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

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 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
Bozeman, Montana

August, 1986

APPROVAL

of a thesis submitted by

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.

8/5/86
Date

Michael J. Kelley
Chairperson, Graduate Committee

Approved for Major Department

Aug. 11, 1986
Date

Dwane H. Miller
Head, Major Department

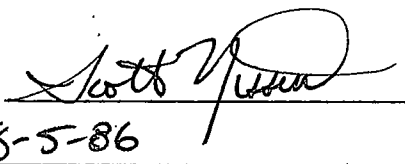
Approved for College of Graduate Studies

August 13, 1986
Date

Henry L. Carsons
Graduate Dean

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a doctoral degree at Montana State University, I agree that the Library shall make it available to borrowers under rules of the Library. I further agree that copying of this thesis is allowable only for scholarly purposes, consistent with "fair use" as prescribed in the U.S. Copyright Law. Requests for extensive copying or production of this thesis should be referred to University Microfilms International, 300 North Zeeb Road, Ann Arbor, Michigan 48106, to whom I have granted "the exclusive right to reproduce and distribute copies of the dissertation in and from microfilm and the right to reproduce and distribute by abstract in any format."

Signature 

Date 8-5-86

ACKNOWLEDGMENTS

I would like to thank the members of my committee, Drs. Pete Fay, Samuel Rogers, Gary Strobel, and Ron Lockerman for their help and advise during the course of my Ph.D. program and especially my advisor, Dr. Mike Foley, for providing just enough encouragement to ensure steady progress. For helpful assistance above and beyond the call of duty, I gratefully acknowledge Drs. Richard Stout and James Bauder for their editorial expertise.

I have been in residence at MSU for six years, long enough to have a lengthy list of fond memories and friendships. I would like to acknowledge a number of individuals for their contributions to the social and intellectual aspects of my graduate education: Dennis Cash, Dave Hadley, Dan Biggerstaff, Mike Bruce, Mike Wille, Dave Withman, Monica Juhnke, Bill Dyer, Bruce Maxwell, Tim Chicoine, Dan Burkhart, Cel Lacey, Lee Coble, Gary Fellows, Larry Hicks, Denny Hall, Tim Gutormson, Dave Buss and Dan Roddy. Excellent technical assistance was provided by Wanda Foley, Renee Humphrey and Ken Worthan. Special thanks to Cheryl Thull for her help and loyalty during my extension career and her continued friendship.

Special thanks also goes to Leslie Harrison for her support and encouragement over the past year, and also for several excellent ideas which were used in my thesis research.

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.

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 napthaleneacetic 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-triiodobenzoic 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 (Euphorbia esula 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 carbohydrate 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

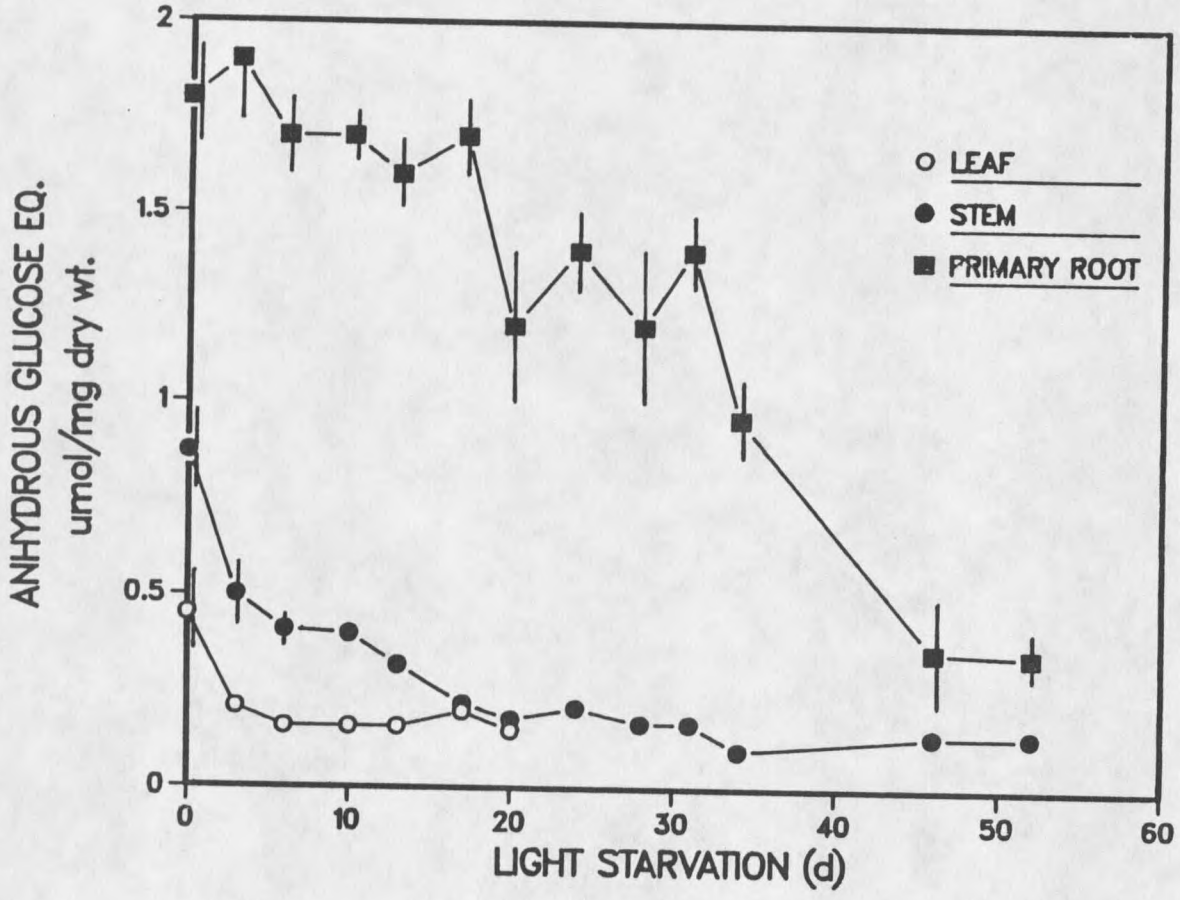


Figure 1. Starch utilization by E. esula during 52 d light starvation period.

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

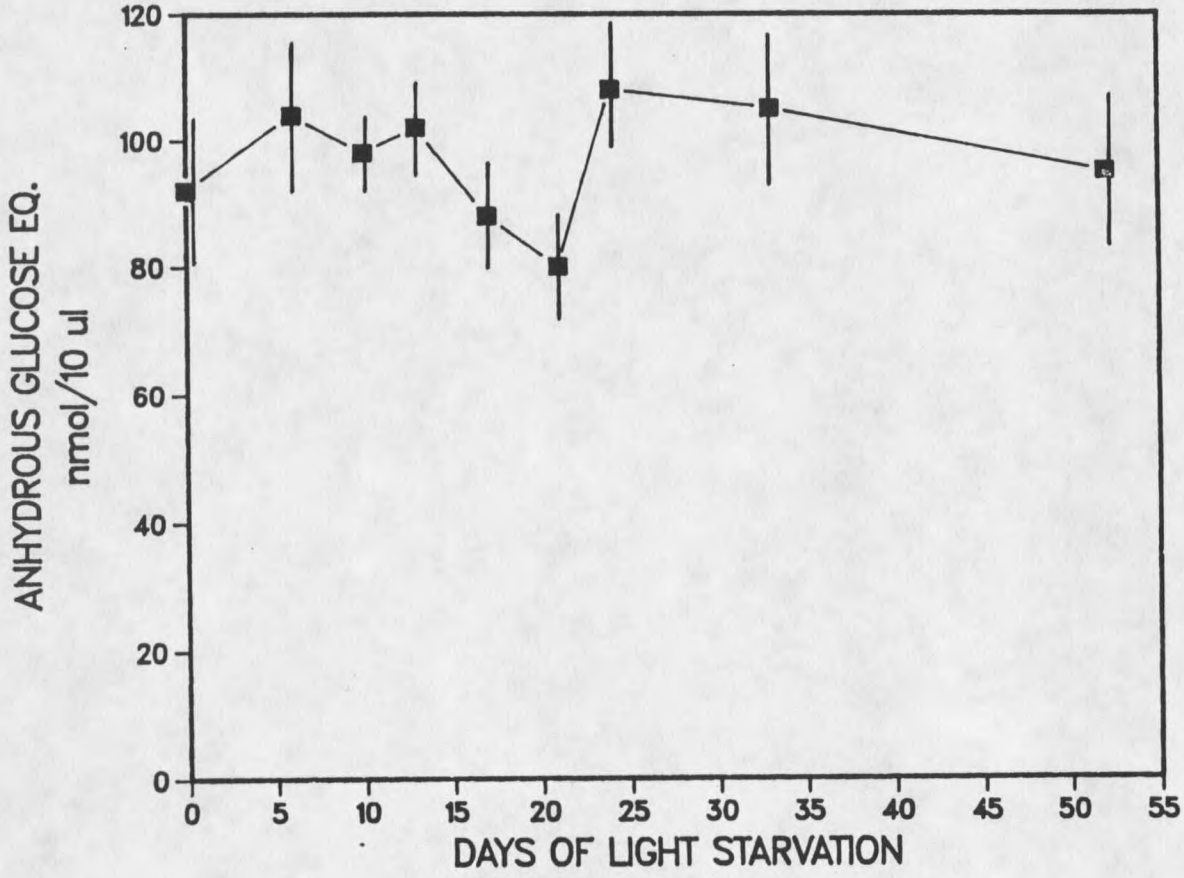


Figure 2. Latex starch levels measured over 52 day light starvation period. Each value represents the mean of triplicate samples.

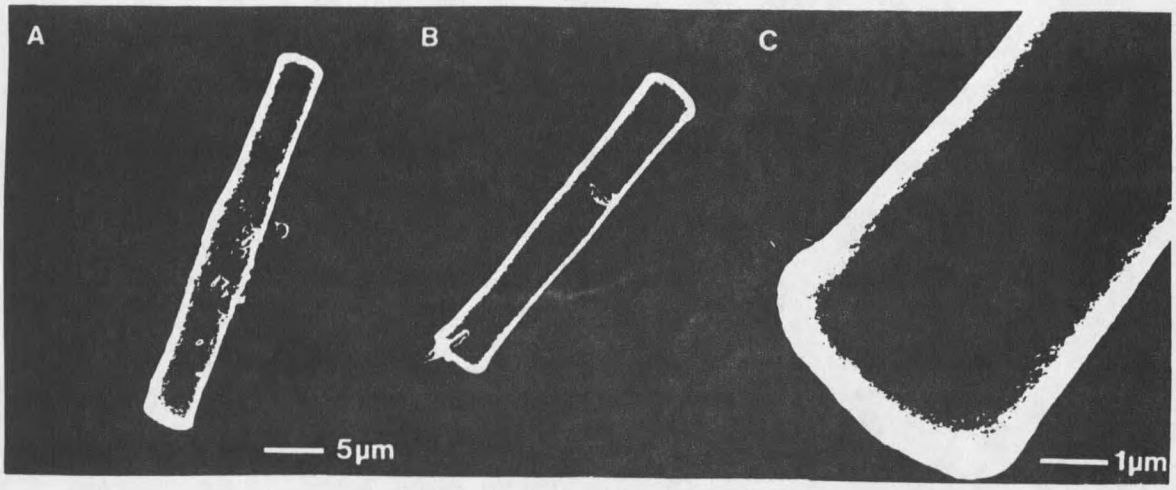


Figure 3. Scanning electron micrographs of latex starch grains from (a) plant grown under normal photoperiod (16 h), (b) grown without light for 52 days, and (c) high magnification SEM of latex starch grain from plants grown without lights for 52 days.

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 photoperiod 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.

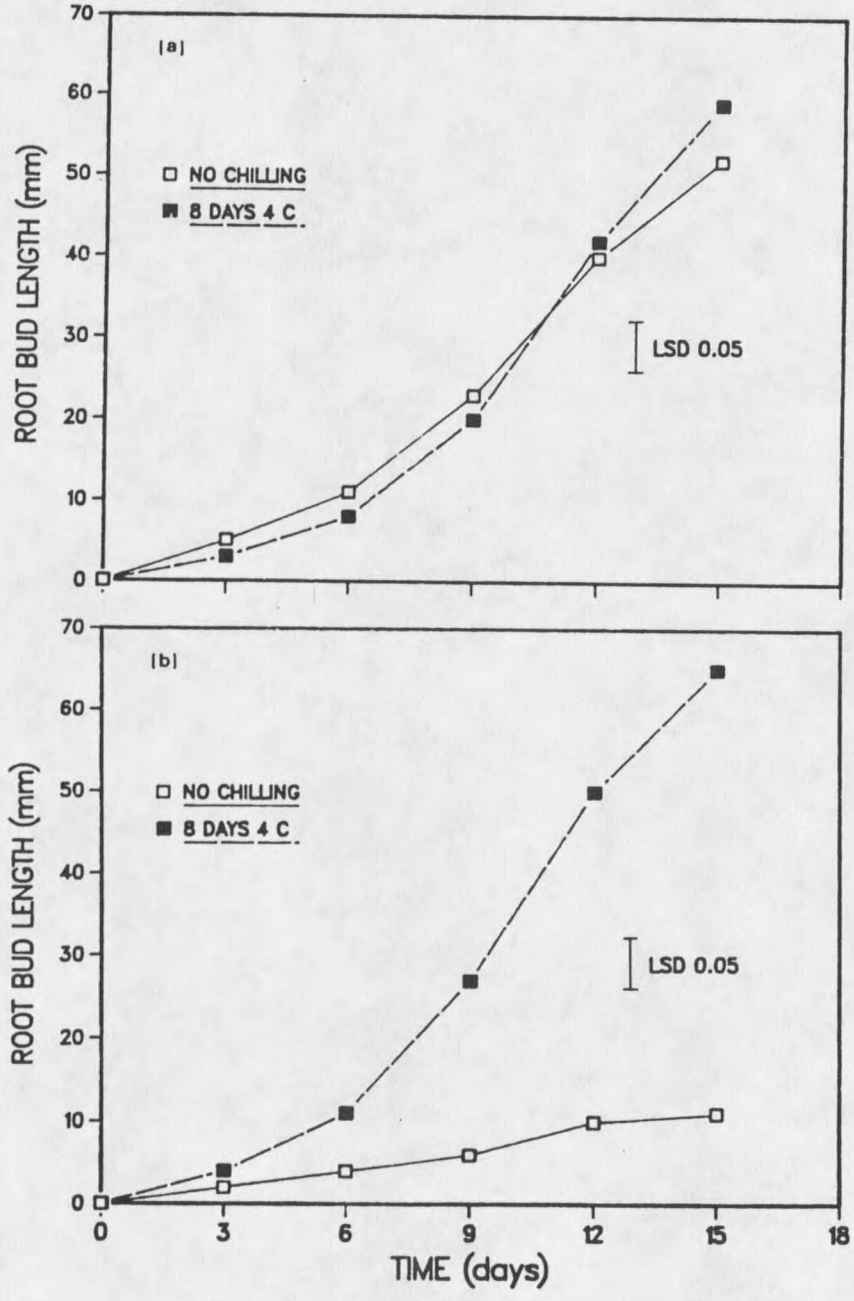


Figure 4. Elongation of leafy spurge root buds excised from plants with and without exposure to chilling temperatures. Root buds were removed from plants in various growth stages, (a) average of response from plants in vegetative, bract formation, early flower and late summer regrowth stages, (b) response 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

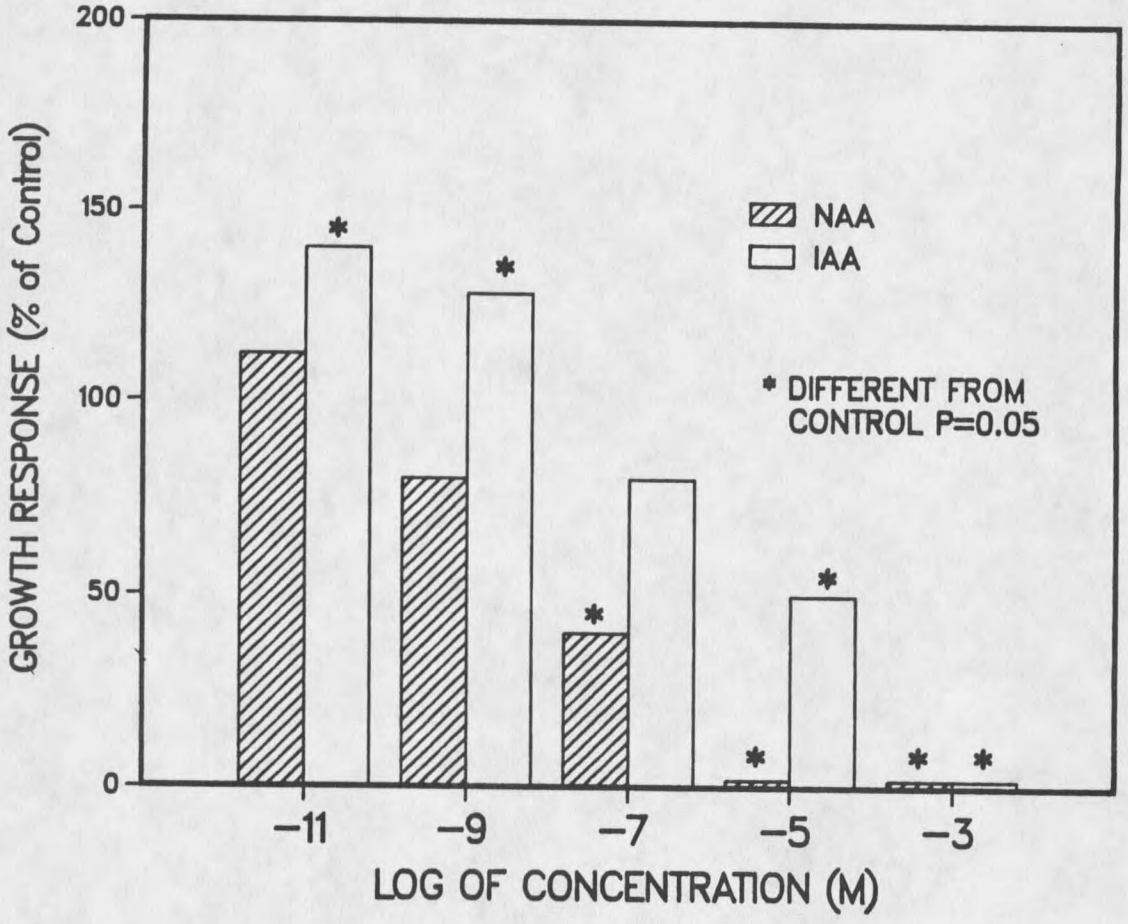


Figure 5. Growth response of leafy spurge root buds on 2 cm long root sections after 9-day exposure to various levels of napthaleneacetic acid and indole-3-acetic acid. Data from four experiments were combined. Bars marked with "*" are significantly different from the control as determined by LSD (P=0.05).

Table 3. Response of leafy spurge root buds to various applications of TIBA (1 mM). Treatments were applied to intact plants.

Location of TIBA application	Increase in root buds > 3 mm[a]
	(%) Control
Control[b]	3.2 a
Stem	14.0 b
Root	32.2 c
Stem + root	26.0 c

[a] Number of root buds longer than 3 mm was determined before and after treatment application. The differences in starting and ending values were expressed as a percent increase and compared by Newman-Kauls sequential studentized range (P=0.05). Means followed by the same letter are not significantly different.

[b] Combination of root and stem treatment controls which were treated in an identical manner but without TIBA.

root applications resulted in a 14 and 32% increase in percent of root buds longer than 3 mm, respectively. The response of root buds following combined stem and root treatment was not significantly different than growth following root treatment alone.

Since early reports on the effects of TIBA on basipetal IAA transport (Niedergang-Kamien & Skoog, 1956), this compound and others have been used to indicate the primary role of IAA in correlative control of axillary buds (Phillips, 1975). Kefford and Caso (1972) demonstrated the ability of TIBA to reverse inhibitory auxin effects on shoot production in rush skeletonweed root sections. Eliasson (1971b) used TIBA applied in lanolin to aspen stems, plus mechanical and steam girdling to demonstrate the role of shoot produced auxin on bud formation. Our results agree with those of previous investigators and provide additional evidence for the role of shoot-produced IAA in root bud correlative inhibition of leafy spurge.

It has been suggested that leafy spurge root buds are quiescent because of internal nutrient and water balances which favor movement of existing resources to the main shoot (McIntyre, 1972, 1979). However, root bud growth in a number of perennial plants has been manipulated by exogenous auxin applications (Budd, 1973; Eliasson, 1961; Emery, 1955; Kefford & Caso, 1972). In all cases auxin concentrations between 10^{-5} and 10^{-3} M have strongly inhibited adventitious shoot growth. Although these auxin concentrations are probably higher than endogenous levels, our results and those of other investigators (Budd, 1973; Eliasson, 1961; Emery, 1955; Kefford & Caso, 1972) indicated inhibition of root

bud growth in plants such as leafy spurge results predominantly from hormonal regulation.

Factors such as plant water and nutrient status, day length, and temperature have been shown to affect endogenous hormone levels (Goodwin et al., 1978); therefore, the correlation between these factors and root bud growth is not unexpected. Measuring endogenous-free and conjugated IAA levels during the growing season or after experimental manipulation could provide important information about hormonal regulation of root bud growth. Findings from such work might indicate areas of research which would eventually lead to long term control of leafy spurge and other perennial weeds.

CHAPTER 4

LEAFY SPURGE (Euphorbia esula L.) ROOT AND ROOT BUD INDOLE-3-ACETIC ACID LEVELS AT THREE PHENOLOGIC STAGES

Introduction

Euphorbia esula L. is one of many plant species which has the capacity to produce adventitious shoots from root buds. Several plants with this capacity are horticulturally important, while others represent persistent and undesirable plants which interfere with the production of food and fiber. It was previously demonstrated that E. esula shows a significant seasonal variation in the capacity of root buds to produce new shoot growth (Nissen & Foley, 1986; Raju et al., 1964). Root buds show the highest regenerative capacity in spring and fall with a significant reduction in regenerative capacity associated with full flower. A number of other plants, which reproduce vegetatively by root buds, show a similar reduction in the roots capacity to produce new shoots during flowering (Cuthbertson, 1972; Dore, 1953; Eliasson, 1971a; Emery, 1955; Marston, 1972; Raju et al., 1964; Rosenthal et al., 1968; Sterrett et al., 1968).

It has been suggested that this reduction in shoot regenerative capacity during flowering is related to increased IAA levels in the root (Eliasson, 1971a; Emery, 1955; Schier, 1973; Sterrett et al., 1968). Early attempts were successful in demonstrating a significant increase in root extractable IAA associated with flowering and active

shoot growth in Populus tremula L. (Eliasson, 1971a) and P. tremuloides L. (Schier, 1973). However, these studies did not utilize highly specific and sensitive techniques now available for IAA analysis and root bud IAA was not measured. No attempt was made to examine conjugated IAA levels which may also fluctuate with phenologic stage.

The present study was undertaken to examine IAA levels of E. esula primary root and adventitious root buds at three phenologic stages; vegetative, full flower, and post flower. Since the capacity of E. esula root buds to produce new shoots appears related to the plants phenologic stage (Nissen & Foley, 1986; Raju et al., 1964), we hypothesized that root and/or root bud IAA content might also show significant changes during these stages.

Materials and Methods

Instrumentation

IAA quantitation was done by HPLC coupled with fluorescence detection. The HPLC was a Kratos 430 gradient former, 400 pump, 970 fluorometer and Shimadzu R-C3A computing integrator. Excitation was at 220 nm and emitted light was monitored using a 350 nm bandpass filter before the photomultipler tube. Results of HPLC fluorescence procedure were verified by analyzing several duplicate samples by GC/MS using $^{13}C_6$-[benzene ring]-indole-3-acetic acid (gift from Dr. Jerry Cohen, USDA, Beltsville, MD) as internal standard. The mass spectrometer was a 70 eV VG 7070E-HF coupled to a Varian 3700 GC. The GC was equipped with a 30 m x 0.025 mm DB-5 capillary column with a helium velocity of

30 cm/sec and temperature programmed to hold at 50 C for 4 min and increased to 220 C at 5 C/min. Splitless mode injections were made. The mass range scanned was 100-250 m/e at 1/2 sec/decade.

Plant Material

Euphorbia esula L. plants were cloned by root cutting from a single plant and propagated as previously described (Nissen & Foley, 1986). After 3 months plantlets were transferred to polyvinyl chloride tubes 0.1 m diameter and 1 m long filled with 50:50 (v/v) mixture of potting soil and sand. Plants used in these experiments were at least a year old with root systems extending the entire length of the tube. Plants were grown under greenhouse conditions, watered daily, and fertilized once each week with commercial liquid fertilizer (Pete's Professional, 20-20-20). Supplemental lighting was provided during winter months.

Experimental Design

Free and conjugated IAA levels were determined for root bud tissue of E. esula at three phenological stages: vegetative, full-flower, and post-flower. Primary root free IAA was also measured. At each growth stage root buds and primary root tissue were collected from three to four plants and analyses were run in triplicate. Experiments were repeated with similar results. Tissue samples were collected at approximately the same time each day. Root bud sample sizes ranged from 200 to 300 mg fresh weight (FW), while primary root samples ranged from 2.7 to 3.0 g.

Quantitation of IAA

The same procedure was used for isolation of IAA for both HPLC and GC/MS. Freshly harvested samples were weighed and frozen immediately in liquid nitrogen. Frozen tissue was ground to a fine powder in a mortar and pestle. A 2 ml volume of 80% methanol/water (v/v) was added along with 11,000 dpm of methylene [^{14}C]-IAA (Amersham, 59 mCi/mmol) which was equivalent to 15 ng of IAA. For samples to be analyzed by GC/MS, 632 ng of [$^{13}C_6$]-IAA was added instead of radioactive IAA. The tissue was homogenized for 1 min before being quantitatively transferred to test tubes with an additional 13 ml of 80% methanol. Tissue samples were extracted overnight in the dark at 4 C.

Samples were evaporated to the aqueous phase under a stream of nitrogen at 40 C. The procedure of Bandurski and Schulze (1977) was used to hydrolyze esterified and peptidyl IAA. The aqueous phase, containing free or free plus conjugated IAA, was transferred to 250 ml erlenmeyer flasks by rinsing the test tubes with 100 ml of 0.075 M K_2HPO_4. The pH was lowered to 2.7 with 8.4 N H_3PO_4. The procedure of Liu and Tillberg (Liu & Tillberg, 1983) was then used to partition IAA using dialysis. The only modification made in this procedure were volume reductions of the three phases. Phase I extraction buffer (0.075 M K_2HPO_4, pH 2.7) was reduced to 100 ml, phase II (diethyl ether plus BHT[3]) was reduced to 130 ml and phase III dialysis tubing buffer (0.1 M K_2HPO_4, pH 9.0) was reduced to 15 ml. Samples were dialyzed in the dark for 12-15 hours at room temperature.

After partitioning, phase III dialysis tubing buffer was transferred to 20 ml test tubes and acidified to pH 2.7 with 8.4 N H_3PO_4. IAA was extracted from the acidified dialysis tubing buffer using 6 ml high capacity C_{18} solid phase extraction (SPE) columns (J.T. Baker, Phillipsburg, NJ). SPE columns were first conditioned with 6 ml of HPLC grade methanol and followed by 6 ml of 0.1 M K_2HPO_4, pH 2.7. An additional 6 ml of buffer was added with the vacuum turned off. Sample reservoirs (15 ml) were attached and acidified dialysis tubing buffer added. Samples were aspirated through columns over a 5 min. period with vacuum. Columns were washed with 2 ml of 20% methanol/water (v/v) pH 2.7 and air dried with vacuum for 3 min. IAA was eluted with two-1 ml aliquots of 80% methanol/water (v/v). The IAA fraction was collected in 2 ml conical centrifuge tubes and evaporated to dryness under a stream of nitrogen at 40°C. Samples were resuspended in 100 ul of HPLC grade methanol for HPLC quantitation or 20 ul for purification by HPLC and quantitation by GC/MS.

HPLC solvents used were acetonitrile (American Burdick and Jackson, Muskegon, MI) and HPLC grade water (EM Science, Cherry Hill, NJ) containing 0.1% (v/v) HPLC grade glacial acetic acid (J.T. Baker) pH 3.3. The column was a 250 mm by 4.6 mm 5 um Bakerbond C_{18} held at 30°C by a recirculation water bath. The gradient former was programmed to hold for 1 min at 95/5 water/acetonitrile (v/v), followed by a 5 min linear gradient to 80/20 water acetonitrile (v/v) and hold for 20 min. At a flow rate of 1.5 ml/min IAA had a retention volume of 22.5 ml and K' of 6.9. Plate number/meter calculated for IAA under conditions described was 45,000. The injection volume was 10 or 20 ul depending

on IAA concentration. IAA quantitation was by comparison of peak area to standard curves of authentic IAA and corrected for losses by isotope dilution (Rittenberg & Foster, 1940). Actual samples were also spiked with known amounts of IAA to check the validity for the standard curve. The limit of detection under our conditions was 0.25 ng/10 ul injection, however this was not the limit of instrument sensitivity.

Samples quantified by GC/MS were purified by HPLC using the same conditions described above. The entire 20 ul sample was injected. IAA peaks were collected with fluorometer excitation beam momentarily interrupted by a shutter to avoid any photodecomposition. One ml C_{18} SPE columns (J.T. Baker) were used to extract IAA from the 80% aqueous HPLC solvent by first diluting the IAA fraction with 5 ml of HPLC grade water acidified to pH 2.7 with 8.4 N H_3PO_4. This step was necessary to reduce the concentration of acetonitrile below 5% (v/v). One ml columns were first conditioned with 2 ml of HPLC grade methanol, followed by 2 ml of 96/3 water/acetonitrile (v/v) pH 2.7. Vacuum was turned off and 1 ml of 96/3 water/acetonitrile (v/v) was added. Sample reservoirs were attached. IAA fractions were added to reservoirs and aspirated through the columns at a flow rate of 2 to 3 ml/min. Since no buffer salts were present columns were not washed, but allowed to air dry under vacuum. IAA was eluted with two 100 ul volumes of HPLC grade methanol collected in 2 ml conical centrifuge tubes. Diethyl ether was added to the centrifuge tubes and IAA was methylated with diazomethane.

Quantitation by GC/MS was done by comparison of the ratio of mass 130/136 (base peak of endogenous IAA and $[^{13}C_6]$-IAA internal standard)

and verification of the analysis by the ratio of 189/195 (molecular ion of IAA-methyl ester and [$^{13}C_6$]-IAA methyl ester) as suggested by Cohen et al. (1986). Comparison of the mass spectra of authentic IAA and [$^{13}C_6$]-IAA indicated that the internal standard was 90% [$^{13}C_6$]-IAA. Calculation of endogenous IAA reflect this difference.

Results

Typical HPLC chromatograms of primary root and root bud free IAA extracts are shown in Figure 6. IAA eluted without interfering peaks and samples spiked with known amounts of authentic IAA increased in area proportional to the amount of IAA added. GC/MS verified the presence of IAA in the HPLC peak and IAA levels determined by GC/MS showed good agreement with those analyzed by HPLC. For example, post flower root tissue free IAA levels were determined to be 13.6 ng/g FW by GS/MS and 15.3 ng/g FW by HPLC (Table 4). Because of the limited availability of root buds, verification by GC/MS was most often performed on root tissue samples. IAA levels determined by GC/MS are indicated in Table 4. Figure 7 shows a typical mass spectra from which free IAA of a post flower root tissue sample was calculated. The ratio of 130/136 is .072 and the ratio of 189/195 is .072 verifying the analysis (Cohen et al., 1986). Based on the amount of internal standard added (632 ng x .9 = 568 ng) and the sample weight (3 g) the endogenous IAA content is calculated to be 13.6 ng/g FW. Although additional clean-up and derivation were required, $^{13}C_6$[benzene ring]-indole-3-acetic acid work very well for purposes of verification as suggested by Cohen et al. (1986).

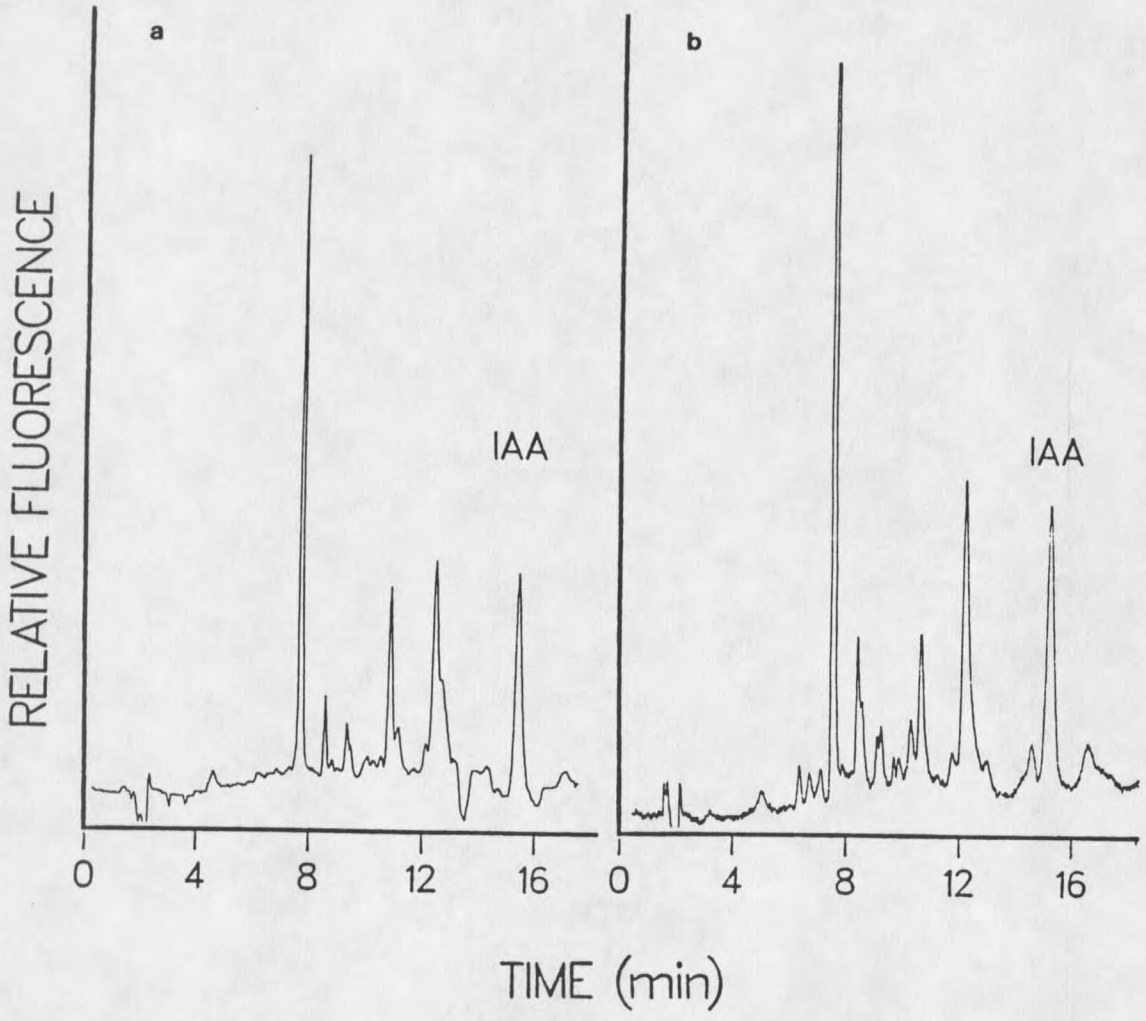


Figure 6. Typical HPLC/fluorescence chromatograms of leafy spurge tissue extracts analyzed for indole-3-acetic acid; (a) root bud tissue, (b) primary root tissue. The IAA peak was identified by cochromatography with authentic-IAA, spiking tissue samples and GS/MS.

Table 4. Levels of free and bound forms of indole-3-acetic acid in primary root and root bud tissue of E. esula L. at 3 different phenological stages.

Tissue and IAA Form	Phenological Stage	[1]IAA
		ng/g f. wt.
Root Bud Free IAA		
	Vegetative	74.8 (7.4)
	Full Flower	103.8 (10.9)
		94.9 (2.5)[2]
	Post Flower	80.5 (10.1)
Root Bud Esterified IAA		
	Vegetative	N.D.[3]
	Full Flower	15.5 (0.4)
	Post Flower	44.5 (13.3)
Root Bud Peptidyl IAA		
	Vegetative	N.D.[3]
	Full Flower	66.5 (8.0)
	Post Flower	136.0 (6.7)
Primary Root Free IAA		
	Vegetative	35.0 (1.3)
	Full Flower	34.3 (0.1)
	Post Flower	15.3 (0.2)
		13.6 (1.7)[2]

[1] Values listed are the means (se) of triplicate samples.

[2] Levels of IAA determined by GC/MS.

[3] N.D. indicates none detected i.e., the levels of IAA determined after treatment with 1N and 7N NaOH were not significantly different than the amount determined for free IAA.

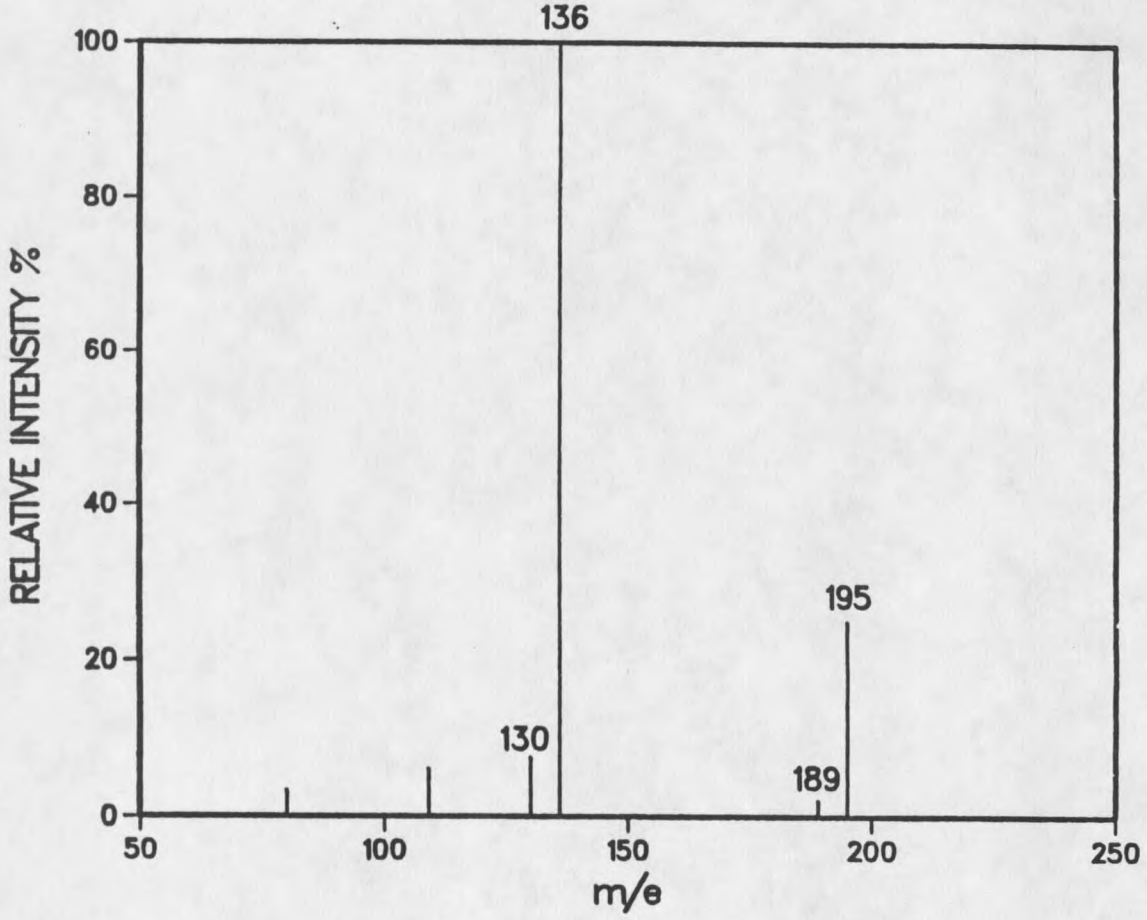


Figure 7. Typical mass spectra of post flowering root tissue sample spiked with $^{13}[C_6]$-IAA as internal standard. The base pike and molecular ion of endogenous IAA-methylester and $^{13}[C_6]$-IAA methylester are shown. The ratio of 130/136 and 189/195 is .072. Sample weight 3g and 632 ng of internal standard was added.

Free IAA levels were significantly higher in root buds of E. esula plants in full flower (Table 4) as compared to vegetative and post flower plants. IAA conjugates were undetectable in root buds of vegetative plants, while esterified and peptidyl IAA was found in root buds of full flower and post flower plants. Highest levels of conjugated IAA were found in post flower root buds. The ratio of esterified to peptidyl IAA resembled that of legumes in which peptidyl conjugates are most abundant (Bandurski & Schulze, 1977). Root tissue free IAA levels were highest in vegetative and full flower plants and decreased by 50% in post flower plants.

Discussion

There is little available information concerning free and conjugated IAA levels in root buds of perennial plants. Hillman et al., (1977) determined the IAA content of Phaseolus vulgare L. axillary buds from intact and decapitated plants. Free IAA levels in these axillary buds were reported to range from 60 to 130 ng/g dry wt. Expressed on a dry weight basis, E. esula root bud free IAA levels would be 3 to 4 times higher (average water content of root buds is 77%). Chrysanthemum morifolium shoot tip IAA levels ranged from 15.6 to 29.0 ng/g FW, which is also considerably lower than E. esula root bud free IAA levels. On the other hand, E. esula primary root free IAA levels were very similar to levels of free IAA reported for primary root tissue of Zea mays (Rivier & Pilet, 1983), vegetative tissue of Zea mays, Pisum sativum (Allen et al., 1981; Bandurski & Schulze, 1977) and Avena sativa (Bandurski & Schulze, 1977) and also similar to free

IAA levels in stem cutting of Chrysanthemum morifolium (Weigel et al., 1984).

The results of the present study suggest that the reduced elongation of E. esula root buds during flowering (Nissen & Foley, 1986; Raju et al., 1964) was associated with significant increases in root bud free IAA levels. The possible existence of this relationship has been theorized (Emery, 1955; Marston & Village, 1972) and this theory supported by evidence from studies utilizing Avena bioassays to quantify IAA levels in roots to Populus tremula (Eliasson, 1971a) and P. tremuloides (Schier, 1973). Our work represents the only attempt to examine the relationship of plant phenology to root bud endogenous IAA levels using highly specific and sensitive techniques like HPLC/fluorescence and GC/MS. Harrison and Kaufman (1983) attempted to demonstrate a relationship between free and conjugated IAA and zeatin levels and tiller bud release in Avena sativa associated with influorescence emergence. No relationship to IAA levels was found. However, since stem segments which included a tiller bud were analyzed, rather than tiller buds directly, it is possible that differences in IAA levels occurred but were masked by the large amount of vegetative tissue included in the sample. E. esula root tissue free IAA levels were found to be considerably lower than that of root buds, and had large amounts of vegetative tissue been included in estimates of root bud IAA, changes in IAA levels could have been masked.

IAA conjugates did not appear related to phenologic variation in E. esula root bud growth. Esterified and peptidyl IAA levels increased dramatically in full flower and post flower root buds. Root buds of

post flower plants had the highest levels of conjugated IAA, however, root buds excised from these plants elongate rapidly (Nissen & Foley, 1986). This seems to indicate that conjugated IAA is not directly related to shoot production by root buds. It has been suggested that IAA conjugates have four possible functions; 1) storage, 2) transport, 3) protection from enzymatic hydrolysis, and 4) regulation of free IAA levels (Cohen & Bandurski, 1982). The function of IAA conjugate in root buds of E. esula was not evident from our experiments.

Primary root free IAA levels appeared to reflect the growing state of the main shoot but did not appear directly related to adventitious shoot production. Highest free IAA levels were associated with very active stem growth of vegetative and full flower plants. Post flower plant stems show considerable axillary bud growth indicating reduced IAA translocation from the shoot apex. The 50% reduction in primary root free IAA levels would appear to reflect this decrease in auxin production and the overall reduction in plant vigor which occurs at this phenologic stage.

Little information is available on the endogenous IAA levels in root buds and their possible relationship to root bud growth. However, research utilizing exogenously applied IAA provides evidence implicating IAA as a factor in control of adventitious shoot production by root buds. Exogenous IAA applications at concentration greater than 10^{-5} M significantly reduced root elongation in P. tremula (Eliasson, 1961), Chondrilla juncea L. (Kefford & Caso, 1972), Chamaenerion angustifolium L. (Emery, 1955) and E. esula (Budd, 1973; Nissen & Foley, 1986). In addition, auxin transport inhibitor studies indicate that IAA moving

basipedally from the shoot is involved (Eliasson, 1971b; Nissen & Foley, 1986). In Rubus idaeus L., root cuttings will not produce adventitious shoots when plants are flowering. Marston (1972) demonstrated that if the shoot apex and axillary buds were removed three weeks before root cuttings were taken adventitious shoot production was significantly increased. This treatment's effectiveness may result from reducing the amount of IAA translocating from shoot to root.

The suggestion was previously made that E. esula root buds might be an excellent source of material for studies of apical dominance using exogenous plant growth regulators (Budd, 1973). The present work further indicates that they would also provide an excellent system for the study of endogenous IAA regulation in meristemic tissue.

LITERATURE CITED

LITERATURE CITED

Allen, J.R., L. Rivier, and P.E. Pilet. 1981. Quantification of indole-3-acetic acid in pea and maize seedlings by gas chromatography-mass spectrometry. Phytochem. 21:525-530.

Alley, H.P., N. Humberg, J.K. Fornstrom, and M. Ferrell. 1984. Leafy spurge repetitive herbicide treatments. Research in Weed Science. Univ. of Wyoming Ag. Exp. Sta. Res. J. 192:90-93.

Arny, A.C. 1932. Variations in organic reserves in underground parts of five perennial weeds from late May to November. Univ. of Minnesota Agric. Exp. Stn. Tech. Bull. 84. 28 pp.

Bandurski, R.S. 1984. Metabolism of indole-3-acetic acid. Pages 183-200 in Crozier and Hillman, eds. The Biosynthesis and Metabolism of Plant Hormones. Cambridge University Press, Cambridge, U.K.

Biesboer, D.D., and P.G. Mahlberg. 1978. Accumulation of non-utilizable starch in laticifers of Euphorbia heterophylla and E. myrsinites. Planta 143:5-10.

Biesboer, D.D., and P.G. Mahlberg. 1981. A comparison of alpha-amylases from the latex of three selected species of Euphorbia (Euphorbiaceae). Amer. J. Bot. 68:498-506.

Blake, T.J., D.M. Reid, and S.B. Rood. 1983. Ethylene, indoleacetic acid and apical dominance in peas: a reappraisal. Physiol. Plant 59:481-487.

Bonner, J., and A.W. Galston. 1947. The physiology and biochemistry of rubber formation in plants. Bot. Rev. 13:543-566.

Budd, R.W. 1973. An excellent source of vegetative buds for use in plant hormone studies of apical dominance. Plant Physiol. 52:82-83.

Cohen, J.D., and R.S. Bandurski. 1982. Chemistry and physiology of the bound auxins. Ann. Rev. Plant Physiol. 33:403-430.

Cohen, J.D., B.D. Baldi, and J.P. Slovin. 1986. $^{13}C_6$-[Benzene ring]-indole-3-acetic acid: A new internal standard for quantitative mass spectral analysis of indole-3-acetic acid. Plant Physiol. 80:14-19.

Coupland, R.T. and J.F. Alex. 1955. The reproductive capacity of vegetative buds on the underground parts of leafy spurge (Euphorbia esula L.). Can. J. Agric. Sci. 35:477-484.

Cuthbertson, E.G. 1972. Chondrilla juncea in Australia IV. Root morphology and regeneration from root fragments. Aust. J. Exp. Agric. Anim. Husb. 12:528-534.

Dore, J. 1953. Seasonal variation in the regeneration of root-cuttings. Nature 172:1189.

Dosland, J.G. 1969. Studies relating to bud dormancy and auxin catabolism in Euphorbia esula L. Ph.D. Thesis, North Dakota State University, Fargo, North Dakota.

Dunn, P.H. 1985. Origins of leafy spurge in North America. Pages 7-13 in A.K. Watson, ed. Leafy Spurge, Weed Sci. Soc. of Amer., Champaign, Illinois.

Eliasson, L. 1961. The influence of growth substances on the formation of shoots from aspen roots. Physiol. Plant. 14:150-156.

Eliasson, L. 1971. Growth regulators in Populus tremula. III. Variation of auxin and inhibitor level in roots in relation to root sucker formation. Physiol. Plant 25:118-121.

Eliasson, L. 1971. Growth regulators in Populus tremula. IV. Apical dominance and suckering in young plants. Physiol. Plant 25:263-267.

Emery, A.E.H. 1955. The formation of buds on the roots of Chamaenerion angustifolium (L.) Scop. Phytomorphology 5:139-145.

Gallant, D., A. Derrien, A. Alimaitre, and A. Guilbot. 1973. In vitro degradation of starch: Studies by transmission and scanning electron microscopy. Starke 24:56-64.

Goodwin, P.B., B.I. Gollnow, and D.S. Letham. 1978. Phytohormones and growth correlations. Pages 215-249 in D.S. Letham, P.B. Goodwin and T.J.V. Higgins, eds. Phytohormones and Related Compounds-A Comprehensive Treatise, Elsevier/North Holland Biomedical Press, New York.

Hangarter, R.P., and N.E. Good. 1981. Evidence that IAA conjugates are slow-release sources of free IAA in plant tissue. Plant Physiol. 68:1424-1427.

Harrison, M.A., and P.B. Kaufman. 1983. Estimates of free and bound indole-3-acetic acid and zeatin levels in relation to regulation of apical dominance and tiller release in oat shoots. J. Plant Growth Regul. 2:215-223.

Hillman, J.R., V.B. Math, and G.C. Medlow. 1977. Apical dominance and the levels of indole acetic acid in Phaseolus lateral buds. Planta 134:191-193.

Hudson, J.P. 1955. Propagation of plants by root cuttings. II. Seasonal fluctuation of capacity to regenerate from roots. J. Hort. Sci. 30:242-251.

Kefford, N.P. and O.H. Caso. 1972. Organ regeneration on excised roots of Chondrilla juncea and its chemical regulation. Aust. J. Biol. Sci. 25:691-706.

Liu, S., and E. Tillberg. 1983. Three-phase extraction and partitioning with the aid of dialysis--A new method for purification of indole-3-acetic and abscisic acids in plant materials. Physiol. Plant 57:441-447.

Lym, R.G., and G.G. Messersmith. 1983. Control of leafy spurge with herbicides. North Dakota Farm Research 40:16-19.

Mahlberg, P.G. 1975. Evolution of the laticifer in Euphorbia as interpreted from starch grain morphology. Amer. J. Bot. 62:577-583.

Marston, M.E., and P.J. Village. 1972. Regeneration of raspberries from root cuttings in response to physical treatment of the shoots. Hort. Res. 12:177-182.

McIntyre, G.I. 1972. Developmental studies on Euphorbia esula. The influence of the nitrogen supply on the correlative inhibition of root bud activity. Can. J. Bot. 50:949-956.

McIntyre, G.I. 1979. Developmental studies on Euphorbia esula. Evidence of competition for water as a factor in the mechanism of root bud inhibition. Can. J. Bot. 57:2572-2581.

Niedergang-Kamien, E., and F. Skoog. 1956. Studies on polarity and auxin transport in plants. I. Modification of polarity and auxin transport by triiodobenzoic acid. Physiol. Plant. 9:60-63.

Nielsen, P.E., H. Nishimura, J.W. Otvos, and M. Calvin. 1977. Plant crops as a source of fuel and hydrocarbon-like materials. Science 198:942-944.

Nissen, S.J., and M.E. Foley. 1986. Dormancy and correlative inhibition in root buds of Euphorbia esula. Weed Sci. (In Press).

Noble, D.L., P.H. Gunn, and L.A. Andres. 1979. The leafy spurge problem. Pages 8-15, in Proc. Leafy Spurge Symposium, Bismark, North Dakota, June 26-27, 1979. North Dakota State Univ. Coop. Ext. Serv. 84 pp.

Nooden, L.D. and J.A. Weber. 1978. Environmental and hormonal control of dormancy in terminal buds of plants. Pages 221-268 in M.E. Clutter, ed. Dormancy and Developmental Arrest. Academic Press Inc., New York.

Outlaw, W.H., Jr., and J. Manchester. 1979. Guard cell starch concentration quantitatively related to stomatal aperture. Plant Physiol. 64:79-82.

Phillips, I.D.J. 1975. Apical dominance. Ann. Rev. Plant Physiol. 26:341-367.

Pillary, I., and I.D. Railton. 1983. Complete release of axillary buds from apical dominance in intact, light grown seedlings of Pisum sativum L. following a single application of cytokinin. Plant Physiol. 71:972-974.

Raju, M.V.S., T.A. Steeves, and R.T. Coupland. 1964. On the regeneration of root fragments of leafy spurge (Euphorbia esula L.). Weed Res. 4:2-11.

Rittenberg, D., and G.L. Foster. 1940. A new procedure for quantitative analysis by isotope dilution, with application to the determination of amino acids and fatty acids. J. Biol. Chem. 133:737-744.

Rivier, L., and E. Pilet. 1983. Simultaneous gas chromatographic-mass spectro-metric determination of abscisic acid and indol-3-yl-acetic acid in the same plant tissue using 2H-labelled internal standards. In A. Frigerio, ed, Recent Developments in Mass Spectrometry in Biochemistry, Medicine, and Environmental Research. Elsevier Scientific Publishing Company, Netherlands.

Rosenthal, R.N., R. Schirman, and W.C. Robocker. 1968. Root development of rush skeletonweed. Weed Sci. 16:213-217.

Sachs, T., and K.V. Thimann. 1967. The role of auxins and cytokinins in the release of buds from dormancy. Amer. J. Bot. 54:136-144.

Sanders, P. 1978. Phytohormones and bud dormancy. Pages 423-441, in D.S. Letham, P.B. Goodwin, and T.J.V. Higgins, eds. Phytohormones and Related Compounds--A Comprehensive Treatise. Elsevier/North Holland Biomedical Press, New York.

Schier, G.A. 1873. Seasonal variation in sucker production from excised roots of Populus tremuloides and the role of endogenous auxin. Can. J. For. Res. 3:459-461.

Selleck, G.W., R.T. Coupland, and C. Frankton. 1962. Leafy spurge in Saskatchewan. Ecol. Monogr. 32:1-29.

Sperlich, A. 1939. Das trophische Parenchym B. Exkretionsgewebe. In K. Linsbauer, ed., "Handbuch der Pflanzenanatomie", Borntraeger, Berlin, p. 38

Sterrett, J.P., W.E. Chappell, and G.M. Shear. 1968. Temperature and annual growth cycle effects on root suckering in black locust. Weed Sci. 16:250-251.

Waldecker, M.A., and D.L. Wyse. 1985. Chemical and physical effects of the accumulation of glyphosate in common milkweed (Asclepias syriacia) root buds. Weed Sci. 33:605-611.

Walker, G.J., and P.M. Hope. 1962. The action of some α-amylases on starch granules. Biochem. J. 86:452-460.

Weigel, U., W. Horn, and B. Hock. 1984. Endogenous auxin levels interminal stem cuttings of Chrysanthemum morifolium during adventitious rooting. Physiol. Plant 61:422-428.

Yang, S.F. 1980. Regulation of ethylene biosynthesis. Hort. Sci. 15:238-243.

Zimmerman, R.H., M. Lieberman, and O.C. Broome. 1977. Inhibitory effect of a rhizobitoxine analog on bud growth after release from dormancy. Plant Physiol. 59:158-160.

APPENDIX

APPENDIX

Ethylene Synthesis Inhibitors

Experimental Procedure

Root piece and whole plant experiments were conducted using the ethylene synthesis inhibitor (aminooxy)acetic acid (AOA) to examine the possible role of IAA induced ethylene in correlative inhibition of leafy spurge root buds. IAA experiments outlined in Chapter 3 (page 21) were repeated with 30 μM AOA incorporated into the buffered IAA solutions. The controls were buffer alone and buffer plus 30 μM AOA. Root sections were incubated in sterile sand as previously described in Chapter 3 (page 18). Elongation was measured after 9 days and compared to the control response.

For whole plant experiments, shoots of cone-tainer plants were sprayed to incipient run-off with 30 μm AOA plus 0.05% (v/v) Tween 20 [oxysorbic (20 POE)(polyoxyethylene sorbitanin)] in water. Root systems were treated by soaking in the same concentration for 3 h.

Results

Incorporation of AOA into the buffered IAA solutions did not change the response of root buds to exogenous IAA (data not presented). Root buds showed no response to whole plant root or shoot treatments. This indicates that IAA could have a direct effect on the root bud

rather than the indirect effect of stimulating ethylene production (Yang, 1980). There is also the possibility that the concentration of AOA used was not sufficient to eliminate ethylene production. However, the concentration used has been shown to completely inhibit the production of ethylene in other plant tissues (Yang, 1980).

Exogenous Cytokinins

Experimental Procedure

Two synthetic cytokinins were tested for their ability to reduce correlative inhibition in the intact plants. Kinetin (K) and 6-benzyl-aminopurine (BAP) were applied to root buds of intact plants at concentrations of 1, 2 and 3 mM in a solution of 50% ethanol (v/v) and 5% polyethylene glycol 4000 (PEG) (v/v) according to published methods (Pillary & Railton, 1983; Sachs & Thimann, 1967). Plants were removed from cone-tainers and roots were washed free of soil with tap water. Five root buds were measured per plant and five plants were used per treatment. Cytokinins were applied in a single 10 µl drop directly to root buds. Control plants were treated with water, 50% ethanol and/or 5% PEG. Plants were then repotted in sterile sand and returned to the growth chamber for 10 days (growth chamber conditions are described in Chapter 3, page 18). Buds were remeasured after 9 days and elongation was compared by analysis of variance.

Results

Direct cytokinin applications have been shown to release axillary buds of pea, apple, crabapple, and apricot from correlative inhibition (Pillary & Railton, 1983; Sachs & Thimann, 1967; Zimmerman et al., 1977). Both natural and synthetic cytokinins have shown this response. However, root buds of leafy spurge showed no response (data not presented) to the same concentrations of K and BAP used in experiments on axillary buds of peas. This lack of response could be due to the concentration of cytokinin used. Budd (1973) demonstrated that high levels of K (10^{-3} M) could inhibit leafy spurge root bud elongation. However, rush skeletonweed root bud formation was stimulated by increasing K levels, with the greatest stimulation at a concentration of 0.3 mM (Kefford and Caso, 1972). Significant increases in root bud elongation have also been produced by 1 mM applications of BAP to root buds of milkweed (Waldecker & Wyse, 1985).

Other Exogenous Chemicals

Experimental Procedure

A variety of other chemicals were applied to intact cone-tainer leafy spurge plants in an attempt to release root buds from correlative inhibition. These chemicals included the following: 1) PCIB [a-(p-chlorophenyoxy)isobutyric acid], a compound which binds to IAA receptors but does not produce a response, 2) ethephon (2-chlorethyl-phosphonic acid), a compound which stimulates ethylene production, 3) sodium azide, a compound which inhibits mitochondrial electron

transport at cytochrome oxidase and has been shown to stimulate germination of dormant wild oat seeds.

PCIB was applied to plants at a concentration of 0.1 mM as a foliar spray and root soak (3 h). Ethephon was applied to roots of leafy spurge at a concentration of 1 mM by soaking alone (3 h). Sodium azide (2 mM) was applied to roots of leafy spurge by soaking for 3 hours and by watering with azide on four consecutive days. Leafy spurge plants used for these experiments were removed from cone-tainers and roots were washed free of soil with tap water. Four to six representative root buds were measured on each of three plants before treatment application. Ten days after treatment application selected root buds were remeasured. The amount of elongation was determined and compared to the control by analysis of variance.

Results

No significant increase in root bud growth resulted from the treatment application. The elongation of control buds as well as treated buds was approximately 0.31 mm over the ten day period.

Day Length and Chilling Temperature Response

Experimental Procedure

One year old leafy spurge plants growing in 0.1 m by 1.0 m PCV pipe were placed under two different light regimes. One light regime was natural light from November 1, 1984 to December 12, 1984; the other was natural light supplemented with fluorescent and incandescent for a

total photoperiod of approximately 16 hours. After growing under these conditions for 1½ months, one plant of each set was exposed intact to 4 C for 8 days. The other plant was removed from its PVC tube, roots washed free of dirt with tap water, and root buds harvested on 2 cm root sections as previously described in Chapter 3. At the end of the eight day chilling treatment the other plant was removed from its PVC tube and root buds harvested as before. Root bud growth was measured after 15 days and compared by analysis of variance.

Results

Plants growing under short day conditions showed significantly less root bud elongation than root buds from plants growing under 16 h photoperiod. Short day plants which did not receive chilling temperatures showed the least amount of elongation, followed by short day plants which did receive chilling temperatures. Long day plants with and without chilling temperatures had the same elongation rate (see Table 5). While statistically significant differences in elongation occurred, these differences may not be biologically significant. Root buds from all four treatments showed a significant amount of elongation after release from correlative inhibition.

Table 5. Average elongation of excised root buds under long and short day conditions.

Treatment	15 Day Elongation[a]
	(mm)
Short day	35.6 ± (3.0) a
Short day + chilling	43.2 ± (2.2) b
Long day	48.7 ± (2.2) c
Long day + chilling	48.5 ± (1.9) c

[a] Numbers followed by the same letter are not significantly different as determined by LSD (P=0.05).

Thidiazuron Application

Experimental Procedure

Thidiazuron is a chemical used as a harvest aid in crops like cotton. Relatively low rates of this compound (0.25 kg/ha) caused complete defoliation of crop plants in 5-6 days. Our interest in trying this compound on leafy spurge was to further examine the influence of ethylene on correlative control of leafy spurge root buds. The mode of action of thidiazuron involves the rapid and continuous evolution of ethylene from treated tissue. Before treatment leafy spurge were removed from their cone-tainers and root buds marked and measured. Three buds were measured per plant and three plants were used per treatment. Treatments consisted of a control (sprayed with 0.5% Tween 20), thidiazuron applied as a 100 uM solution with 0.5% Tween 20, and plants which were defoliated by hand. Hand defoliation

was included to distinguish the effects of defoliation from other effects the chemical might be having on the plant. Plants were sprayed to incipient runoff and then placed in a growth chamber for three weeks (conditions previously described). At the end of three weeks, plants were removed from their cone-tainers and root buds remeasured.

Results

Based on the increase in root bud length of measured root buds thidiazuron had no effect. Hand defoliation did not cause a significant increase in root bud growth. However, visually rating the two treatments it appeared that root bud growth was increased in the thidiazuron treated plant over the control or hand defoliated control (pictures available).

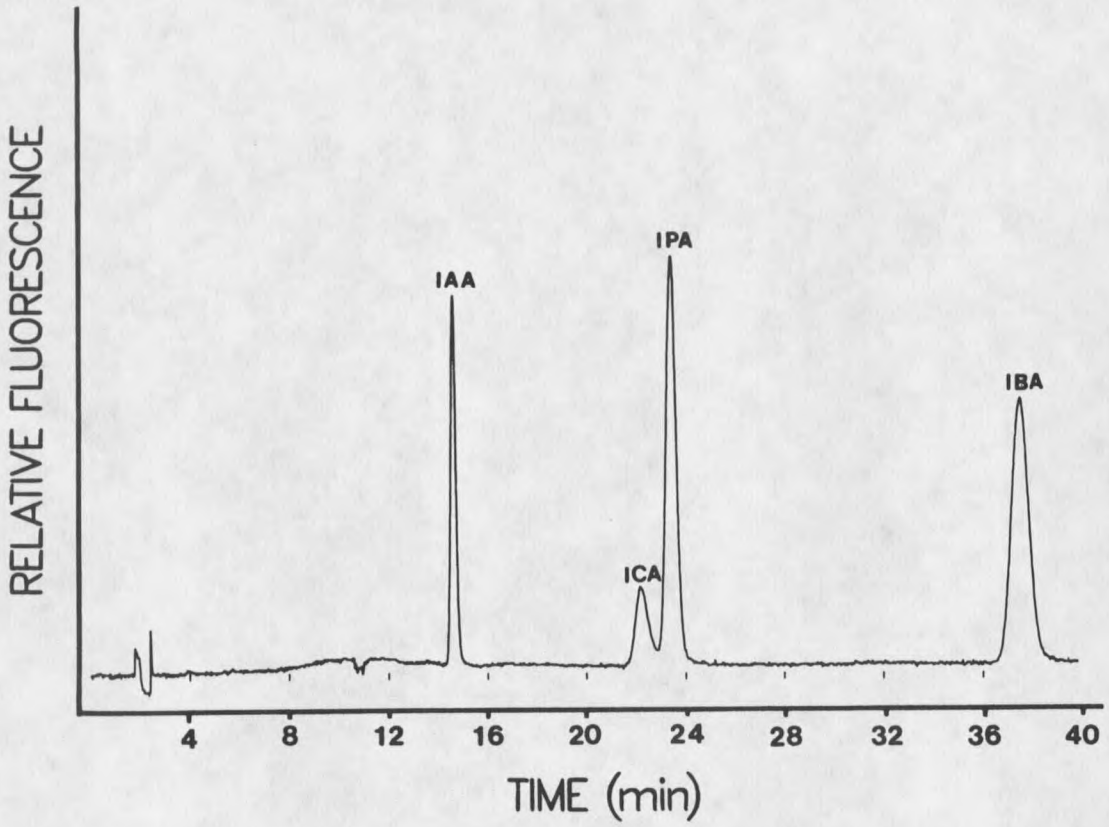


Figure 8. Chromatogram showing the separation of various indole compounds by reverse phase HPLC coupled with fluorescence detection. HPLC conditions are identical to those described in Chapter 4. Chromatogram shows indole-3-acetic acid (IAA); indole-carboxylic acid (ICA); indole-proponic acid (IPA); indole-butyric acid (IBA).

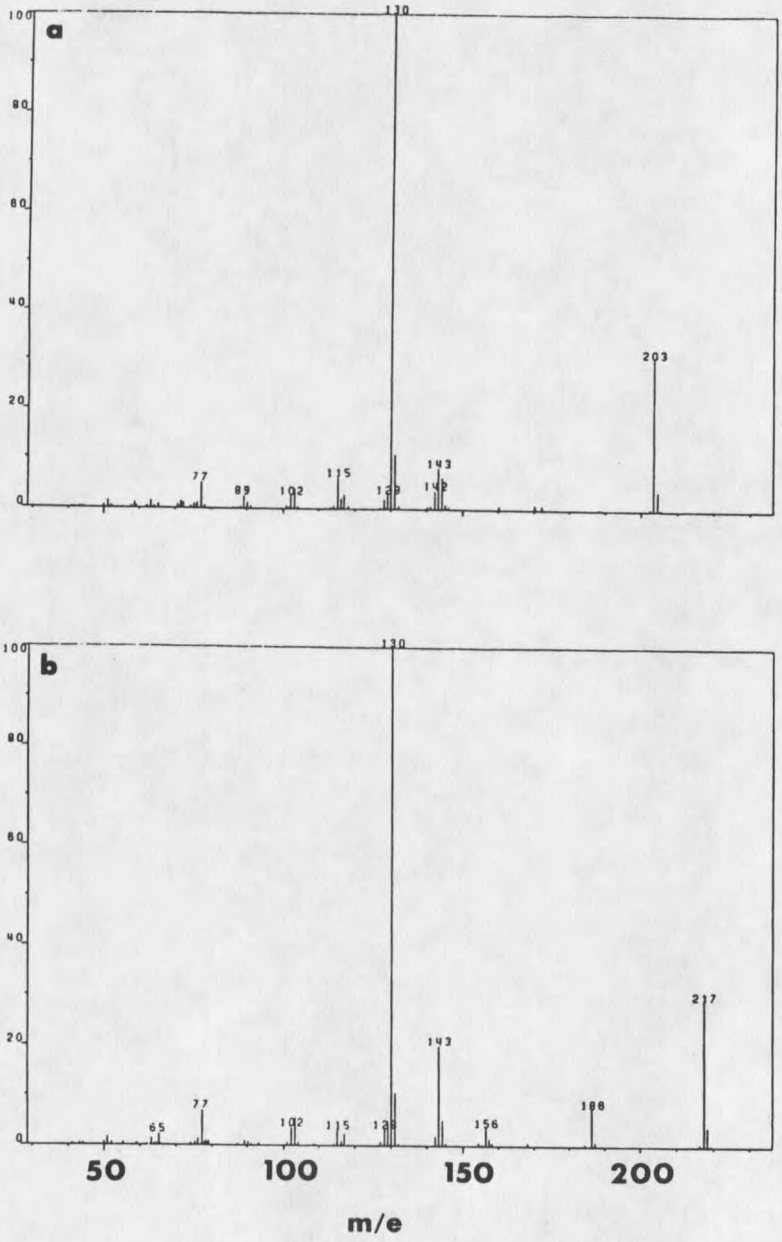


Figure 9. Mass spectra of a) indole-proponic acid and b) indole-butyric acid.

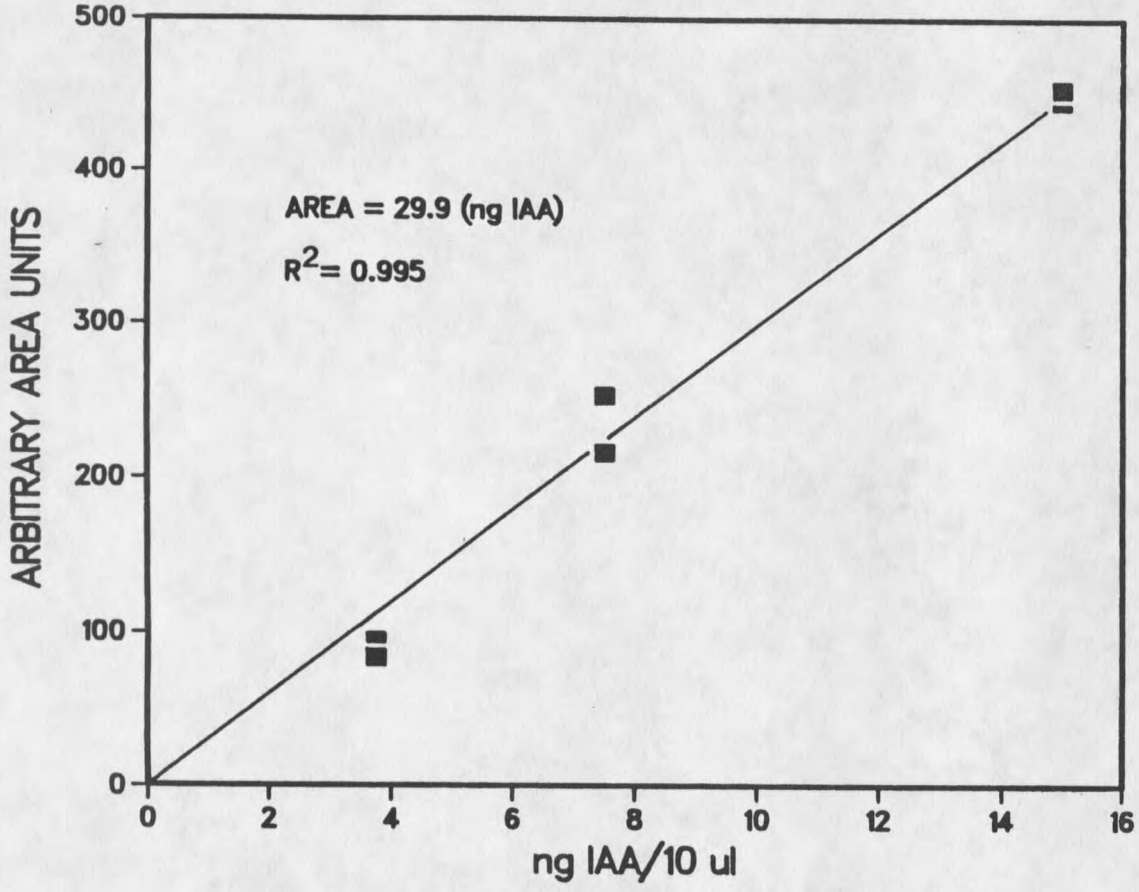


Figure 10. Calibration curve used in 4.0 to 15.0 ng IAA range without bandpass filter. Fluorometer sensitivity set at 428 and 0.1 μA range.

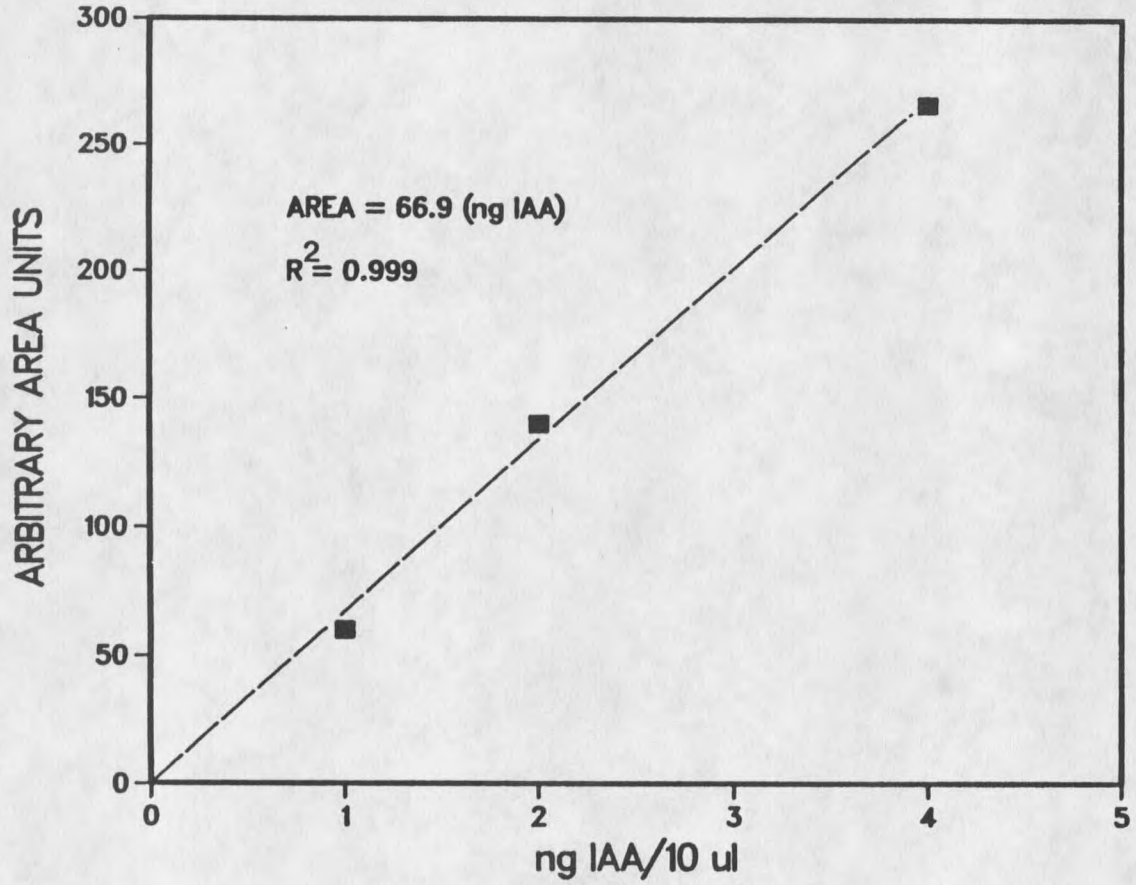


Figure 11. Calibration curve used in 0.25 to 4.0 ng range with bandpass filter conditions described in Chapter 4.

D378
N636
cop.2