy which occurred during that growth stage. No fall dormancy was present in root buds, since root buds from plants in late summer regrowth stage elongated rapidly without chilling treatments. Dosland (1969) found that leafy spurge crown buds had a definite fall dormancy period which was overcome by an accumulation of days with average temperatures below 5 C. These data indicate that crown and root buds are somewhat different physiologically.
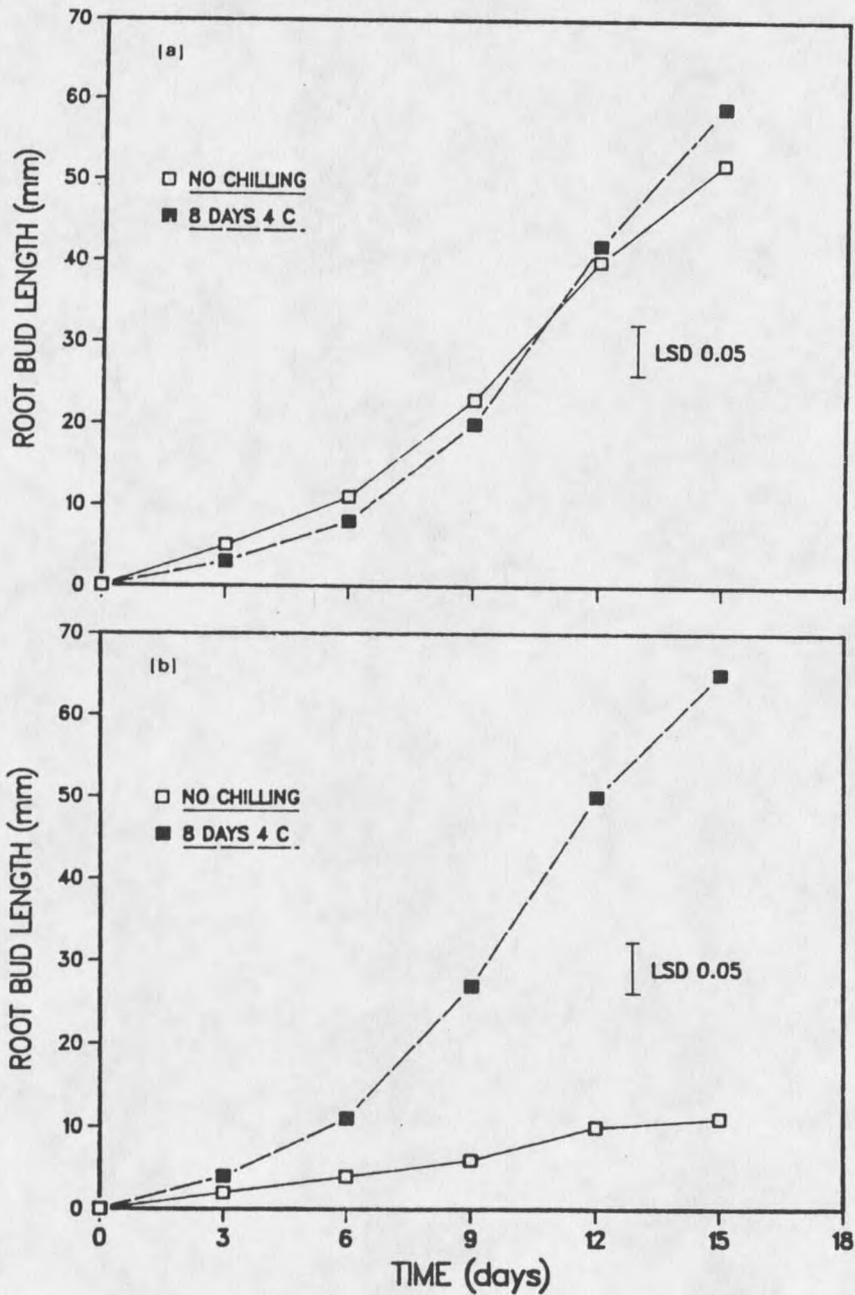
Figure 4. Elongation of leafy spurge root buds excised from plants
with and without exposure to chilling temperatures. Root
buds were removed from plants in various growth stages,
(a) average of response from plants in vegetative, bract
formation, early flower and late summer regrowth stages,
(b) response from plants in full flower. Intact plants were
chilled at 4 C for 8 days and root buds excised on 2 cm long
root sections. Data represents average of 1984 and 1985
field seasons.

Exogenous Auxins

Root bud growth was significantly higher than the control at 10^{-9} M and 10^{-11} M IAA whereas 10^{-3} M IAA completely eliminated root bud elongation (Figure 5). Growth suppression at 10^{-3} M and 10^{-5} M IAA was similar to that reported by Budd (1973). However, Budd did not observe stimulation of leafy spurge root bud growth at 10^{-9} M, and did not test a concentration of 10^{-11} M IAA. In addition, Budd applied IAA in 1.5% agar rather than in aqueous solution which could account for the difference in response at low concentrations.

NAA did not significantly increase root bud growth at 10^{-9} and 10^{-11} (Figure 5) or 10^{-13} M (data not shown). NAA eliminated root bud elongation at 10^{-5} M, a concentration 100 times lower than IAA concentration required to stop root bud elongation. NAA was found to be significantly more inhibitory than IAA to shoot production in roots of rush skeletonweed (Kefford & Caso, 1972) and European aspen (Eliasson, 1961). The physiological basis for this difference is not known, however a synthetic auxin like NAA would seem to be less susceptible to enzymatic hydrolysis. Differences in rates of conjugation do not seem likely since both NAA and IAA applied exogenously are readily conjugated in plant tissue (Bandurski, 1984) (for additional data see Appendix).

TIBA experiments

TIBA applications significantly increased root bud growth in intact plants 10 days after treatment application (Table 3). Stem and