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ABSTRACT

GRAFT SUCCESS AS INFLUENCED BY ENVIRONMENTAL CONDITIONS AFFECTING PHYSIOLOGICAL CHANGES IN JUNIPERUS L.

By

Ronald Lee Spangler

Juniperus horizontalis 'Andorra', J. chinensis 'Hetzi' and J. chinensis 'Pfitzer' were self-grafted following varying temperature storage treatments to the scion and understock. Treatments consisted of two temperatures, greenhouse stored (18°C) and cold dark storage (2°C). The storage periods for the cold treatment were four, nine or twelve weeks. Different scion/stock combinations were made in the grafting studies.

Graft survival data indicated decreasing order of clonal survival was Andorra, Hetzi, and Pfitzer. Decreasing order of graft survival for temperature-storage treatments was nine, four and twelve weeks of cold storage. Data from the scion/stock treatments indicated whenever greenhouse (18°C) scion or stock was involved in the graft treatment, graft survival was greater than if (2°C) scion or stock material was involved regardless of the length of cold treatment.

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Application of different concentrations of auxin:gibberellin:kinetin solutions resulted in equally poor graft survival.

Physiological studies were designed to characterize the growth cycle of the juniper clones when the above mentioned environmental treatments were given. The purpose of these studies was to correlate the growth cycle of a clone to its self-graft take potential.

Root activity determined by percent white root tips on the root system of a plant was evaluated for greenhouse plants, and plants exposed to four and nine weeks of cold prior to being moved into the greenhouse. Root activity in greenhouse plants declined from October through December and then plateaued. Root activity for all clones exposed to four or nine weeks of cold prior to being moved to the greenhouse increased within one month in the greenhouse but then declined and plateaued in the second and third month.

Shoot growth, measured by the total length of one randomly selected shoot from each of ten plants, was recorded biweekly for plants under long (16 hours), natural and short (9 hours) days. Long days caused plants to grow continuously. Under natural days, shoot growth response resembled a sigmoidal curve, while short days caused a cessation of growth from November through February. Growth resumed in short-day treatments when the greenhouse day temperature was approximately 26°C.

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Plants given four, nine or twelve weeks exposure to cold prior to being moved into the greenhouse began growth soon after entering the greenhouse. The growth rate of the different clones was different according to treatment. In Andorra, four weeks of cold storage did not affect total growth when compared to greenhouse natural day length plants. Nine and twelve weeks of cold caused the growth rate of Andorra to be greatly reduced when compared to growth of natural day length. In comparison to Andorra, Hetzi and Pfitzer shoot growth rate was accelerated by increasing the cold storage period to nine or twelve weeks.

Changes in growth promoters were determined by the mung bean bioassay. Preliminary characterization studies from methanol extracts indicated that the most active region of the chromatogram was in the region R_f 0.80-0.93. A second region investigated was R_f 0.26-0.40. By partitioning the crude methanol extract into its acidic, basic and neutral ether fractions only R_f 0.80-0.93 was found to be active from the mung bean bioassay. This region was designated cofactor 4 which was found to be present in the shoots and roots of all clones.

Changes in level of cofactor 4 were determined for outdoor shoots, greenhouse shoots and roots, and for shoots and roots of plants exposed to four and nine weeks of cold storage. In Andorra, the pattern of change in relative concentration of cofactor 4 for greenhouse plants from October

through May was found to be very similar to the pattern of change of Andorra outdoor plants from April through November. When these curves were superimposed on each other a shift of six months by the greenhouse plants was noted. A shift of one month and two months was noted for Hetzi and Pfitzer, respectively. All clones exposed to four weeks of cold showed a decline in cofactor 4 concentration after being moved into the greenhouse for one month. Plants exposed to nine weeks of cold increased in cofactor 4 concentration after one month in the greenhouse. No differences were noted in the roots of greenhouse plants for all clones from October through May. For Andorra and Pfitzer after four weeks of cold the cofactor levels for the roots and shoots followed almost identical patterns in change.

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INTRODUCTION

Grafting of evergreens is practiced by many nurserymen who desire to propagate specific clones that do not root easily or do not come "true" from seed. Nurserymen may also practice grafting to produce plants with hardier or more disease-resistant roots, to change the growth rate of the plant, or to change shoot variety.

Grafting of evergreens in the United States was practiced only by a few nurserymen until recent years, despite the long history of grafting dating back to the Chinese writings of 1560 B.C. (see Roberts, 1949). Early nurserymen kept their grafting techniques closely guarded and passed their secrets within each family from generation to generation. Today, however, the once closely guarded techniques are becoming common knowledge (being published or shared) among nurserymen (Pinney, 1970; Hill, 1953).

Although grafting techniques are improving, surprisingly little research has been done on the process (on the basis of scientific principles). With scarcely more than intuition and experience many propagators have continued to improve their techniques by trial and error. When a new cultural practice results in successful grafts the practice is

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usually adopted; conversely, when a cultural practice is unsuccessful the practice is rejected.

It is not understood why graft-take for particular species is often inconsistent from year to year. Evidently, graft-take is influenced by many factors that are as of yet not recognized as being important in the grafting practice. Improved graft-take in the junipers will require a complete study of this genus. In addition to grafting, rooting studies may also provide information on juniper growth and development. Lanphear (1962) studied rooting of woody ornamentals including junipers, and Nuss (1967) observed rooting of J. chinensis 'Glaucia Hetz'. Their purpose was to improve rooting of cuttings by application of growth compounds and to describe the cyclic pattern of rooting potential. Their studies provide excellent background material for studies in this genus.

Evans (1969) studied the anatomical sequence of graft healing of self and interclonal grafts. He selected clones which had varying (relative) degrees of wound healing potential.

Graft-take may be influenced by many factors: activity of the root system; activity of the understock, shoot or scion wood at the time of grafting; temperature and moisture at the graft; presence and concentration of hormones at the union and within the understock and scion; and age of understock and scion wood. The scope of this research was

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limited to the following objectives: 1) to determine the effect of day length and chilling on the graft-take potential of junipers; 2) to determine if exogenously applied growth substances to the graft union would improve wound healing; 3) to compare graft success between two species and within two clones of one species; 4) to quantify growth regulators and determine if cyclic patterns exist within the clones for specific environmental conditions; and 5) to further understand the physiology of juniper growth and development.

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LITERATURE REVIEW

Junipers

The name juniper has been traced to the Latin words juvenis (young) and the verb parere (to produce) (Coltman-Rogers, 1920). The genus was presumably so named because in many species two entirely different looking sets of leaves are on the same tree--namely, awl-shaped, acicular young or juvenile leaves and the mature, appressed-to-the-stem, adult foliage. The genus contains about 60 species of evergreen trees or shrubs distributed over the Northern Hemisphere from the Arctic Circle to Mexico, the West Indies, Azores, Canary Islands, North Africa, Abyssinia, the mountains of East Tropical Africa, Himalaya, China and Formosa (Dallimore and Jackson, 1967).

Cytological studies within Juniperus L. have been conducted on 14 species or clones. Evans (1969) compiled the documented chromosome numbers for the various Juniperus L. species or clones. Although in the Juniperus L. genus the common chromosome number is $2n = 22$, the chromosome number varies both between and within species. A description of three clones will be briefly sketched giving the chromosome number and description of the clone.

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Juniperus horizontalis Moench. ($2n = 22$) is a native of North America found on sea cliffs, gravelly slopes and in swamps from the coast of Maine to British Columbia, ranging south to Massachusetts, Western New York, Illinois and Montana (Dallimore and Jackson, 1967). The clone 'Andorra' is a low, slow-growing evergreen with feathery foliage turning from light green to purple in the fall (Wyman, 1969).

Juniperus chinensis ($3n = 33$) is a species that originated in China and Japan and was first introduced to Europe by William Kerr, who sent it from Canton, China to England in 1804. It is polymorphic and is known for its tall columnar bush or small tree form (Wilson, 1916).

Juniperus chinensis 'Hetzi' is a male seedling mutation of the common J. chinensis. The origin of 'Hetzi' is controversial. Den Ouden and Boom (1965) indicated that 'Hetzi' was first clonally propagated and introduced in 1920 by the Fairview Evergreen Nurseries, Fairview, Pennsylvania. Leiss (1966), on the other hand, believed that 'Hetzi' was discovered before 1948 in a batch of seedlings from the West Coast, and received by Hetz Nurseries in Fairview, Pennsylvania. He suggested that 'Hetzi' is a cross between J. virginiana 'Glaucua' as the seed plant and J. chinensis 'Pfitzeriana' as the pollinator. 'Hetzi' has upright branches which grow about 15 feet tall and wide with very dense, light bluish colored foliage.

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Juniperus chinensis 'Pfitzeriana' or 'Pfitzer' ($4n = 44$) is another clone of J. chinensis. Dallimore and Jackson (1967) reported that it originated in Spaeths Nursery, Berlin, Germany, but van Melle (Dallimore and Jackson, 1967) postulated its origin to be the Ho Lan Shan Mountains of Inner Mongolia. 'Pfitzer' is a densely branched shrub with long branches and slightly drooping branchlets with awl-shaped, slightly glaucous leaves.

In addition to the genetic and phenotypic differences among the three previously described junipers these clones also display varying degrees of rooting and grafting potential. These clones provide the researcher with ideal material for comparisons to be made between species and between two clones within a species.

Graftage

Graftage is a recognized means of propagating plant materials which are either difficult or impossible to obtain from seeds or cuttings. Other important reasons for grafting include: obtaining special forms of plant growth; obtaining benefits of certain rootstocks; changing varieties of established plants; and repairing damaged parts of trees (Hartmann and Kester, 1968; Roberts, 1949).

Current theories and practices of grafting are discussed by Hartmann and Kester (1968) and Mahlstedt and Haber (1957).

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A simple definition of grafting is "the art of joining parts of plants together in such a manner that they will unite and continue to grow as one plant" (Hartmann and Kester, 1968). Roberts (1949) reviewed the ancient literature on grafting, dating back to the Chinese writings of 1560 B.C. The basic grafting and budding techniques of today were first described by Lawson in 1660 and illustrated by Sharrock in 1672 (Roberts, 1949).

Research studies of compatibility, transport of materials across the union, scion/stock and interstock relationships, and anatomical changes occurring at the graft union have been reported (Evans et al., 1961; Evans, 1969; Copes, 1969; Dana, 1963). Studies on the environmental and physiological factors affecting graft-take are few.

Grafting success is often inconsistent and many propagators have found grafting discouraging. Environmental and physiological factors known to influence the healing of the graft union include: supply of endogenous hormones and growth substances; time of year and stage of development; temperature; and moisture (Kester, 1965).

Maintaining the proper environment around the graft union during callus formation is necessary for healing to take place. Temperatures in the range of 25°C to 30°C resulted in nearly 90 percent callusing; higher or lower temperatures reduced the amount of callus in walnut (Sitton, 1930). Brierley (1955) found that only three percent of the

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grafts callused where temperatures ranged from 5°C to 22°C. Harmon and Weinberger (1967) reported a positive correlation between success of grafting and development of the callus at the union with shading of newly planted grafts and length of scion wood. Hansen and Hartmann (1951) found that white-washing the scion and stock improved walnut graft-take, while addition of growth regulating chemicals did not.

Nenjuhin (1965) measured changes in scion weight after grafting of Pinus sylvestris, P. sibirica, P. contorta, and Picea abies. After 10 days, weight loss was stabilized and the initial weight was regained about 30 days after grafting.

Shippy (1930) studied the effect of humidity on healing of apple tree grafts. Air moisture levels below saturation inhibited callus formation. Presence of a film of water against the callusing surface resulted in more callus than just maintaining the air at 100 percent relative humidity. Graft-take of Rhododendrons, juniper and beech (Fagus sp.) was poorer under mist or humidification than under double glass (van Doesburg and Ravensberg, 1962).

Stage of development of the stock plant is important for some propagation methods. "Slipping" of the bark, an indication that vascular cambial cells are actively dividing and producing thin-cells on each side of the cambium, is required before T-budding and bark grafting can be performed (Hartmann and Kester, 1968).

Juglans and Acer are plants which have high root pressure in the spring when they are actively growing. Because of the excessive sap flow or "bleeding" the graft union often will not heal. Grafting must therefore be performed at some other time of the year (Hartmann and Kester, 1968).

Potted rootstock plants such as junipers and Rhododendrons that are dormant and brought into a warm greenhouse in winter must be allowed to begin active growth prior to grafting. Pinney (1970) suggested that grafting should be delayed until the rootstock begins to form new roots. Plants held for several weeks at 15°C to 18°C in a greenhouse would begin to form new roots, and the rootstock would be physiologically active enough for nutrients to move to the union for healing to begin.

The optimum time for grafting is debatable. Klapsis (1964) observed that if rooted understock was brought into the greenhouse about November 1, root activity as indicated by white root tips began around December 1. Once the white roots were present grafting was begun. Wagner (1967) suggested that the best time for grafting of junipers was the months of January and early February. Willard (1968) found that optimum time of grafting of Picea 'Kosteriana' occurred in January (95 percent success). Wells (1955) reported that grafting of pine (Pinus sp.) and spruce (Picea sp.) during January and February resulted in high percent graft-take.

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Choi (1966) reported that conifer scions kept in cold storage showed poor survival. Madden (1968) found that pecan (Carya sp.) scions cut and stored several days resulted in reduced graft success.

Nienstaedt (1958) hypothesized that grafting success was a function of speed and effectiveness of union formation and these in turn depended on the amount of cambial activity of the rootstock and scion at the time of grafting and immediately thereafter. To test his hypothesis he attempted to control plant activity by altering day length and chilling in fall grafting of spruce. From his studies he found that fall grafting was feasible (76.5 percent); however, to obtain maximum survival the grafts had to be exposed to cold or long day conditions.

The use of growth substances to promote healing of the graft union has been suggested (McQuilken, 1950; Davis, 1949). However, as a result of the inconsistent effects no growth substance has been found effective (Brierley, 1955; Hansen and Hartmann, 1951; McQuilken, 1950). Tissue culture studies however demonstrate a definite relationship between callus formation and the levels of certain endogenous growth substances--particularly cytokinins and auxin (Murashige and Skoog, 1962; van Overbeek, 1966). Homes (1965) concluded that induction of the differentiation process in vascular bundles in the lower part of the scion was caused by growth substances moving basipetally.

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Evans (1969) studied the effect of leaf-stem extract of one juniper applied to the cut surfaces of other juniper self-grafts. Graft survival data revealed that extract treated self-grafts responded differently from non-treated self-graft controls. The extracts produced a marked influence upon scion vigor.

There have been a number of studies on healing of the graft union in woody plants (Mergen, 1954; Sharples and Gunnery, 1933; Copes, 1969; Evans, 1969). Anatomical studies of the developing graft union have been studied on Douglas Fir (Pseudotsuga menziesii) (Copes, 1969) and junipers (Evans, 1969). Copes (1969) described a general model for the sequence of graft union development for most conifers as follows: contact layers or isolation layer formation; cell enlargement; callus formation; phellogen formation; and vascular cambium formation.

Evans (1969) described the healing process in three juniper clones--Juniperus horizontalis 'Fountain', J. chinensis 'Hetzi', and J. chinensis 'Pfitzeriana Kallay'. Anatomical and sequential development for interspecific grafts were studied at 10 day intervals for 60 days. No basic anatomical or developmental differences were noted; however, the rates at which the developmental sequence occurred were variable. In general, voids between the stock and scion began to fill with callus between the 10th and 20th day following grafting. This change was first

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noted in 'Fountain' and last in 'Pfitzeriana Kallay'. Iso-diametric cells from the uninjured cambia of the stock appeared by the 20th day except in grafts with 'Pfitzeriana Kallay' as the scion which did not appear until after 30 days. Newly formed tissue in a typical graft arose primarily from the stock prior to the 50th day following grafting. After 60 days contribution of new tissue was nearly equal from the stock and scion.

Anatomical definitions and descriptions of graft union terminology are found in the text by Esau (1965).

Callus and Wound Healing

The healing of a graft union may be likened to the healing of a wound. The difference between a wound and a graft union is that in the wound only one individual is involved and healing of a graft union involves two individuals--scion and understock.

Shippy (1930) studied the influence of temperature and moisture on the callusing of apple tree grafts. Callusing occurred between 0°C and 40°C. The time required for a specific volume of callus decreased as temperature increased from 5°C to 32°C. Above 32°C injury occurred. Moisture below saturation inhibited callusing and a water film enclosing the cutting appeared to provide the most favorable moisture conditions for callusing.

When wounding occurs the normal functions of the plant are disrupted and new events occur to restore the previous functions. Generally, a wound causes either a localized burst of cell division or a change in the growth pattern causing new cells to form and cover the wound (Galston and Davies, 1970). To fully understand wound healing, studies should include the physiological and anatomical changes which precede and follow the meristematic activity which occurs in cells and tissues in the immediate vicinity of the wound. Brown (1937) found that the greater the amount of living bark distal to a wound, the greater the cambial activity was promoted by the presence of developing buds and leaves distal to the wound.

Cambial activity in the spring has been correlated with high auxin concentration moving down the cambium from expanding buds. The complexity of the stimulation was shown by Gouwentak (1941) who reported that dormant cambium must be in a reactive or sensitive condition before it can be stimulated into activity by auxin application.

"The facility with which callus is produced from cambium is seasonally parallel with the normal activity of the cambium and possibly is determined by prevailing auxin concentrations" (Audus, 1963). As Audus explained, "it is difficult to understand that the mere act of wounding should cause such a marked local stimulation of cambial activity if auxin is the only factor concerned. Therefore, another

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hormone produced from damaged tissue could be the initial cambial stimulant".

The isolation of such a hormone from certain plant tissues has been described. Hammett and Chapman (1938) suggested that the characteristics of a wound hormone would be the ability to stimulate cell division and to be liberated by the trauma rather than by disintegration. The ability of a compound to promote tissue growth is not sufficient proof that it is a wound hormone.

Block (1941, 1952) in a review of the literature stated that certain metabolic changes in the cells abutting on the cut surface are accompanied by increased oxygen absorption and carbon dioxide production, and that oxygen is essential for healing. Shippy (1930) also pointed out that even though moisture was necessary, the film of water must allow oxygen exchange. Goodwin and Goddard (1940) measured oxygen consumption in thin sections of tissues from trunks of ash (Fraxinus nigra) and maple (Acer rubrum) using Fenn volumetric microrespirometers. Oxygen consumption before bud break was higher in the cambial region than in the secondary phloem and xylem. After bud break oxygen consumption was essentially the same in the cambium, phloem, and heartwood of ash. In the newly formed differentiating xylem, oxygen consumption exceeded the cambial rate.

Davis (1949a,b) tested several growth substances (3-indolebutyric acid (IBA), 2,4-dichlorophenoxyacetic

acid, traumatic acid, glutathione, o-chlorophenoxypropionic acid and p-chlorophenoxyacetic acid and cysteine hydrochloride) for promotion of wound healing of sugar maple (A. saccharum). Only glutathione and cysteine hydrochloride were found to be stimulative. Davis suggested that -SH-containing compounds might be of practical importance when incorporated into the wound dressings.

McQuilken (1950) applied several substances as dressings to numerous tree wounds with and without incorporation of growth regulators and other chemicals. He found no chemical which significantly increased the rate of wound healing in any tree species tested. Dressing of the wound with lanolin prevented desiccation and callus was formed promptly at the wound edges.

Periodicity

The potential for a plant to heal after wounding may depend upon the physiological activity of the plant at time of wounding (Nienstaedt, 1958). Growth of woody plants, determined by activity of the roots, shoots, and cambium, often occur in recurrent cycles alternating with periods of dormancy or relative inactivity. Such growth cycles are generally annual but intraseasonal cycles are common (Reed, 1928). Root growth is determined by the number of white root tips or by measuring increase in diameter or length. Shoot growth may be determined by several means, one of

which is by increase in shoot length. During a flush of growth only certain shoots elongate and in any one shoot, growth may occur in one to several flushes. Temperate Zone trees often show both seasonal and diurnal periodicity (Kramer and Kozlowski, 1960). Intermittency in growth has been demonstrated in Pinus sylvestris, P. resinosa, P. strobus, and Picea abies (Farnsworth, 1955).

Periodicity phenomenon are often related to physiological factors affected by environmental conditions (Wilcox, 1954). Shoot growth began in early spring and concluded before cambial growth or root elongation was completed. Kozlowski and Ward (1957) reported that gymnosperm seedlings varied in length of growing season. In an average frost-free season of 148 days, the number of days to complete 90 percent of the seasonal growth in various species was: Picea abies, 57; Abies balsamea, 82; Tsuga canadensis, 93; Picea glauca, 99; Pinus resinosa, 103.

Nienstaedt (1959) induced two flushes of growth on spruce in the greenhouse in the winter by manipulating day length and chilling. His regime included 4 weeks of long days followed by 2 weeks of short days, followed by 8 weeks of chilling and finally long days.

Growth may be affected by day length, chilling or moisture. Moisture may cause day to day variations in growth but is not too important in long term growth studies.

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Day length and chilling treatment, on the other hand, affect growth over a longer period of time.

Day Length

Downs and Borthwick (1956) reported the effects of day length on the growth of several woody species. Catalpa, elm, birch, red maple and dogwood growth continued when a constant day length of 16 hours or more was given. Nitsch (1957a) classified several woody plants according to their response to day length. Junipers were representative of a group of plants that grow under both long and short days.

Waxman (1955, 1957) found that for Thuja occidentalis L. var. Lutea Kent and Juniperus 'Andorra' shoot elongation was continuous under long and short days. In the conifers loblolly pine (Pinus faeda) and northern white cedar (Chamaecyparis thyoides), short days prevented shoot elongation. Once day length began to increase in the spring, shoot elongation began to increase (Phillips, 1941). He suggested that long days promoted vegetative growth whereas short days inhibited growth and induced dormancy.

Hellmers (1959) reported that dormant Colter pine (Pinus coulteri) and Douglas fir seedlings under short days (9 hours) failed to break dormancy within six to eight weeks during which plants under long days broke dormancy.

Light intensity studies have been reported for several plants (Wareing, 1950; Waxman, 1957). In herbaceous plants light intensities as low as 0.3 foot-candles (ft-c) can

produce an effect (Nitsch, 1957b). This intensity is well below that level required for photosynthesis. In woody plants the minimum light intensity required for a physiological response is not known. Matzke (1936) reported that one ft-c was sufficient to delay autumn coloration and abscission of leaves on trees near street lights. In other experiments woody plants responded to light intensities as low as 3.5 ft-c (Garner and Allard, 1923); 20 ft-c (Wareing, 1950); and 10 ft-c (Waxman, 1957).

In addition to the interaction of temperature and light the quality of light varies with the light source used (Fisher and Watson, 1956). Downs (1958) found that shoot growth was accelerated by incandescent supplemental light. The incandescent light furnished more of the far red wavelengths than fluorescent light of the same intensity.

Thermoperiodism

Temperature can modify the day length response for some plants. Nitsch (1957b) reported that for some plants day length is operative within a given temperature range only. Waxman (1957) noted that some plants exhibited definite day length responses only when the minimum night temperature was 21°C. At 10°C longer days were required to produce the same response. For example, at 10°C maximum growth resulted with continuous light.

White spruce (Picea glauca) and Norway spruce (P. abies) differ in response to chilling and day length. Nienstaedt

(1959) found that white spruce breaks dormancy more readily than Norway spruce after chilling but Norway spruce responds more favorably to long days.

Although the relationship of dormancy to chilling and day length has been studied in many tree species, the actual chilling requirements have been studied for only a few species (Kramer and Kozlowski, 1960). According to Samish (1954), the chilling requirement for different varieties of peaches varies from 200 hours below 7°C to over 1100 hours for other varieties. In Georgia, accumulation of 1000 hours below 7°C would break dormancy for most peach varieties (Weinberger, 1950). Long days compensated for the lack of chilling in many species (Kramer and Kozlowski, 1960). Nienstaedt (1966) demonstrated that chilling requirements can be compensated for in white spruce by exposure to long days. Such compensation has also been observed in eastern hemlock (Tsuga sp.) (Olsen and Nienstaedt, 1957) and Scotch pine (Pinus sylvestris) (Wareing, 1951a). Abies species when grown for a year in the greenhouse abort their terminal buds and force the lateral buds to grow continuously (Worrall and Mergen, 1967).

Cambial Activity

Cambial activity is cyclic, with periods of activity alternating with periods of rest or relative inactivity. In addition to shoot growth, photoperiod may affect the cambial activity of plants. Long days (more than 15 hours of

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light) maintained cambial activity longer in Pinus sylvestris than plants receiving short days (less than 10 hours of light) (Wareing, 1951b). In the spring, if new shoot growth was allowed to develop, then cambial activity occurred under both long and short days. Exposure to short days brought about a cessation of cambial activity in some species of trees, while exposure to long days prolonged it (Wareing, 1956; 1957). Wareing (1951a) demonstrated that cambial activity in Scotch pine seedlings was maintained longer under 15 hours of light than under 10 hours of light and that it could be prolonged in the autumn by supplementary illumination to provide a 15-hour day. He concluded that natural changes in day length in the autumn affected the duration of cambial activity of this species.

Hormonal Control of Growth

Endogenous growth regulators in woody plants are important in the regulating mechanisms of plant growth and development. In Aesculus and Malus cambial activity began at the level of the terminal buds and progressed basipetally in the stems (Avery and Burkholder, 1937). Wareing (1958) concluded from girdling experiments that cambial activity continued longer than stem extension growth in early spring. Priestly (1930) showed that continued cambial activity was dependent upon continued extension growth, and when the latter ceased, cambial activity ceased soon after. The inner bark of Pinus sylvestris L. contained an acidic growth

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promoter which stimulated elongation of wheat coleoptiles (Wodzicke, 1968). Allen (1960) reported that acidic growth promoters and inhibitors regulated the winter rest period of longleaf pine buds.

Kramer and Kozlowski (1960) discussed the hypothesis that auxin from opening buds moved down the stem causing activity to begin. Cambial activity progressed downward so rapidly that diameter growth began almost simultaneously at both ends of the tree. Mirov (1941) reported that in Pinus torreyana distribution of diffusible auxin in xylem yielded more auxin. Maximum diffusible auxin was not located in the tip but occurred lower in the new shoot and diminished toward the previous year's growth. In Ponderosa pine (Pinus ponderosa) there was little difference in the hormone concentration of the leader as compared to side shoots; whereas, in slow growing trees the leader always contained more auxin than side shoots.

Auxin has an active and perhaps specific role in controlling the differentiation of cambial derivatives. The evidence was in favor of its participation in xylem differentiation, in wound healing and root culture (Torrey, 1953). The role of auxin in phloem differentiation was less convincing, and there was reason to believe that a number of growth substances may be involved in cambial activity.

Ladefoged (1952), Fraser (1958), and Wareing (1958) have shown that cambial division began first in the twigs

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immediately below the swelling buds and spread to the base of the branches and downward.

The maintenance of cambial activity might depend upon the continued production of auxin in mature leaves under suitably long day lengths (Wareing and Roberts, 1956).

Zimmerman (1936) investigated the auxin content of dormant buds of 11 different species of hardwoods and conifers and failed to obtain any diffusible hormone. However, as soon as the buds began to swell he was able to collect diffusible auxin. The amount of auxin increased rapidly and reached a peak during the elongation of the new shoot and had already started to decrease slowly when elongation was completed. A much lower hormone content was found in the newly developed terminal buds at the end of the current season, and this amount diminished progressively to the low values of the dormant winter bud.

Snow (1933) reported that leaves promoted the growth of the cambium beneath them, and this stimulus traveled basipetally. Later Snow (1935) activated cambial activity with synthetic hetero-auxin and concluded that normal cambial growth was activated by the same growth hormone which was formed by the leaves and promoted cell extension in the stems.

In Betula, inhibitor concentrations increased with increasing short days and decreased in concentration with increasing day length. Greatest inhibitory activity

occurred in the growing point while the least was found in the roots (Kawase, 1961).

Hatcher (1959) suggested that auxin production was in the stem apex and young developing leaves of deciduous plants. The peak of diffusible auxin was found several internodes below the apex in the region where the leaves attained full size. The peak was suggested to be due to accumulated auxin delivered from the growing zones.

Root Studies

The root system also displays patterns of growth periodicity. In order for a shoot to continue growth, to be provided with moisture and nutrients, and to be held in an upright position the root system must continue to grow throughout the life of the plant. Romberger (1963) and Whittington (1968) reviewed much of the research about root initiation and growth. Studies showed that roots may elongate during any month of the year and that changes in elongation rate coincided with environmental changes (Kramer and Kozlowski, 1960). Individual roots did not all grow at one time even though all roots displayed periodicity in growth. A common pattern reported was a burst of growth in the spring, a midsummer low, and renewed activity in the fall (Wilcox, 1962).

In Juniperus excelsa, Cupresus sempervirens, Quercus pubescens, Quercus ilex, shoot and root growth were most active from the end of April to the beginning of July



(Jaroslovec, 1964). Root growth reached a second peak of activity between November and December.

The relationship between roots and shoots may be seen in a number of physiological patterns. Went (1938, 1943) suggested the existence of root hormones which controlled shoot growth. Hess (1962a) showed evidence of rooting cofactors present in easy to root species which were absent in difficult to root species. Growth substances, according to Went (1938), may be produced in the roots and transported to the shoots where they become active.

Rooting potential for many woody plant cuttings also follows a periodic pattern (Lanphear and Meahl, 1966; Vietez and Pena, 1968). Vietez and Pena followed the rooting success of Salix atrocinerea at monthly intervals for one year. Rooting potential was greatest from January to May when 96-100 percent of the Salix cuttings rooted. Rooting potential was also high from July to September (86-94 percent). Low rooting potential occurred in June (58 percent) and declined from October through December from 76-40 percent.

Seasonal variation in rooting potential of cuttings of J. horizontalis 'Plumosa' was reported by Lanphear and Meahl (1963, 1966). They found that cuttings taken during the fall and winter resulted in high root-forming capacity which was independent of the low seasonal temperatures since greenhouse stock plant cuttings during this same time also demonstrated high root forming capacity. Long days reduced the

rooting potential when 'Plumosa' had been previously subjected to a root-breaking chilling period.

In addition to rooting studies, Lanphear and Meahl (1963, 1966) tested for the presence and change in levels of rooting cofactors. They found no relationship between the rooting cofactor level and the rooting response of juniper.

A rooting response may be counteracted by applying growth regulators to cuttings prior to insertion in the propagation bench. Cuttings of J. chinensis 'Glaucia Hetzi' and Taxus cuspidata 'Nana' resulted in increased rooting percentage if IBA was applied to cuttings under long days (Lanphear and Meahl, 1963). Chadwich and Kiplinger (1939) reported that J. chinensis 'Pfitzer' displayed a much greater response to IBA when cuttings were taken in January instead of November or December. Hitchcock and Zimmerman (1939) found that Taxus cuspidata required a higher concentration of IBA for rooting when the cuttings were taken in October and November than in succeeding months.

MATERIALS AND METHODS

General

The following studies were designed to examine the relationship of graft-take to some environmental and physiological conditions between two species of Juniperus L. and between two clones within a Juniperus sp. Five major areas of research were followed: 1) environmental and physiological factors affecting graft-take; 2) changes in root activity of 2-year-old plants submitted to various environments; 3) changes in shoot growth under different climatic regimes; 4) changes in growth substance as influenced by environment; and 5) preliminary characterization of endogenous growth substances.

The overall experimental design is shown in Table 1. To understand the various studies on the basis of growth and development of the juniper and at the same time to relate this information to two species and two clones within a species required that several studies be conducted simultaneously. Therefore, root activity level, endogenous growth substances as measured by the mung bean bioassay, and graft survival of all three clones were determined in one year. The root activity study and mung bean bioassays

TABLE 1.--Overall experimental design for grafting and physiological studies. Three juniper clones were used--Andorra, He21, and Pfitzer.

1969-1970	Outdoor	Greenhouse	4 Weeks, 2°C	9 Weeks, 2°C	12 Weeks, 2°C	Graft Studies
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TABLE 1.--Overall experimental design for grafting and physiological studies. Three juniper clones were used--Andorra, Hetzi, and Pfitzer.

1969-1970	Outdoor	Greenhouse	4 Weeks, 2°C	9 Weeks, 2°C	12 Weeks, 2°C	Graft Studies
September ¹	-- MB ²	SG ³				
October	-- MB	-- SG, MB, RA ⁴	SG			
November	-- MB	-- SG, MB, RA	-- SG, MB, RA	SG		4 Week Graft
December	-- MB	-- SG, MB, RA	-- SG, MB, RA	-- SG, MB, RA	SG	
January	-- MB	-- SG, MB, RA	-- SG, MB, RA	-- SG, MB, RA	-- SG, MB, RA	9 Week Graft
February	-- MB	-- SG, MB, RA	-- SG, MB, RA	-- SG, MB, RA	-- SG, MB, RA	12 Week Graft
March	-- MB	-- SG, MB, RA	-- SG	-- SG, MB, RA	-- SG, MB, RA	
April	-- MB	-- SG, MB, RA	-- SG	-- SG	-- SG, MB, RA	
May	-- MB	-- MB, RA				
June	-- MB					
July	-- MB					
August	-- MB					

¹Month the sample was taken or graft made.

²Sample for mung bean bioassay study (MB).

³Shoot growth measurement study (SG).

⁴Sample for root activity study (RA).

were conducted using three single plants in both determinations for all treatments except outdoor treatment, in which one large plant of each clone was used. The shoot elongation study was conducted the following year under similar conditions. Grafting studies were conducted for three years with certain treatments being repeated in all years.

Plant Material

Two species of junipers and two clones within a single species were selected to represent different stable chromosomal clones within the genus. The clones studied were Juniperus horizontalis 'Plumosa' (Andorra), $2n = 22$; Juniperus chinensis 'Hetzi', $3n = 33$; and Juniperus chinensis 'Pfitzeriana', $4n = 44$. Hereafter, the clones will be referred to as Andorra, Hetzi, and Pfitzer, respectively.

The plant material consisted of vigorously growing potted cuttings approximately 8-12 inches in height obtained from two nurseries.¹ Evergreen shoots used for the outdoor treatment in the growth regulator studies were obtained from individual 10-to 15-year old shrubs. Shoot samples were collected from the same shrub at monthly intervals for one year.

¹1969, Lincoln Nursery, Grand Rapids, Michigan; 1970-1971, Zelenka Evergreen Nursery Inc., Grand Haven, Michigan.

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The plants were removed from peat pots and repotted into 4 inch plastic pots¹ containing a soil mixture of loam soil and coarse sand (1:1). Plants were watered when the soil surface became dry. Water soluble fertilizer (20-20-20) was applied at regular intervals by means of a fertilizer proportioner.

Climatological Data

Climatological data from September, 1969 through April, 1971 are presented in Table 2.

Temperature data at the East Lansing Experiment Station was extracted from "Climatological Data", published monthly by the U.S. Department of Commerce Environmental Science Service Administration. Day length was calculated from the Weather Bureau table "The Time of Sunrise and Sunset for Lansing". Calculations were based on the fifth day of each month.

Analysis of Data

Effects of clones, treatments and sampling dates on Graft-take, root activity, shoot growth and growth promoter activity were evaluated by a single classification analysis of variance (Steele and Torrie, 1960). Preliminary multiple classification analysis of variance indicated no significant interaction between clones, treatments, or sampling dates.

¹Vaughn's Seed Company, Downers Grove, Illinois.

TABLE 2.--Climatological Data, East Lansing, Experiment Station, 1969-1971.

Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.
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TABLE 2.--Climatological Data, East Lansing, Experiment Station, 1969-1971.

	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.
	Temperature (Degrees F)											
	1969-1970											
Average Maximum	73.7	59.7	43.8	32.5	24.8	33.0	39.6	59.7	72.4	78.9	82.0	82.5
Average	62.8	50.0	36.7	26.4	17.0	24.4	30.9	48.9	60.9	66.9	72.1	70.8
Average Minimum	51.8	40.2	29.6	20.3	9.1	15.4	22.1	38.1	49.3	54.9	62.2	59.0
1970-1971												
Average Maximum	74.4	63.4	46.0	35.0	26.5	32.6	38.9	58.7				
Average	64.2	53.7	39.5	27.4	18.9	25.5	30.5	45.8				
Average Minimum	53.9	43.9	33.0	19.8	11.3	18.4	22.0	32.9				
Day Length (All Years)												
(Hours:Minutes)												
5th Day on Month	13:00	11:34	10:09	9:11	9:09	10:08	11:24	11:54	14:06	15:11	15:13	14:21

Grafting Studies

Three grafting studies were designed to determine the effect of various environmental and physiological factors related to graft-take. Parts of each study were repeated for two to three years to evaluate yearly variability. All grafts were self-grafts to avoid interactions due to clonal differences (Evans, 1969).

Prior to cold treatment the root systems were allowed to become established, for four and eight weeks for 1969-1970, and 1970-1971, respectively. The plants were grafted three weeks following the cold treatment.

Grafting Procedure and Subsequent Culture

A modified veneer side graft was used (Hill, 1953; Pinney, 1970). A vertical downward cut was made through the bark and about one-third the way into the wood. A twenty to thirty cm scion having the same diameter as the understock was used; its base was tapered to a wedge. The scion was inserted into the stock and the graft union secured with a ten centimeter grafting strip and covered with damp but not wet sphagnum moss. The plants were placed vertically on a bench under a four mil clear polyethylene tent supported on a wire frame 55 cm above the plants. Inside the tent a Spray Stix¹ mist system was installed. Daily or when

¹Spray Stix is the commercial product of Chapin Watermatics Inc., Watertown, New York.

necessary the plants were misted. Misting was performed when the inside surface of the plastic became dry. This method of humidity control was adopted to prevent overwatering the sphagnum moss and the potted stocks.

Forty days after grafting, half the shoot of the understock was removed; the remaining portion was cut back to the graft union 20 days later.

Graft survival was recorded three to four months following grafting. If the graft was successful the scion would be actively growing.

Understock and Scion Environmental Study - 1969-1970

This study was designed to determine if the understock and/or scion require a period of low temperature rest.

A factorial experiment was designed to elucidate the effect of rest and cold treatment on graft-take. The understocks and plants used as scions were placed in either the greenhouse (18°C) or in a dark, cold storage room (2°C) with a relative humidity of 95-100 percent for varying lengths of time--four, nine or twelve weeks. Four combinations of grafts were made: greenhouse scion onto a greenhouse understock (18°C/18°C); cold storage scion onto cold storage understock (2°C/2°C); greenhouse scion onto cold storage understock (18°C/2°C); and cold storage scion onto greenhouse understock (2°C/18°C).

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Plants held for a given period of time in cold storage were taken to the 18°C greenhouse and allowed to initiate root growth prior to grafting. After approximately three weeks in the greenhouse the grafts were made as previously described. The dates of grafting of randomly selected plants were November 29, 1969, January 5 and January 30, 1970.

Juniper plants similar to those used as understocks were cut above the soil surface. These shoots were used for scion material. By using the same age plants for both stock and scion the diameter of the scion and stock could be closely matched. The 18°C/18°C and 2°C/2°C treatments following four, nine and twelve weeks of cold storage was repeated the second year.

Scion Storage and Environmental Study - 1970-1971

Scion material is often cut in late autumn to avoid freezing and stored in a cool damp room prior to grafting (Hartmann and Kester, 1968). Grafting is performed at a later date when the understock roots are in an active stage of growth.

The purpose of this experiment was to determine if length of storage of the scion wood affected graft-take. Scion wood was collected in the morning on the following dates of 1970: October 1, October 23, and November 18. The scion wood was taken from two-year-old field grown

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material and stored in plastic bags in a 100 percent relative humidity dark cold storage room maintained at 2°C. In addition to the scion wood, moist sphagnum moss was placed inside the plastic bags. Neither fungus nor mold appeared in the bags or on the scion wood during the storage time.

Grafting was performed on November 11, December 17, 1970 and January 8, 1971. Ten single plant replications were grafted per treatment.

Plant Extract Grafting Study

Based on Evans' (1969) plant extract study a graft extract experiment was performed. This study was repeated three times over a three-year-period to determine the effectiveness of the application of plant extracts. The treatments included application of distilled water, Andorra extract, Hetzi extract, or Pfitzer extract on each of the three clones.

For each clone 25 grams of fresh shoots were added to 125 ml of deionized water. The mixture was ground in a Waring Blender at high speed for two and one-half minutes. The liquid paste was strained through four layers of cheesecloth to remove the plant material and stored in glass jars in ice. The pH of the extracts was Andorra, 6.4; Hetzi, 6.1; and Pfitzer, 6.0.

In addition to the extract treatments, auxin:kinetin:gibberellin solution treatments were prepared and applied to

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each of the self-grafted clones. Concentrations of the growth regulators were as follows:

<u>Grafting Period</u>	<u>Auxin</u> (mg/l)	<u>Gibberellin</u> (mg/l)	<u>Kinetin</u> (mg/l)
4 weeks of cold	10	20	10
9 weeks of cold	1	2	1
12 weeks of cold	10	10	2

To determine if the time of extract application with respect to cold storage of the stock affected graft survival the extracts were applied to the nine-week grafted plants in 1969-1970 and to the four- and twelve-week treatments in 1970-1971.

Root Activity Study

The plant propagator begins grafting after examining the root system for root activity or initiation of new growth. Active root growth is defined as the emergence of white root tips from the dormant brown roots (Romberger, 1963).

The treatments and times of sampling for root activity are listed in Table 1. At each sampling date five plants were selected at random and removed from the soil. The entire root mass was then examined for white root tips. The percentage of white root tips were compared to the entire root system using the following rating scale:

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<u>Rating</u>	<u>Description</u>	<u>Per Cent White Root Tips</u>
0	None	0
1	Very Few	15
2	Few	30
3	Few to Moderate	45
4	Moderate	65
5	Moderate to Heavy	85
6	Heavy	100

Shoot Growth Study

Shoot growth of many woody plants is affected by day length. In general, long days (14 hours or more) increase the rate and duration of vegetative elongation of woody plants; short days (9 hours or less) cause a complete cessation of growth (Nitsch, 1957b).

In addition to correlating root growth to graft-take the possibility existed that shoot growth might also be related to graft-take and/or to root growth. This study established the growth pattern of the three clones given the same environmental treatments of cold storage but varying day length treatments.

The treatments for this study were:

1. Greenhouse culture - natural day length (see Table 2), no cold storage.
2. Greenhouse culture - short day (9 hours), no cold storage.
3. Greenhouse culture - long day (16 hours), no cold storage.
4. Cold storage (2°C) - four weeks followed by greenhouse culture, natural day length.

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5. Cold storage (2°C) - nine weeks followed by greenhouse culture, natural day length.
6. Cold storage (2°C) - twelve weeks followed by greenhouse culture, natural day length.

Shoot growth in centimeters was measured biweekly from September 23 through April 7 by selecting randomly one shoot per plant and following its growth. Total height was determined by placing a flat wooden stake across the top of the clay pot and measuring the total length of the selected shoot.

Two-year-old plants were potted in 13 cm clay pots in early September using a sand:peat:soil (1:1:1) mixture.

The plants were fertilized every three weeks with a 20-5-20 fertilizer through the irrigation system. The soluble fertilizer stock was acidified with phosphoric acid to reduce the pH of the solution to approximately 6.7.

Shoot elongation was plotted as cumulative growth over time.

Growth Promoter Studies

To further describe the changes in endogenous growth substances occurring in the outdoor, greenhouse, and cold storage treated plants (Table 1) an endogenous growth regulator study was undertaken to analyze individual shoots and roots by means of the mung bean bioassay. The purpose of this study was to monitor the relative concentration of growth promoters and/or inhibitors in the plants over time.

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The mung bean bioassay was chosen because: 1) Lanphear and Meahl (1966) had reported the presence of a growth promoter in Juniperus cuttings using the mung bean bioassay; 2) the bioassay was capable of providing semi-quantitative data about the specific growth promoters of cofactors 1 through 4; 3) cofactor 4 described by Hess is assumed to be related to auxin, a known growth promoter; 4) the materials and methods of this bioassay procedure were available for a long term experiment; and 5) time and labor required for this bioassay was justified by the amount of information obtained.

Sampling Techniques

At each sampling date (Table 1) five plants were selected at random from each treatment. In all treatments except the outdoor samples, the shoot from each plant was cut and placed in individual plastic bags. For outdoor samples, five shoots from each clone six to eight inches in length were cut and placed individually in plastic bags. Roots from all treatments (except outdoors) were collected and stored. The soil was removed from the root system before being placed in plastic bags.

All samples were then quick-frozen and stored at -15°C . The frozen shoots were cut into small pieces and lyophilized. The individual plants were ground in a Wiley Mill to pass through a 20 mesh screen.

The plants for each treatment were kept separate so that statistical analysis could be performed as a single

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plant analysis; thus variability would be attributed to individual plant variability plus bioassay variability.

A 100 mg sample was extracted three times with methanol at 4-6°C. The methanol extractions were combined and concentrated in vacuo at 37°C. The extract, resuspended in 0.2 ml of methanol, was streaked on Whatman No. 1 chromatography paper and developed (descending chromatography) with 4:1 (v/v) isopropanol:water. The chromatograms were developed uni-directionally for approximately 6.5 hours without prior equilibration until the solvent front was approximately 22.5 cm from the origin. The chromatogram was air-dried and divided into 15 equal sections plus a control taken from above the origin. The control was taken at that point to avoid possible differences between the parts of the chromatograms on which the solvent moved, and those which it did not reach. Each chromatogram section was placed in a vial with 10 ml of 5×10^{-6} M Indoleacetic Acid (IAA).

Mung Bean Bioassay

The level of growth promoting substances in the juniper clones was determined by the mung bean bioassay developed by Hess (1962b, 1964) and modified for juniper plants by Lanphear and Meahl (1966). Mung bean seeds (Phaseolus aureus Roxb.) were treated with a solution of one part sodium hypochlorite (clorox) to six parts water for three minutes. The seeds were rinsed in running tap water for 18-24 hours and planted in moist vermiculite in a 23 x 30 x 6

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cm aluminum pan. The seedlings were grown for eight days at 24°C with continuous light (fluorescent, 450 to 600 ft-c).

Four random mung bean seedlings were cut 3 cm below the cotyledons and placed in each 19 x 65 mm shell vial containing 10 ml of the IAA solution plus the chromatogram section (Figure 1). The mung bean plants remained in the controlled environment room for six days for roots to form after which the number of roots per cutting were counted. The average number of roots per cutting per vial was used to determine the presence or absence of promoters and/or inhibitors.

Ratio Derivation

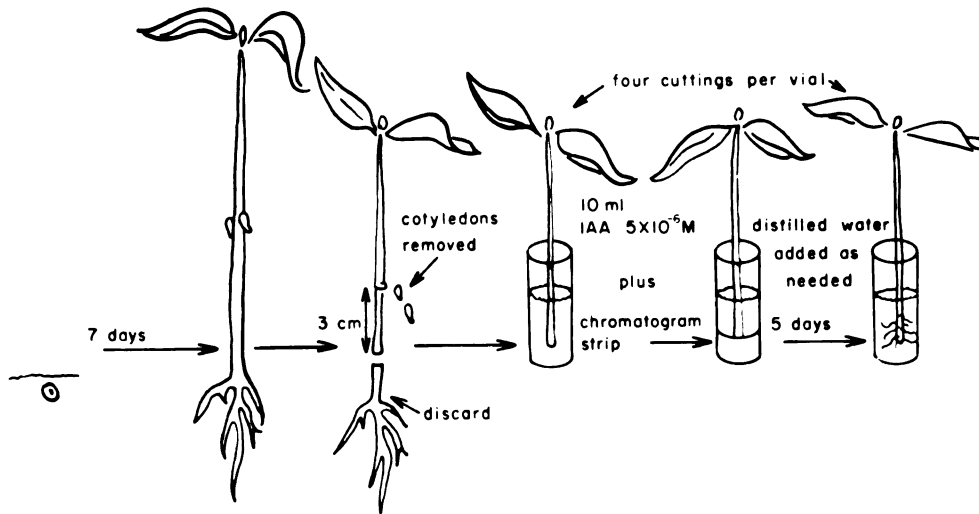
To determine the relative ratio of growth promoter activity a ratio was calculated for each chromatogram strip to account for the variation of the control from one chromatogram to another. The relative ratio was determined by dividing the number of roots per vial by number of roots in the control vial (Figure 1).

Preliminary Characterization of Cofactor 4

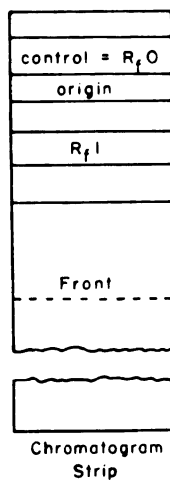
The primary region of activity on the chromatograms for all treatments and clones occurred at R_f 0.80-0.93. This region has been termed cofactor 4 by Hess (1962b). To determine if cofactor 4 is similar in all three clones an extract was prepared and separated by partitioning into

Figure 1.--Diagram of the procedure used for the mung bean bioassay (after Hess, 1962) and the ratio derivation for calculating the relative level of activity for each R_f zone. Ratio calculations based on the number of new roots initiated per mung bean plant.

MUNG BEAN BIOASSAY



RATIO DERIVATION

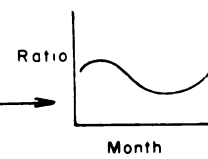


10 roots / mung bean plant

5 roots / mung bean plant

Ratios Calculated

$$R_f 1 = \frac{5}{10} = 500$$



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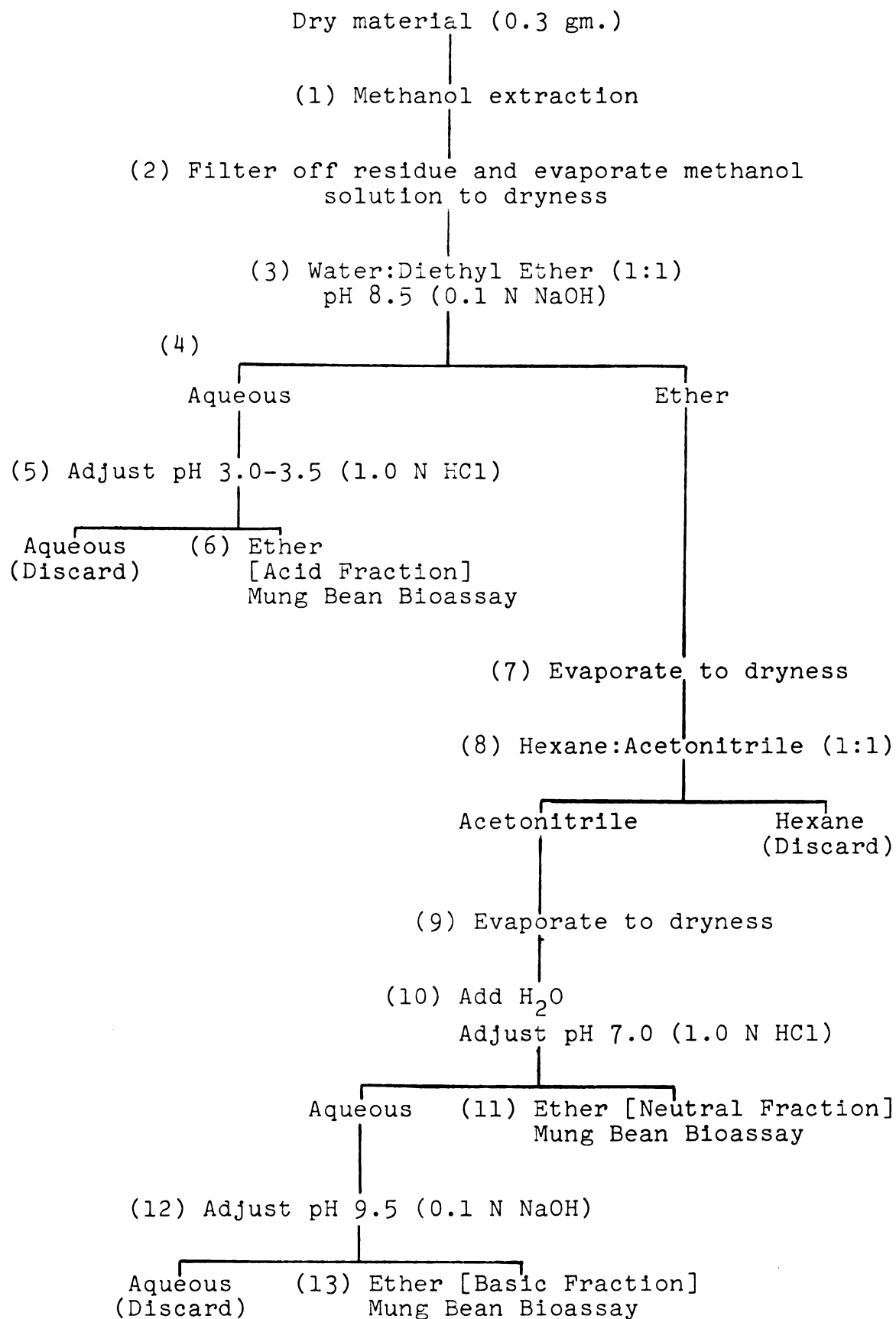
acidic, basic and neutral ether fractions (Figure 2). These fractions were then tested using the mung bean bioassay.

Figure 2 diagrams the procedure followed in the partitioning which is a modified scheme of several researchers suggested by Hopping (personal communication).

The filtered methanolic extract (1) was evaporated under vacuum at 35°C (2). The residue was re-suspended in water and purified diethyl ether and the pH adjusted to 8.5 with 0.1 N NaOH (3). The aqueous residue was extracted three times with ethyl ether. The ether phase (4) was stored for partitioning into basic and neutral fractions. The aqueous fraction (4) was acidified with 1.0 N HCl to pH 3.0-3.5. The acidic aqueous fraction was then extracted with ethyl ether three times. The acidic ether fraction (6) was concentrated and streaked onto Whatman No. 1 chromatography paper.

The ether residue fraction from step four was evaporated to dryness under vacuum (7) and the residue partitioned with 1:1 (v/v) of acetonitrile:hexane (8), using three changes of hexane. The acetonitrile was evaporated to dryness (9) and the residue taken up in ether and water. The pH was adjusted to 7.0 (10) and partitioned into the neutral ether fraction (11). The pH of the aqueous residue was adjusted to 10 with 0.1 N NaOH (12) and partitioned into the basic fraction (13). The neutral and basic ether

Figure 2.--Flow chart of the partitioning procedure for separating the acid, base, and neutral promoter fractions from a crude methanol extract for three Juniperus L. clones. The ether fractions were subjected to paper chromatography in an isopropanol:water (4:1) solvent system. The R_f zones were tested for activity by the mung bean bioassay.



fractions were concentrated and streaked onto Whatman No. 1 chromatography paper.

Chromatography development and mung bean bioassay procedures were performed as previously described.

RESULTS

Grafting Studies

Understock and Scion Environmental Study - 1969-1970

Results for the 1969-1970 graft study are summarized in Table 3. Grafting survival was recorded four months after grafting. Plants receiving nine weeks of cold had 75-100 percent survival while four and twelve weeks of cold resulted in 0-78 percent and 0-44 percent survival, respectively. The order of graft success among the clones was Andorra > Hetzi > Pfitzer.

Graft survival differences are also shown for understock and scion treatments regardless of weeks of cold storage. When greenhouse grown plants were used as scion or stock material the percentage survival was greater than in plants stored at 2°C.

Scion Storage and Environmental Study - 1970-1971

Results for the 1970-1971 graft study are summarized in Table 4. The greatest success for grafting was either four or nine weeks of cold storage of understock. No consistent differences in percent graft survival were noted

TABLE 3.--Survival of self-grafted Juniperus clones following temperature treatments of scion and stock. Grafts made November 29, 1969, January 5, 1970, January 30, 1970. Survival data recorded in May 1970.

Weeks of Temperature Treatment Prior to Grafting ¹	Juniper Clone	Scion Treatment Stock Treatment			
		$\frac{2^{\circ}\text{C}}{2^{\circ}\text{C}}$	$\frac{18^{\circ}\text{C}}{18^{\circ}\text{C}}$	$\frac{18^{\circ}\text{C}}{2^{\circ}\text{C}}$	$\frac{2^{\circ}\text{C}}{18^{\circ}\text{C}}$
		Percent			
4	Andorra	22	78	78	67
	Hetzi	11	45	45	11
	Pfitzer	11	11	11	0
9	Andorra	100	100	100	100
	Hetzi	84	89	100	89
	Pfitzer	75	89	89	78
12	Andorra	11	33	44	22
	Hetzi	11	11	11	11
	Pfitzer	0	11	11	0

¹Understocks placed in greenhouse for three weeks prior to actual grafting.

TABLE 4.--Survival of self-grafted Juniperus L. clones as affected by length of storage of precut scion wood. Grafts were made on November 11, 1970, December 17, 1970 or January 8, 1971. Survival data were recorded in early April, 1971.

Weeks of Cold Treat- ment of Understock ¹	Clone	Scion Treatment				
		Green- house ² 18°C 18°C	Cold Storage (2°C)			
			<u>Whole Plant</u>	<u>Scion Wood</u>		
				Sept.24	Oct.1	Oct.23
Percent						
4	Andorra	40	40	30	70	
	Hetzi	50	10	40	40	
	Pfitzer	30	20	20	50	
9	Andorra	0	30	20	10	60
	Hetzi	20	60	40	30	70
	Pfitzer	50	20	0	10	60
12	Andorra	0	30	0	20	50
	Hetzi	10	20	0	10	10
	Pfitzer	0	40	0	40	50

¹Understocks were placed in greenhouse (18°C) for three weeks prior to actual grafting.

²Scion from whole plant and understock were stored in greenhouse until grafting without exposure to cold treatment.

between the two treatments of whole plants used for scions or among clones. More important than cold treatment in this study was the length of time the scion wood was stored at 2°C prior to grafting. The shorter the interval between scion collection and grafting the higher the percent survival.

Again, least survival occurred among the plants subjected to twelve weeks of cold storage. Consistent with the four and nine weeks of cold graft-take after twelve weeks of cold storage was highest when scion wood was stored the least amount of time.

Plant Extract Study

The amount of callus on the scion and stock for the extract treatment at selected intervals following grafting on March 3, 1969 is summarized in Table 5.

Callus formation increased with time. Twenty days following grafting the amount of callus material formed was about 25 percent for all clones and treatments except for the Hetzi self-graft with Andorra extract which was approximately 35 percent. Neither treatment nor variety exhibited differences in callus formation for the scion.

Forty days after grafting, extract treatment differences began to appear in the degree of callus present on both stock and scion for all clones. Stocks were 30-85 percent covered with callus while 5-20 percent of the scion surface was covered. Generally the water treated graft had

TABLE 5.--Degree of callusing at the graft union at selected time intervals following grafting. At time of grafting the cut surfaces of the scion and understock were coated with a plant extract made from juniper clone. Extract was prepared by grinding shoots in deionized water to form a paste-like slurry. Plants were grafted on March 3, 1969.

Self-Grafted Clone	Plant Extract	DAYS AFTER GRAFTING									
		20 Days		40 Days		60 Days		Stock Callus	Scion Callus	Stock Callus	Scion Callus
		Stock ¹ Callus	Scion ¹ Callus	Stock Callus	Scion Callus	Stock Callus	Scion Callus				
Andorra	H ₂ O	1.2	0	4.0	1.5	3.7	1.0				
	Hetzi	1.3	0	3.2	1.4	3.8	0.7				
	Pfitzer	1.5	0.3	2.7	0.7	3.3	1.0				
Hetzi	H ₂ O	1.3	0.2	3.3	0.4	4.3	0.7				
	Andorra	2.1	0.4	3.1	0.3	3.8	0.3				
	Pfitzer	1.1	0.1	3.0	0.4	3.2	0.3				
Pfitzer	H ₂ O	1.4	0.2	3.3	0.7	2.6	1.7				
	Andorra	1.1	0.1	2.6	0.4	3.5	1.0				
	Hetzi	1.9	0.2	2.5	0.1	4.2	1.2				

¹Rating for callus formation: 0 = no callus, 0%; 1 = some callus, 15%; 2 = average callus, 30%; 3 = above average callus, 60%; 4 = heavy callus, 85%; 5 = complete callus cover, 100%.

the greatest degree of callus on both the stock and scion. Within the Andorra clone a decline in callus formation occurred in the order of water > Hetzi extract > Pfitzer extract. Grafted Hetzi plants showed little difference in stock or scion callus among all treatments. Pfitzer grafts showed a response to plant extract treatments. Treatments for the stock and scion callus formation decreased in the order of water > Andorra extract > Hetzi extract.

Sixty days after grafting, stock callus material increased in most treatments. Only two treatments showed less stock callus material than was present at forty days--Andorra water and Pfitzer water. Callus formation on the scion increased slightly over all treatments, except for Andorra grafts. These decreases may be attributed to sampling differences or to subjectivity of the rater. In Andorra noticeable differences were not evident between water and Hetzi extract treatments. About 80 percent of the stock was covered with callus tissue. The Pfitzer extract showed least callus formation. The water control callus on the Hetzi plants covered about 90 percent of the stock. More callus was produced from the Andorra extract than from the Pfitzer extract, although both were less than the water-treated graft. Scion callus showed little differences. The amount of stock callus formed in Pfitzer self-grafts among the three extract treatments was in the order Hetzi extract > Andorra extract > Pfitzer water.

After 60 days, the graft union had not sufficiently united for graft survival data to be taken.

Table 6 is a summary of plant extract graft success for 1969-1970 and 1970-1971. This study was similar to the first year's study (1969) except the understocks received four, nine or twelve weeks of cold storage prior to grafting. The extract material in 1969-1970 was prepared from plants having nine weeks of cold followed by three weeks in the greenhouse. Extract material in 1970-1971 for the grafts subjected to four and twelve weeks of cold storage was prepared from greenhouse plants under long days. Plant extract treatments did not improve graft-take. Graft survival for the nine-week graft period was low because the extract material had not been strained sufficiently to remove the plant residue from the extract. In the four- and nine-week graft periods the extracts were strained to remove all plant residue. No residue was found between the scion and stock.

The concentrations for the growth regulator applications to the graft union are given in Table 6. The growth regulator solution was inhibitory to graft success. The lowest concentration of the growth regulator solution was least inhibitory which may have resulted from time of grafting (nine weeks of cold storage) or to the actual concentration of growth regulators. Grafted plants treated with growth regulator solutions showed a severe yellow

TABLE 6

Weeks of
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TABLE 6.--Survival of leaf extract treated self-grafted Juniperus clones. At time of grafting the cut surfaces of the scion and understock were coated with a plant extract made from juniper clone or a growth hormone solution. Extract prepared by grinding shoots in deionized water to form a paste-like slurry.

Weeks of Cold ¹ Prior to Grafting	Juniper Clone	Plant Extract Treatments				Growth Hormone Treatment ² Aux.+Gibb. Kin.+
		Control (H ₂ O)	Andorra Slurry	Hetzi Slurry	Pfitzer Slurry	
Percent Graft Survival						
4	Andorra	60	--	20	10	0
	Hetzi	70	60	--	60	10
	Pfitzer	90	50	50	--	10
9	Andorra	100	--	67	67	67
	Hetzi	84	67	--	42	42
	Pfitzer	75	8	8	--	42
12	Andorra	10	--	20	0	0
	Hetzi	20	0	--	10	0
	Pfitzer	50	0	0	--	0

¹Grafting after 4 and 12 weeks of cold performed in 1970-1971; grafting after 9 weeks of cold performed in 1969-1970.

²Concentrations for 4 weeks of cold: auxin 10 mg/l, gibberellin 20 mg/l, kinetin 10 mg/l; 9 weeks of cold: auxin 1 mg/l, gibberellin 2 mg/l, kinetin 1 mg/l; 12 weeks of cold: auxin 10 mg/l, gibberellin 10 mg/l, kinetin 2 mg/l.

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Root Activity Study

The degree of root activity may be qualitatively determined by observing the color of the root tip. Root tips that are brown are in a non-active stage of growth (i.e. dormant); white root tips are in an active stage of growth.

Figure 3 illustrates root activity in greenhouse plants from October through April. Andorra root activity level was higher than in Pfitzer and Hetzi. For all clones the root activity was more or less constant from December through April.

Root activity for Andorra was higher throughout the experiment than for Hetzi and Pfitzer which were almost equal to each other in their relative degree of dormancy (Figure 4). Increasing the cold exposure from four to nine weeks decreased root activity.

Plants moved from cold storage to the greenhouse increased in root activity within one month (Figure 4). The decline in root activity the second month in the greenhouse was greater following four weeks of cold than nine weeks of cold.

Shoot Growth Study

Contrary to earlier reports (Waxman, 1957) that junipers continue to grow under all day lengths, Figures 5-7

Figure 3.--Changes in root activity of Juniperus clones grown in the greenhouse (18°C) throughout the winter. Clones studied were: Andorra, (A); Hetzi, (B); and Pfitzer, (C). Root activity was determined by a rating scale for approximate percent of white root tips observed on a two-year-old plant root system. Rating scale was: 0 = no white root tips, 0%; 1 = very few, 15%; 2 = few, 30%; 3 = few to moderate, 45%; 4 = moderate, 65%; 5 = moderate to heavy, 85% and 6 = heavy, 100%.

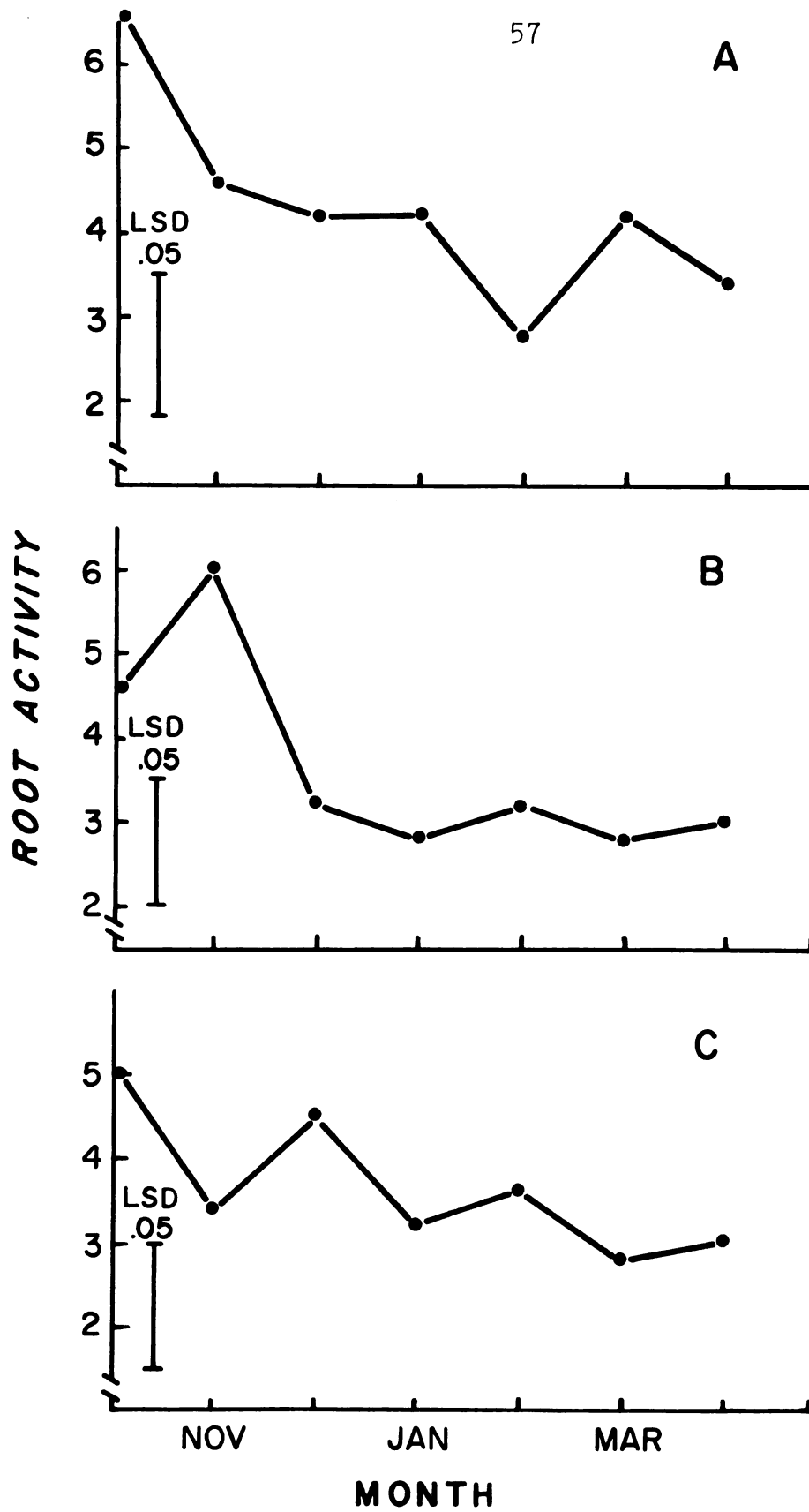
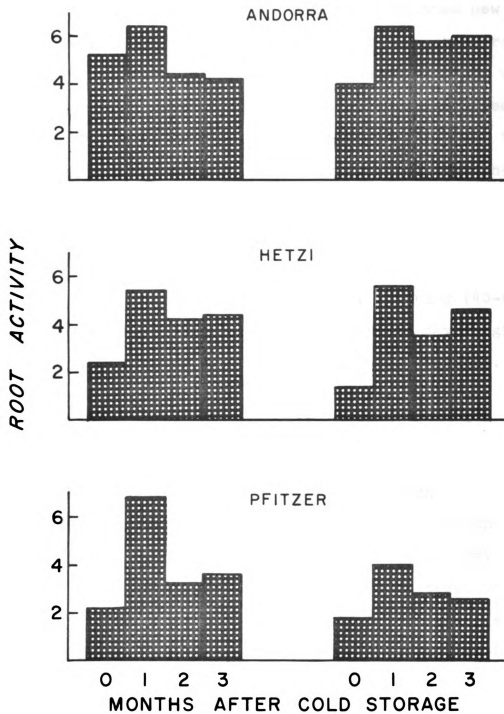


Figure 4.--Changes in root activity of Juniperus L. clones following four and nine weeks of cold. Root activity determined by a rating scale for approximate percent of white root tips observed on a two-year-old plant root system. Rating scale was: 0 = no white root tips, 0%; 1 = very few, 15%; 2 = few, 30%; 3 = few to moderate, 45%; 4 = moderate, 65%; 5 = moderate to heavy, 85% and 6 = heavy, 100%.

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show that for the clones studied short days (9 hours) retarded growth in the greenhouse temperature of 18°C. Andorra and Hetzi (short-day treatments) initiated new growth starting in February when the greenhouse temperature during the day often increased several degrees due to the change in light intensity during this time of year (see Table 2). Pfitzer (short-day treatment) on the other hand, did not begin new growth until nearly a month later when the greenhouse day temperatures increased.

In general, the longer the day length the greater the amount of growth (Figures 5-7).

In Figure 5, Andorra under long-day lighting (40-60 ft-c) grew at a constant rate. Under natural day length Andorra appeared to be growing in a sigmoidal fashion. The rate of growth under natural day length was less from September 23 through January 1 but increased from January through March. A study of longer duration would be required to characterize the growth pattern beyond March.

Under natural and short day lengths Hetzi growth followed a sigmoidal curve (Figure 6). For the long-day treatment the growth rate was constant from October through February. In February the rate changed to a higher level resulting in a double sigmoid curve for the long-day treatment. The higher level may be due to increased temperatures.

Figure 5.--Biweekly shoot elongation of Juniperus horizontalis 'Andorra' under various day lengths. Each point is the average of ten replicates consisting of one shoot per plant. Bars indicate the standard deviation of the mean of the point for accumulative growth.

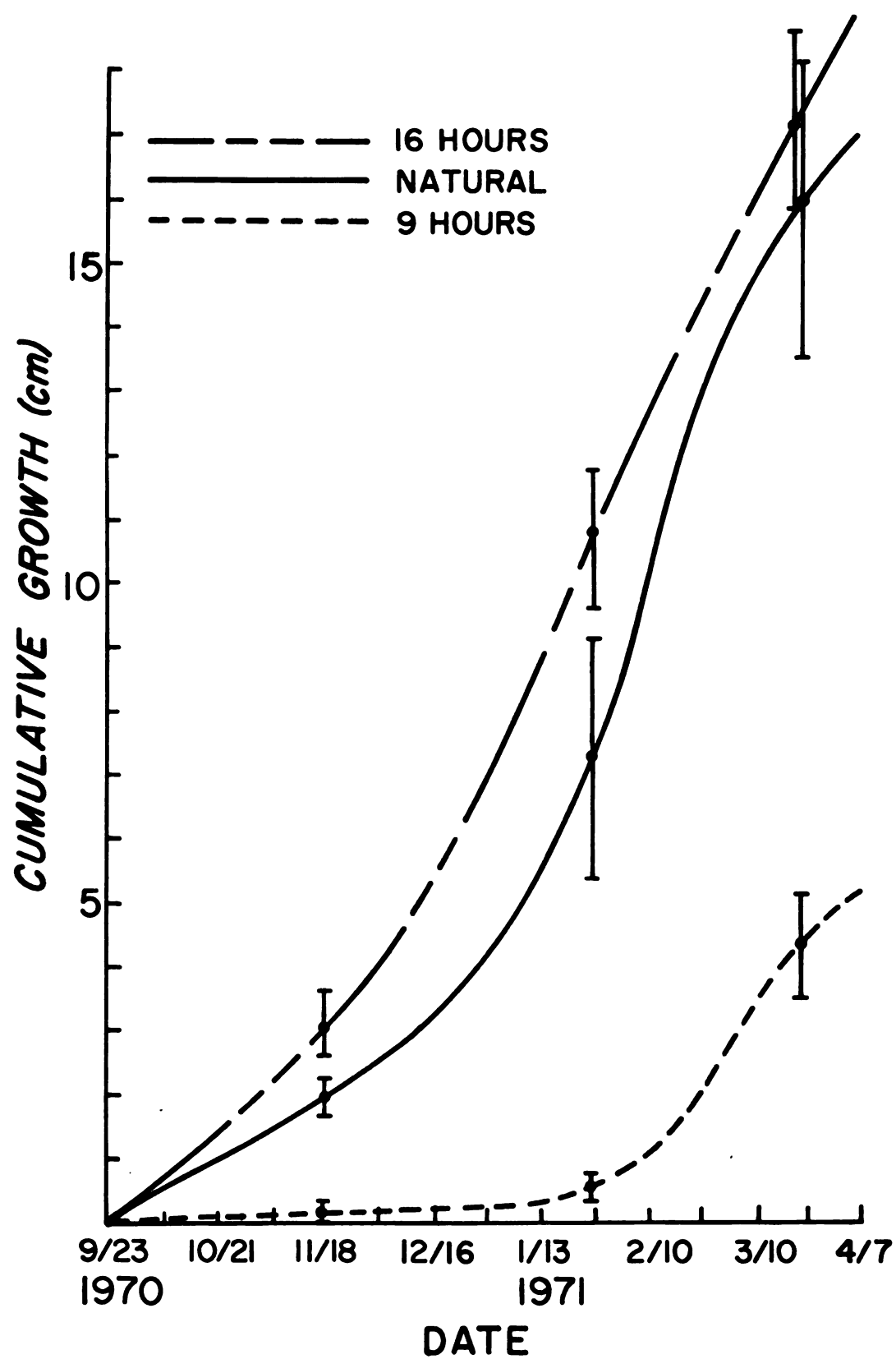


Figure 6.--Biweekly shoot elongation of Juniperus chinensis 'Hetzi' under various day lengths. Each point is the average of ten plant replicates consisting of one shoot per plant. Bars indicate the standard deviation of the mean of the point for accumulative growth.

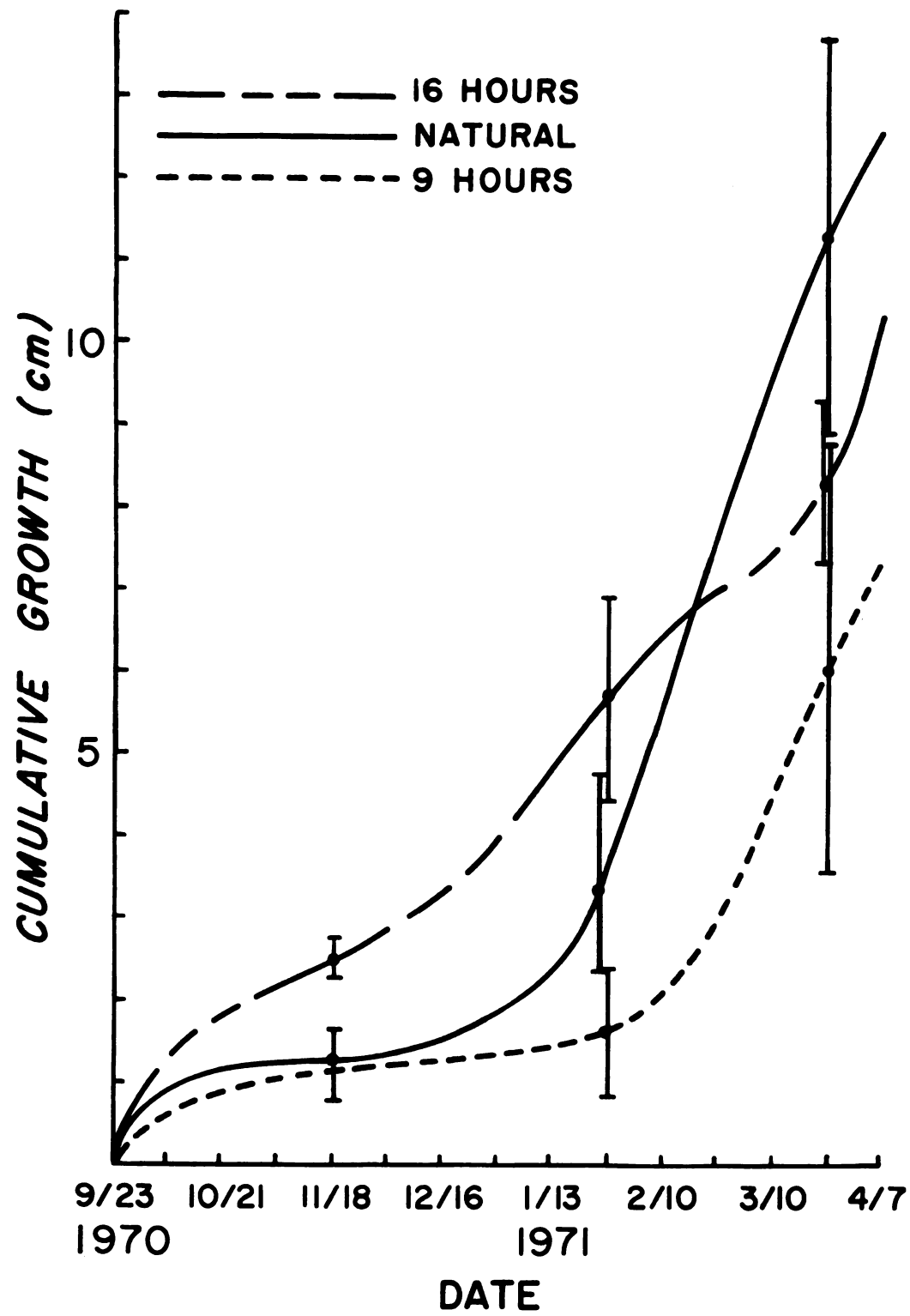
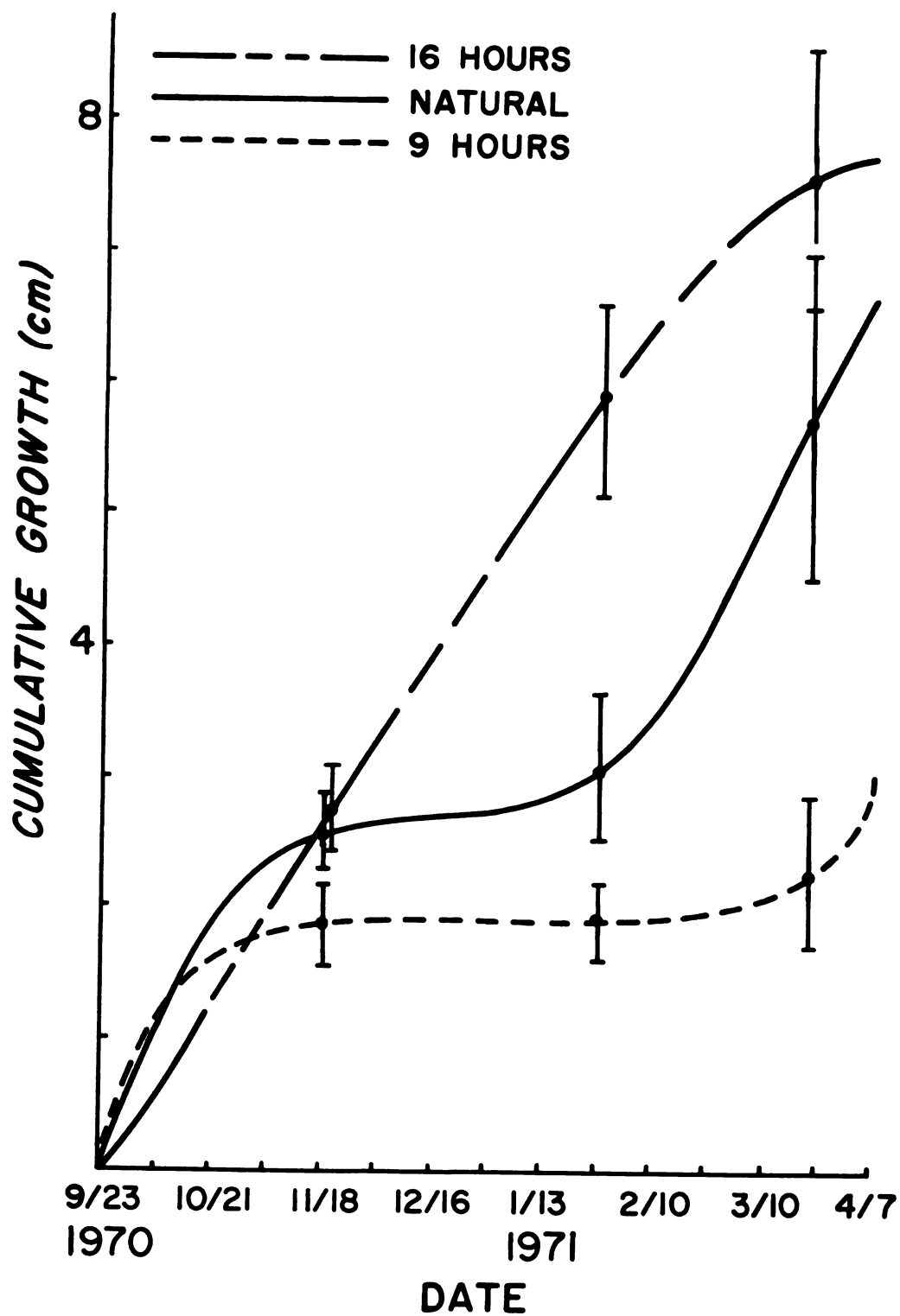


Figure 7.--Biweekly shoot elongation of Juniperus chinensis 'Pfitzer' under various day lengths. Each point is the average of ten plant replicates consisting of one shoot per plant. Bars indicate the standard deviation of the mean of the point for accumulative growth.



Natural day length for Hetzi (Figure 6) showed that beginning in October shoot elongation ceased and began again in December.

Short days (Figure 6) resulted in a decline in shoot elongation to a low rate within two weeks. Growth did not begin again until the middle of February when the greenhouse day temperature began to increase.

Pfitzer plants (Figure 7) also responded to various day lengths. Long days showed a constant rate of growth from September through March. The last two measurements indicated that the growth rate had declined. This sudden decline in growth may indicate that Pfitzer was becoming dormant at this time. Natural day length and short-day growth patterns were sigmoidal. Growth under natural day length continued until the middle of November and then ceased. Growth resumed toward the end of January as day length increased. The slope of the curve from January through April appeared to be nearly parallel to long days.

Short days showed growth for Pfitzer continuing until November 1 when growth ceased. Growth began again around the first of March.

Growth Rate as Affected by Length of Cold Storage

Figures 8-10 represent the growth patterns of Andorra, Hetzi, and Pfitzer, respectively, which were stored for

Figure 8.--Biweekly shoot elongation of Juniperus horizontalis 'Andorra' following zero, four, nine, and twelve weeks of cold storage (2°C). Plants were transferred to a greenhouse (18°C) having natural day lengths. Each point is the average of ten plant replicates consisting of one shoot per plant. Bars indicate the standard deviation of the mean of the point for accumulative growth.

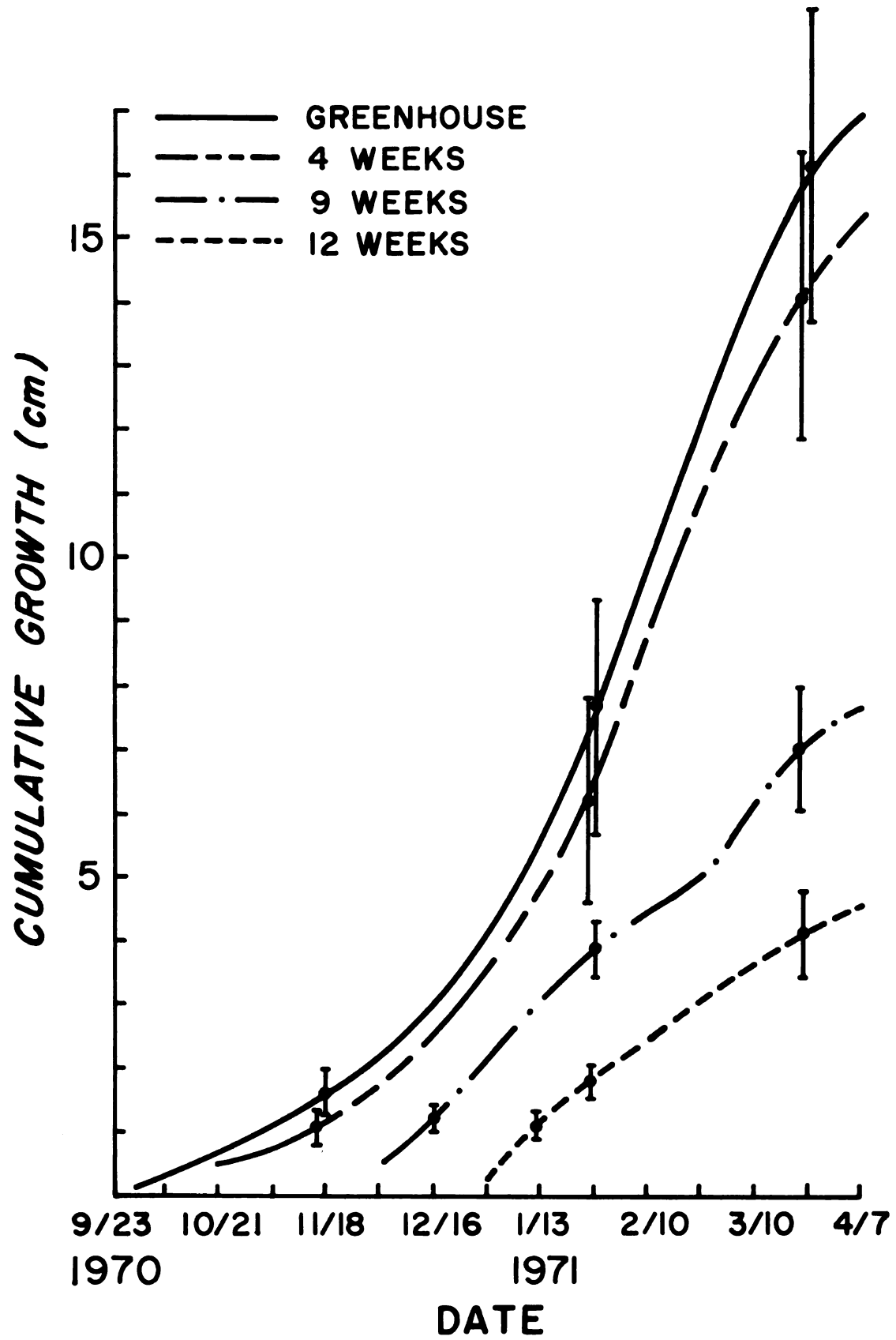


Figure 9.--Biweekly shoot elongation of Juniperus chinensis 'Hetzi' following zero, four, nine and twelve weeks of cold storage (2°C). Plants were transferred to a greenhouse (18°C) having natural daylengths. Each point is the average of ten plant replicates consisting of one shoot per plant. Bars indicate the standard deviation of the mean of the point for accumulative growth.

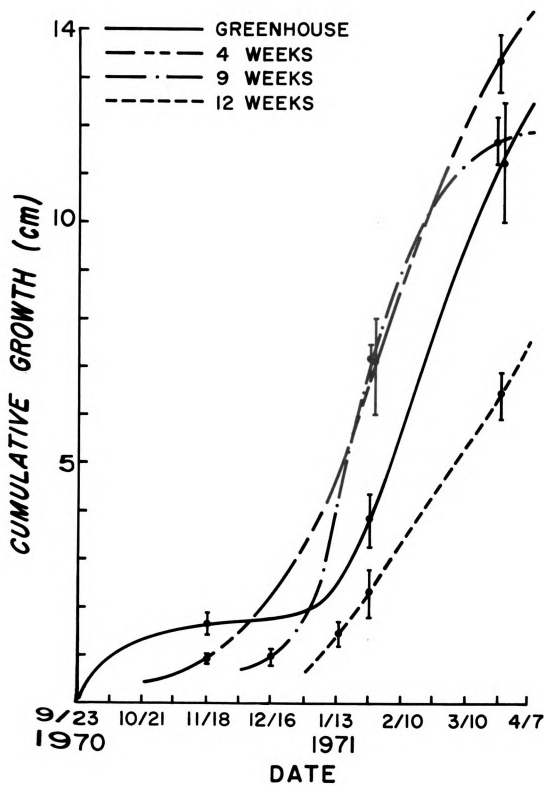
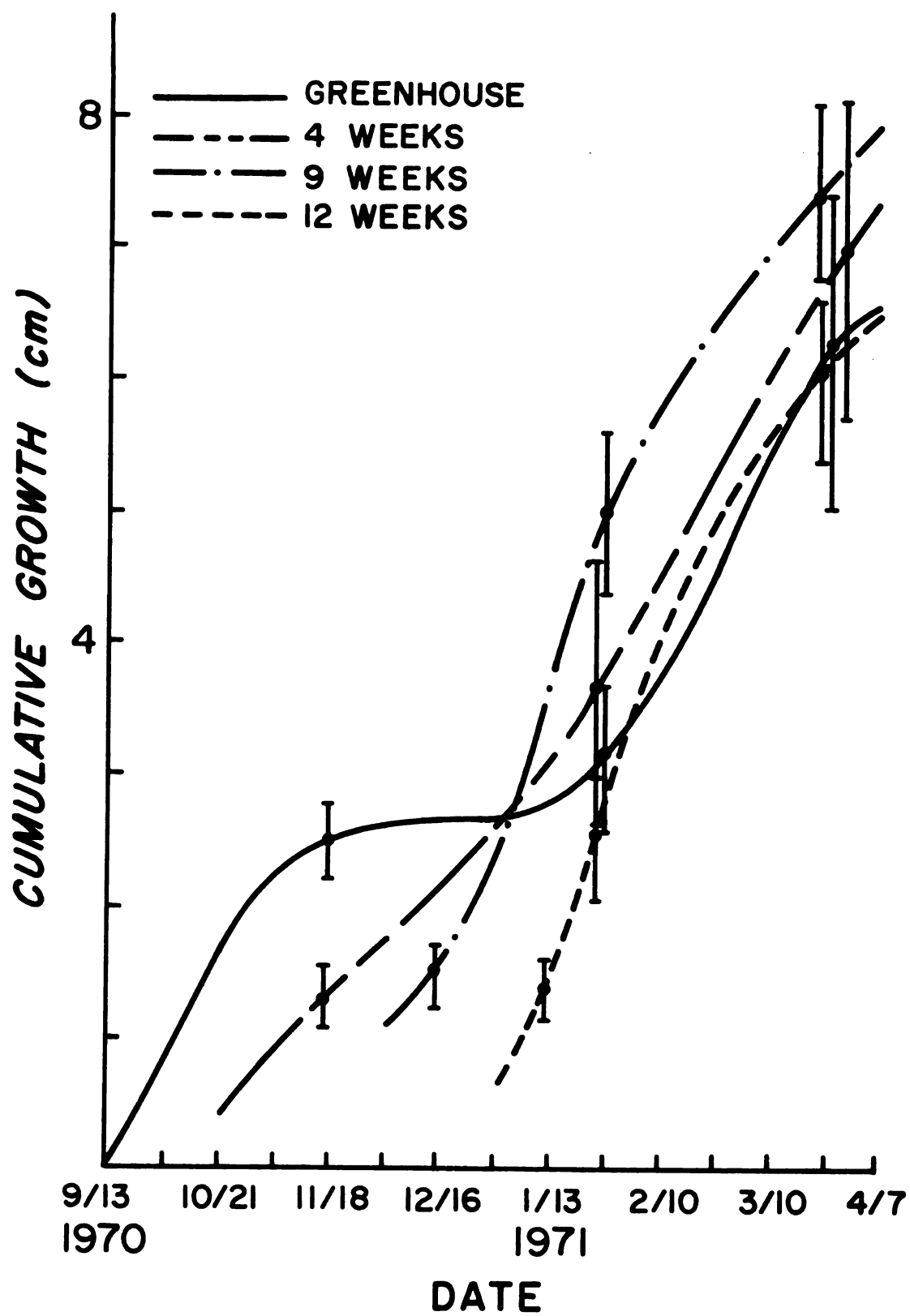


Figure 10.--Biweekly shoot elongation of Juniperus chinensis 'Pfitzer' following zero, four, nine and twelve weeks of cold storage (2°C). Plants were transferred to a greenhouse (18°C) having natural day lengths. Each point is the average of ten plant replicates consisting of one shoot per plant. Bars indicate the standard deviation of the mean of the point for accumulative growth.





zero, four, nine or twelve weeks in the cold (2°C) prior to being moved into a warm greenhouse (18°C). All plants were placed under natural day length in the greenhouse.

Andorra plants after four weeks of cold storage (Figure 8) grew at nearly the same rate as plants growing in the greenhouse. A sigmoid growth curve occurred for both treatments. Following nine and twelve weeks of cold storage Andorra grew at a reduced rate as noted by the change in slope of the curves--twelve weeks being less than nine weeks.

Hetzi (Figure 9) differed in its shoot elongation pattern from Andorra. Greenhouse plants ceased growth from November to January. Starting in January the growth rate increased. Plants which received four or nine weeks of cold also displayed a smaller lag phase in shoot elongation to the extent that shoot elongation in these plants surpassed the non-chilled treatment plants. Hetzi growth after four weeks of cold storage did not begin as fast as after nine weeks cold storage. After twelve weeks of cold storage, Hetzi began to grow almost immediately after being moved into the greenhouse. Apparently, Hetzi either required a dormant period or the cycle of growth was such that by giving a cold storage period the growth cycle was altered to provide for new continued growth.

Pfitzer (Figure 10) grew in yet another pattern from either Andorra or Hetzi. The lag phase in growth for greenhouse treatment occurred much later in the year than for

Andorra or Hetzi. Pfitzer plants receiving varying periods of cold storage resulted in growth patterns having similar slopes. The overall slope of the line increased with continued cold storage. By February all treatments were displaying similar rates of growth although total cumulative growth varied.

Growth Promoters

Figures 11-13 are representative of the chromatogram histograms from the mung bean bioassay for the three clones. In addition to showing regions of growth promotion, the histograms also demonstrate the differences in relative concentrations between months as illustrated by January and August samples.

Changes in the primary growth promoter region (R_f 0.80-0.93) are expressed as a ratio to account for day to day variation of changes in the control (Figures 14-16). The average number of roots per mung bean plant at R_f 0 (control) was 14 ± 4 . The ratios represent a mean of six replications.

Figure 14 illustrates the changes in growth promotion of R_f 0.80-0.93 (cofactor 4) for Andorra shoots in the greenhouse from October through May. Figure 14B illustrates the changes occurring for plants growing outdoors. In both greenhouse and outdoor material, there appears to be definite patterns of activity. When the outdoor cycle is examined there are four peaks in the promoter level during the year. Greenhouse plants also display peaks of activity. In Figure

Figure 11.--Activity from the mung bean bioassay test of chromatograms of methanol extracts from the shoots of Juniperus horizontalis 'Andorra'. Shoots collected from an outdoor plant in January (A); and August (B). Each chromatogram is equivalent to 0.1 gram dry weight. Control (Con.) values are the average number of roots per twelve mung bean plants.

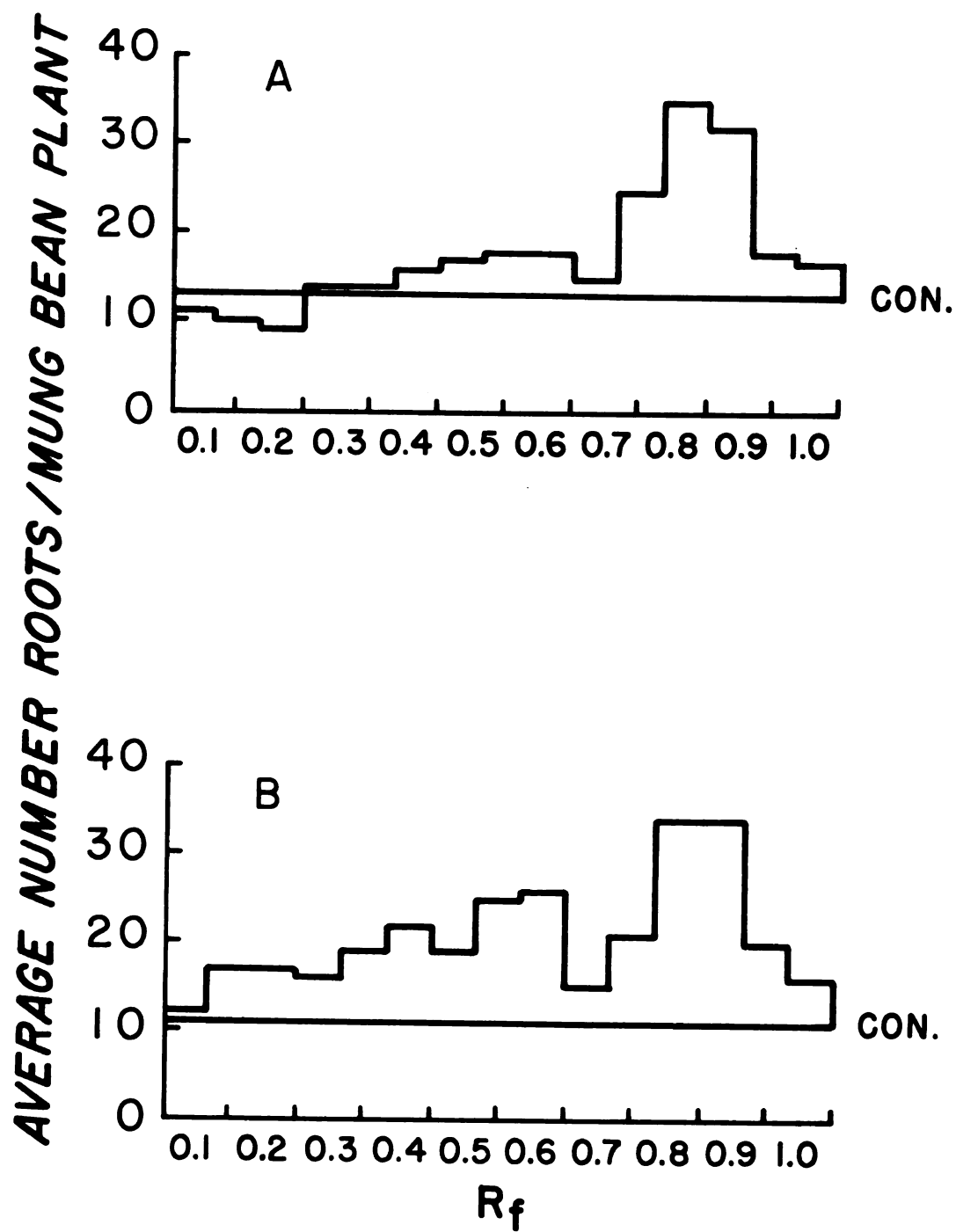


Figure 12.--Activity from the mung bean bioassay test of chromatograms of methanol extracts from the shoots of Juniperus chinensis 'Hetzi'. Shoots collected from an outdoor plant in January (A); and August (B). Each chromatogram is equivalent to 0.1 gram dry weight. Control (Con.) values are the average number of roots per twelve mung bean plants.

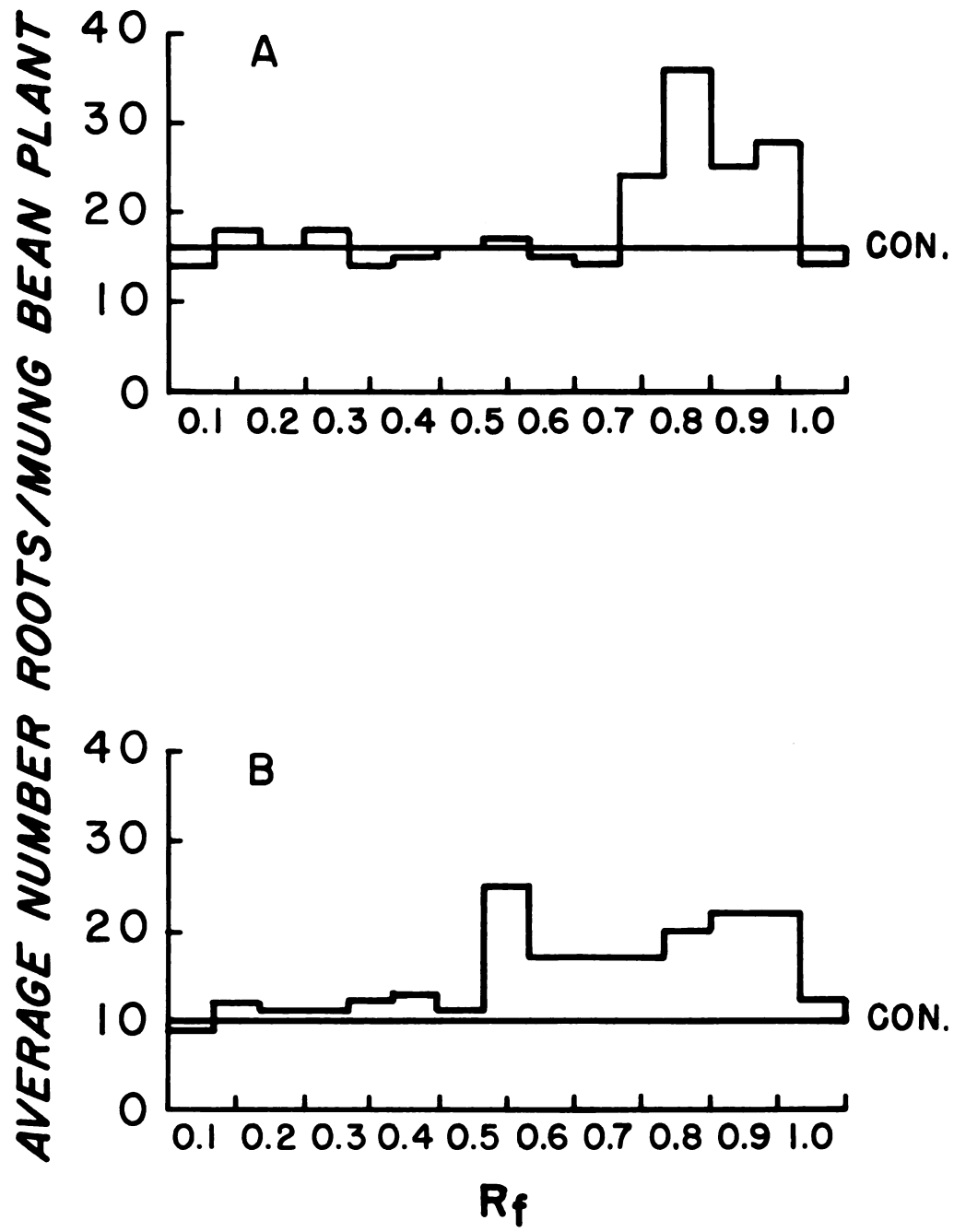


Figure 13.--Activity from the mung bean bioassay test of chromatograms of methanol extracts from the shoots of Juniperus chinensis 'Pfitzer'. Shoots collected from an outdoor plant in January (A); and August (B). Each chromatogram is equivalent to 0.1 gram dry weight. Control (Con.) values are the average number of roots per twelve mung bean plants.

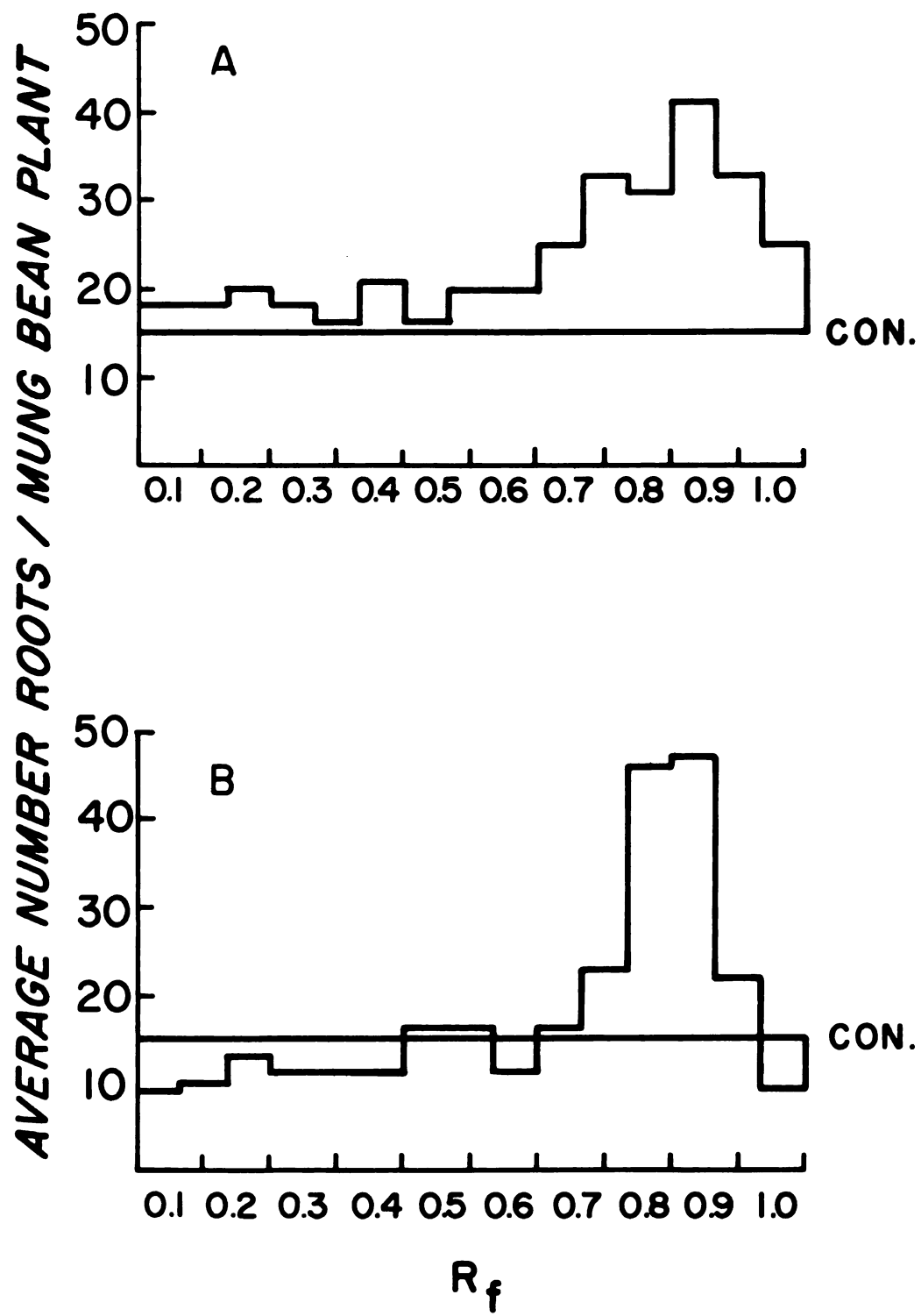


Figure 14.--Cyclic patterns of relative level of co-factor 4 (R_f 0.80-0.93) expressed as a ratio from shoots of greenhouse (A) and outdoor (B) plants of Juniperus horizontalis 'Andorra'. In (C) curves of (A) and (B) are superimposed to illustrate the theoretical shift of the greenhouse cycle to closely match the cycle of the outdoor plants at another time of year.

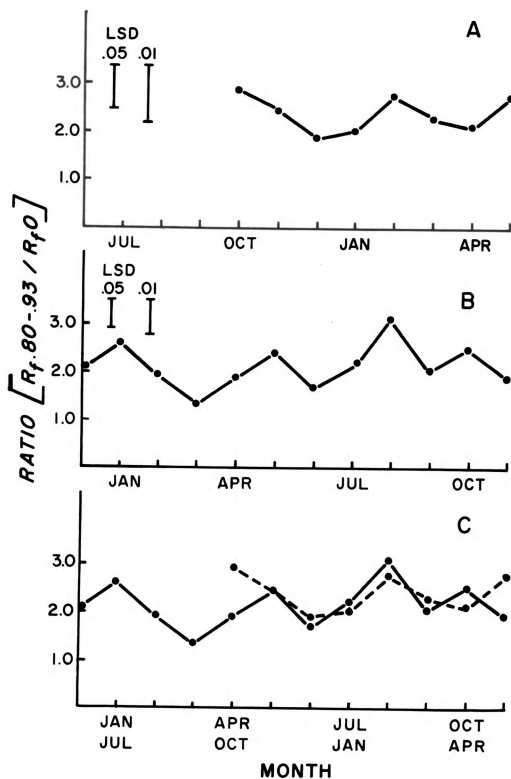


Figure 15.--Cyclic patterns of relative level of co-factor 4 (R_f 0.80-0.93) expressed as a ratio from shoots of greenhouse (A) and outdoor (B) plants of Juniperus chinensis 'Hetzi'. In (C) curves of (A) and (B) are superimposed to illustrate the theoretical shift of the greenhouse cycle to closely match the cycle of the outdoor plants at another time of year.

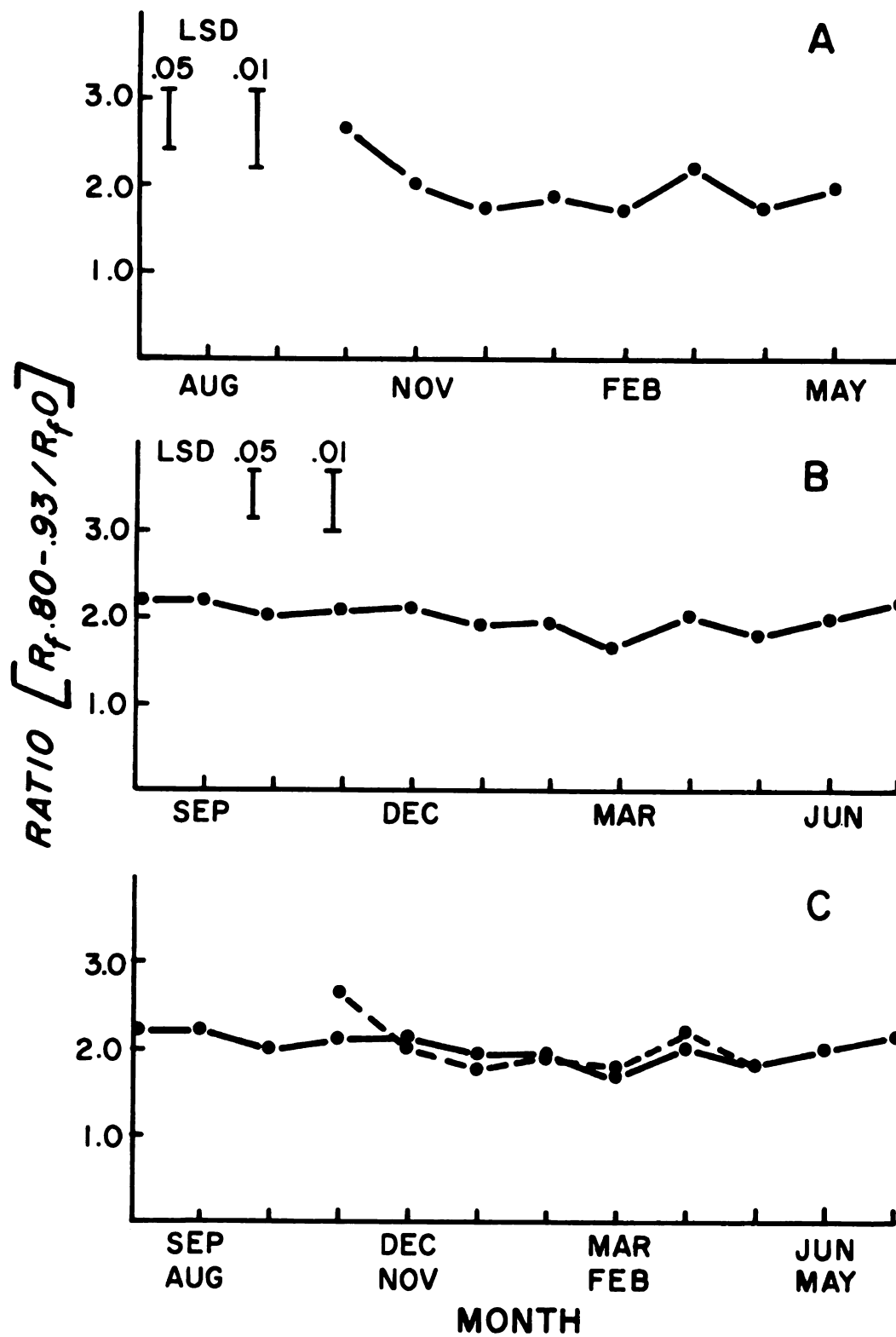
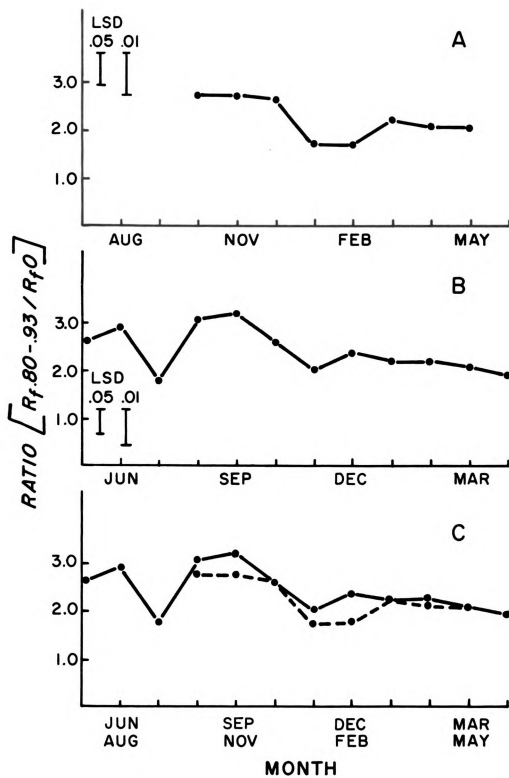




Figure 16.--Cyclic patterns of relative level of cofactor 4 (R_f 0.80-0.93) expressed as a ratio from shoots of greenhouse (A) and outdoor (B) plants of Juniperus chinensis 'Pfitzer'. In (C) curves of (A) and (B) are superimposed to illustrate the theoretical shift of the greenhouse cycle to closely match the cycle of the outdoor plants at another time of year.



14C the promoter pattern for 14A (greenhouse) can be superimposed on the promoter pattern of 14B (outdoor) when the greenhouse curve is shifted by six months from the outdoor curve.

To further illustrate the consistency in this trend it is noted that the same superimposition is possible for Hetzi (Figure 15A-C) and Pfitzer (Figure 16A-C). However, because of the genetic differences in clones the time shift in promoter cycle was not the same; Hetzi was shifted only one month, while Pfitzer was shifted two months. The differences in ratios between months for Hetzi greenhouse and outdoor studies were not significantly different. In addition, no peaks were noted for Hetzi in contrast to the peaks observed for Andorra and Pfitzer. In comparing the three clones for levels of growth promoter, Pfitzer was much higher in both the four and nine week treatments. The lowest activity was in another region (R_f 0.26-0.40) for Andorra. Andorra shoots likewise showed shifts in regulator patterns (Figure 17). The peaks in the region R_f 0.26-0.40 for Pfitzer (Figure 18) were not as pronounced for greenhouse and outdoor studies for R_f 0.26-0.40 as it was for Andorra; however, the time shift of superimposition for both clones was six months.

Changes also occurred for all clones when the four and nine week studies were considered (Figure 19). After one month in the greenhouse following four weeks of cold storage,

Figure 17.--Cyclic patterns of relative level of growth promoter at R_f 0.26-0.40 expressed as a ratio from shoots of greenhouse (A) and outdoor (B) plants of Juniperus horizontalis 'Andorra'. In (C) curves of (A) and (B) are superimposed to illustrate the theoretical shift of the greenhouse cycle to closely match the cycle of the outdoor plants at another time of year.

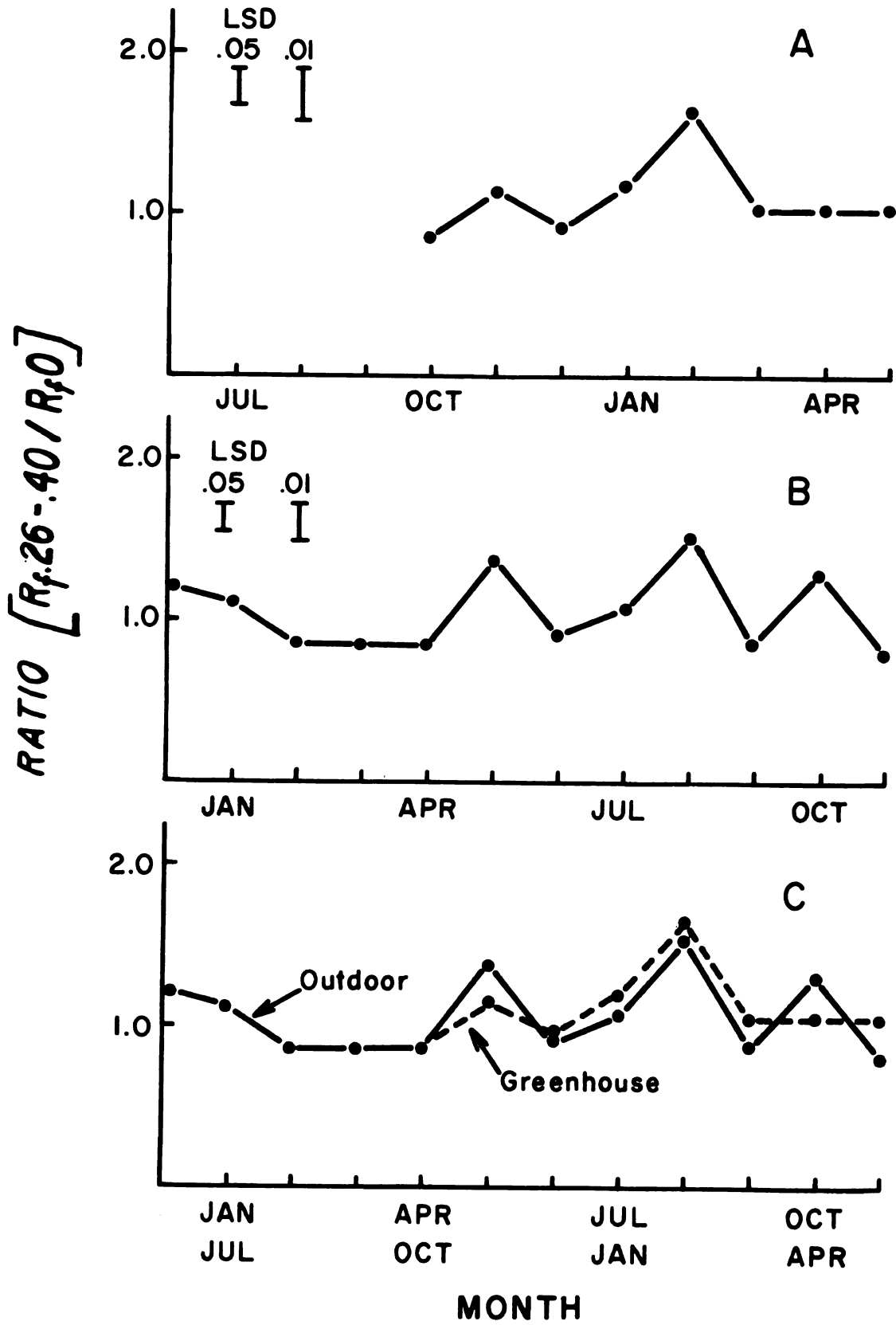
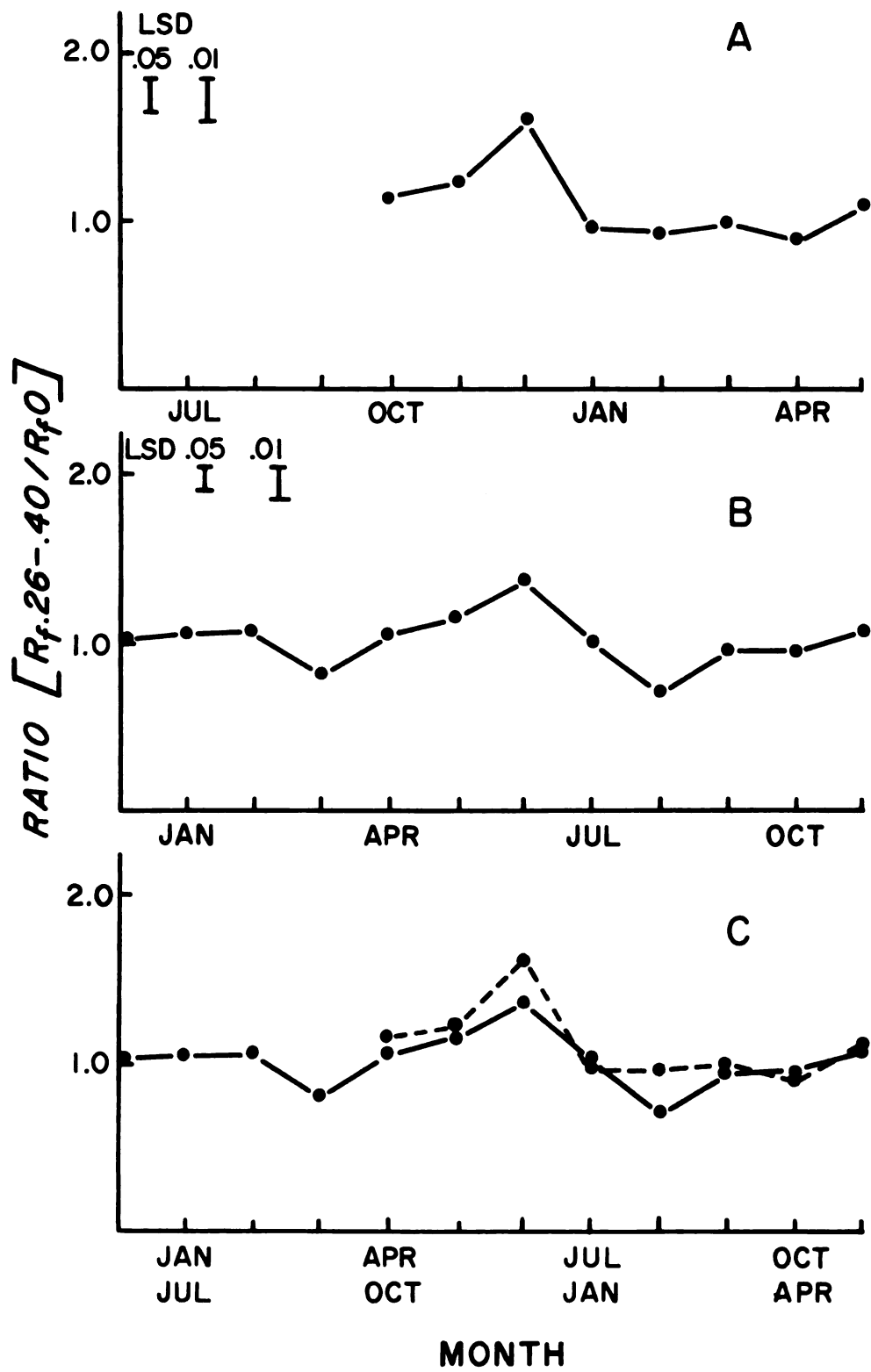
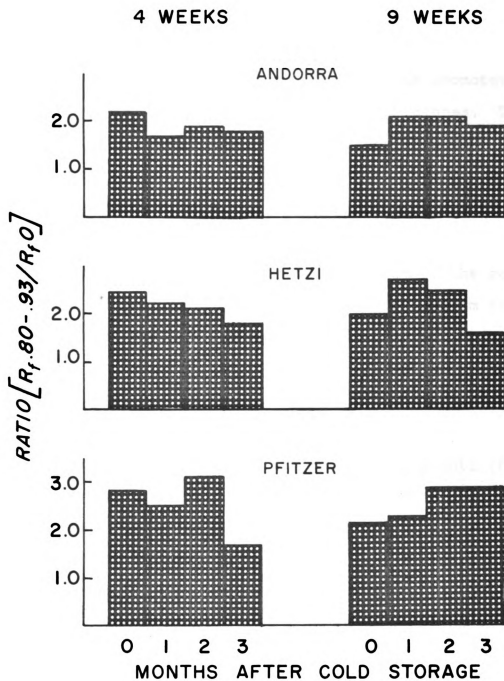


Figure 18.--Cyclic patterns of relative level of growth promoter at R_f 0.26-0.40 expressed as a ratio from shoots of greenhouse (A) and outdoor (B) plants of Juniperus chinensis 'Pfitzer'. In (C) curves of (A) and (B) are superimposed to illustrate the theoretical shift of the greenhouse cycle to closely match the cycle of the outdoor plants at another time of year.



1

Figure 19.--Relative changes in concentration of co-factor 4 in three Juniperus L. clones following four and nine weeks of cold storage (2°C).



there was a decline in growth promoter activity followed in the second month by an increase in promoter activity for Andorra and Pfitzer and a decrease in promoter activity for Hetzi.

After nine weeks of cold, an increase in promoter activity was noted in the first month for all clones. The second and third months then were equal or less than the preceding month.

The relative changes in growth promoter levels in the root system was also studied. Figure 20 presents the histogram for the mung bean bioassay chromatogram of the root system for Andorra and Pfitzer. In the root system the only promotion present occurred at R_f 0.80-0.93. This promotive region corresponded to the main promotive region of the shoots (Figures 13 and 15).

Relative changes in the promotive region of R_f 0.80-0.93 for the roots were noted for greenhouse plants (Figure 21). These plants were the same plants used in the root activity and growth promoter study. Comparison of changes for Andorra and Pfitzer in the growth promoter showed no significant difference between treatments within each clone. In Andorra, there appeared to be a slight increase in the growth promoter level from October through December compared to no difference in Pfitzer.

In Figure 22 cofactor 4 levels for four weeks of cold storage are compared for roots and shoots of Andorra and

Figure 20.--Activity from the mung bean bioassay test of chromatograms of methanol extracts from the roots of (A) Juniperus horizontalis 'Andorra' and (B) Juniperus chinensis 'Pfitzer'. Roots were collected from greenhouse potted plants in January. Each chromatogram is equivalent to 0.1 gram dry weight. Control (Con.) values are the means of three individual root systems with four mung bean plants.

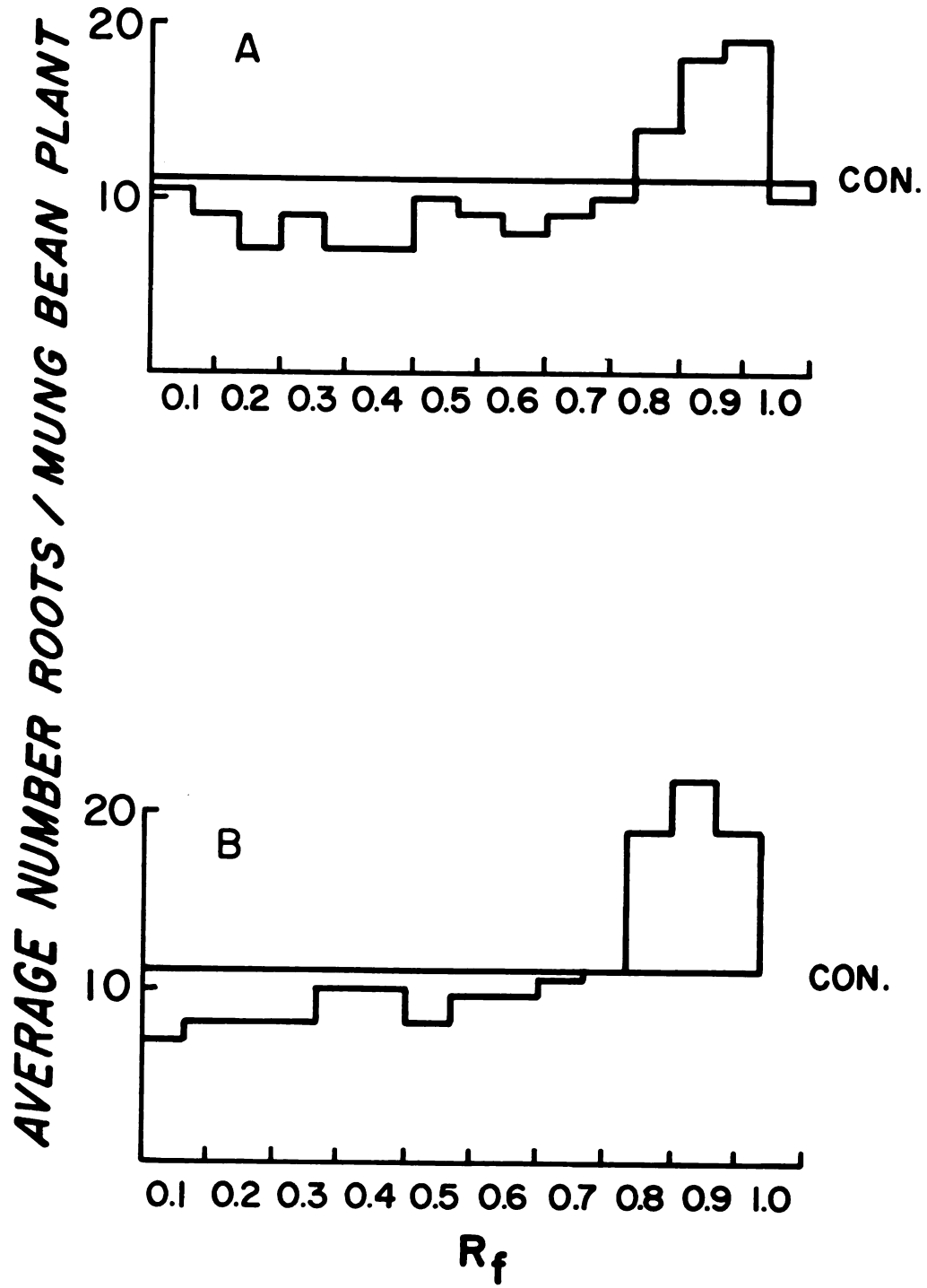


Figure 21.--Cyclic pattern of relative level of co-factor 4 (R_f 0.80-0.93) expressed as a ratio, from the roots of Juniperus horizontalis 'Andorra' (A) and Juniperus chinensis 'Pfitzer'. Plants were grown in the greenhouse during the experiment.

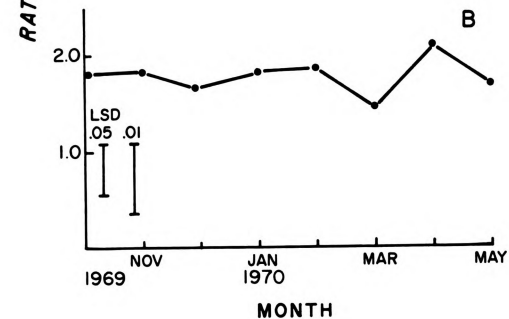
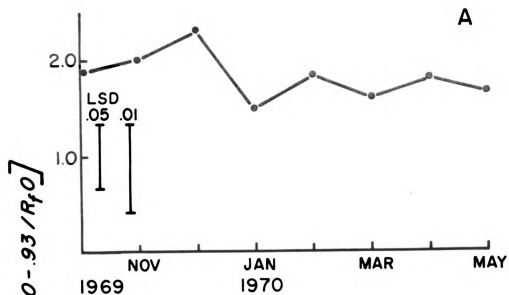
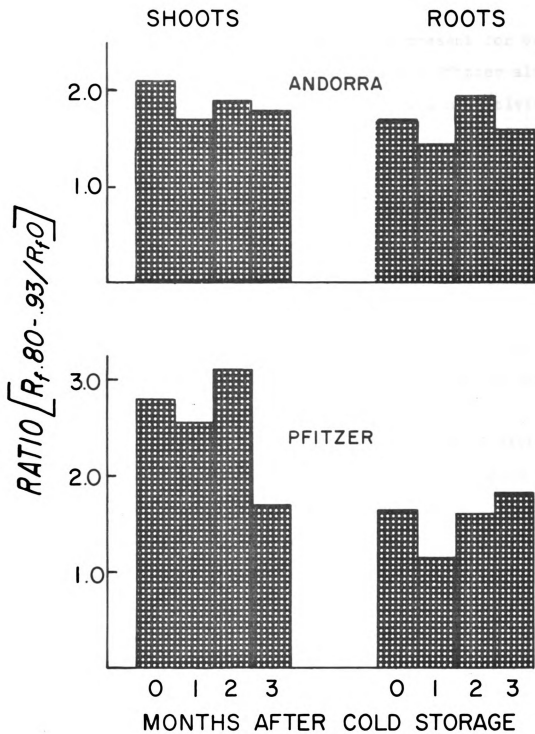


Figure 22.--Comparison of the relative changes in concentration of cofactor 4 (R_f 0.80-0.93) expressed as a ratio, between the shoots and roots of Juniperus horizontalis 'Andorra' and Juniperus chinensis 'Pfitzer' following cold storage (2°C).



Pfitzer. A decrease in activity was noted one month after being placed in the greenhouse. In general, the growth promoter level was less for the roots when compared to the shoots. A higher level of activity was present for the roots of Andorra compared to the roots for Pfitzer although shoots of Pfitzer exhibited a greater level of activity than Andorra shoots.

Characterization of Cofactor 4

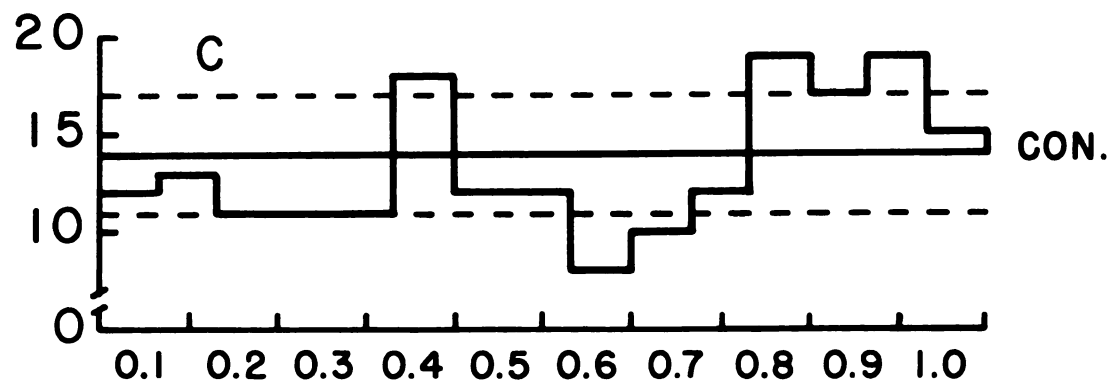
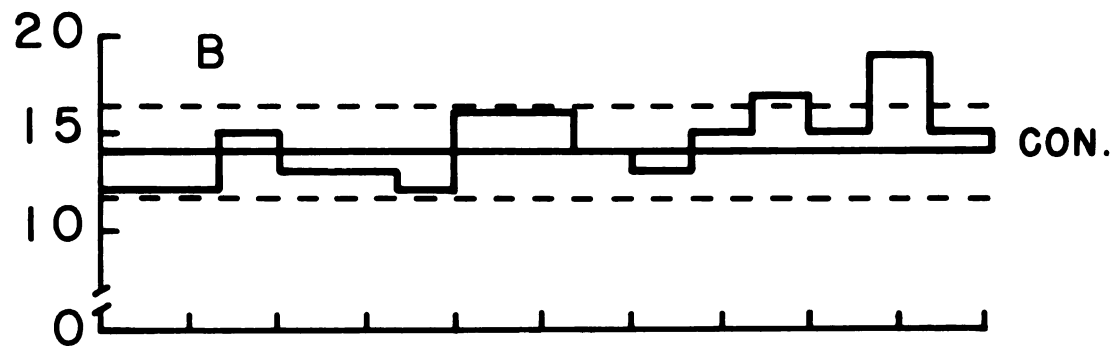
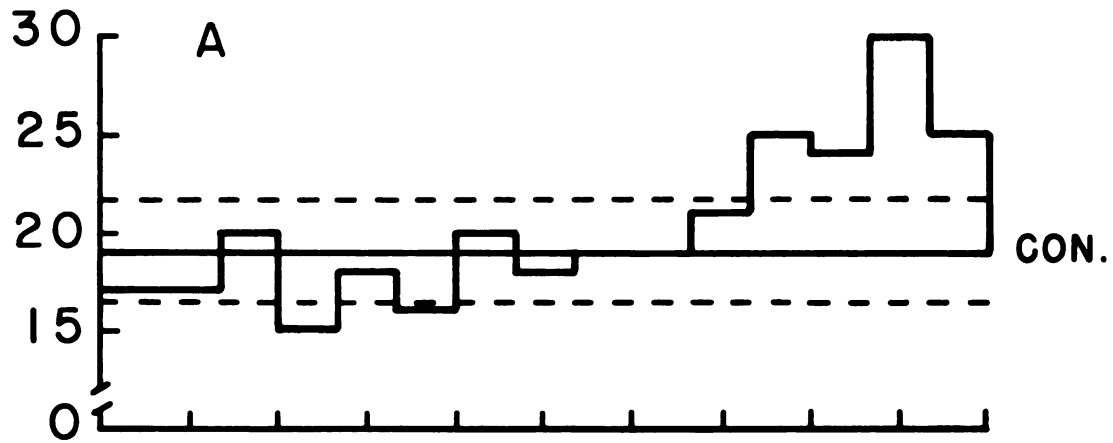
Characterization of growth promoter compounds is essential in following the relative concentration of these compounds through various environmental treatments if correlations are to be made between environments. The changing compounds for one clone are hypothesized to be the same for all clones and the same in roots and shoots.

To examine this hypothesis would require an extensive study. A preliminary study was performed to examine the character of the methanolic extract from all three clones. The acidic, basic and neutral fractions from the methanolic extract for Andorra, Hetzi and Pfitzer, respectively are shown in Figures 23-25. The major activity arose from the acidic fraction (Figures 23A, 24A, and 25A). Similar activity was noted in the neutral fraction of Hetzi (Figure 24C). Very little activity was found in the basic fraction.

Although 0.3 grams dry material was used for characterization studies no activity was discernible in the

Figure 23.--Activity from the mung bean bioassay test of chromatograms of the acidic, (A); basic, (B); and neutral, (C); ether fractions of methanol extracts from the shoots of Juniperus horizontalis 'Andorra'. Shoots collected from an outdoor plant in August. The plant material for the methanol extract was obtained from 0.3 grams dry material. Control (Con.) values are an average number roots per twelve mung bean plants. The dashed lines indicate twice the standard deviation of the mean for the control.

AVERAGE NUMBER ROOTS/MUNG BEAN PLANT



R_f

Figure 24.--Activity from the mung bean bioassay test of chromatograms of the acidic, (A); basic, (B); and neutral, (C); ether fractions of methanol extracts from the shoots of Juniperus chinensis 'Hetzi'. Shoots collected from an outdoor plant in August. The plant material for the methanol extract was obtained from 0.3 grams dry material. Control (Con.) values are an average number roots per twelve mung bean plants. The dashed lines indicate twice the standard deviation of the mean for the control.

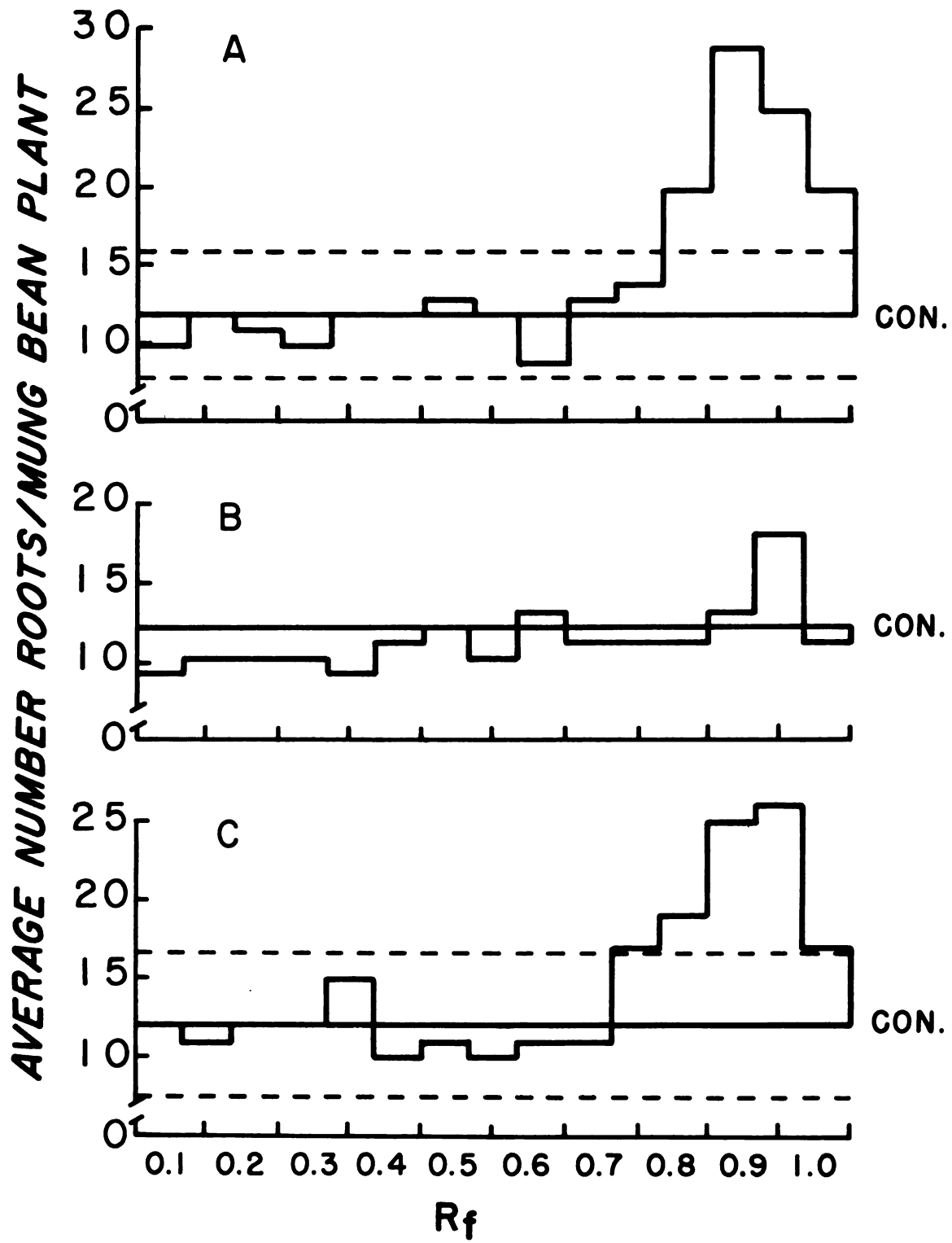
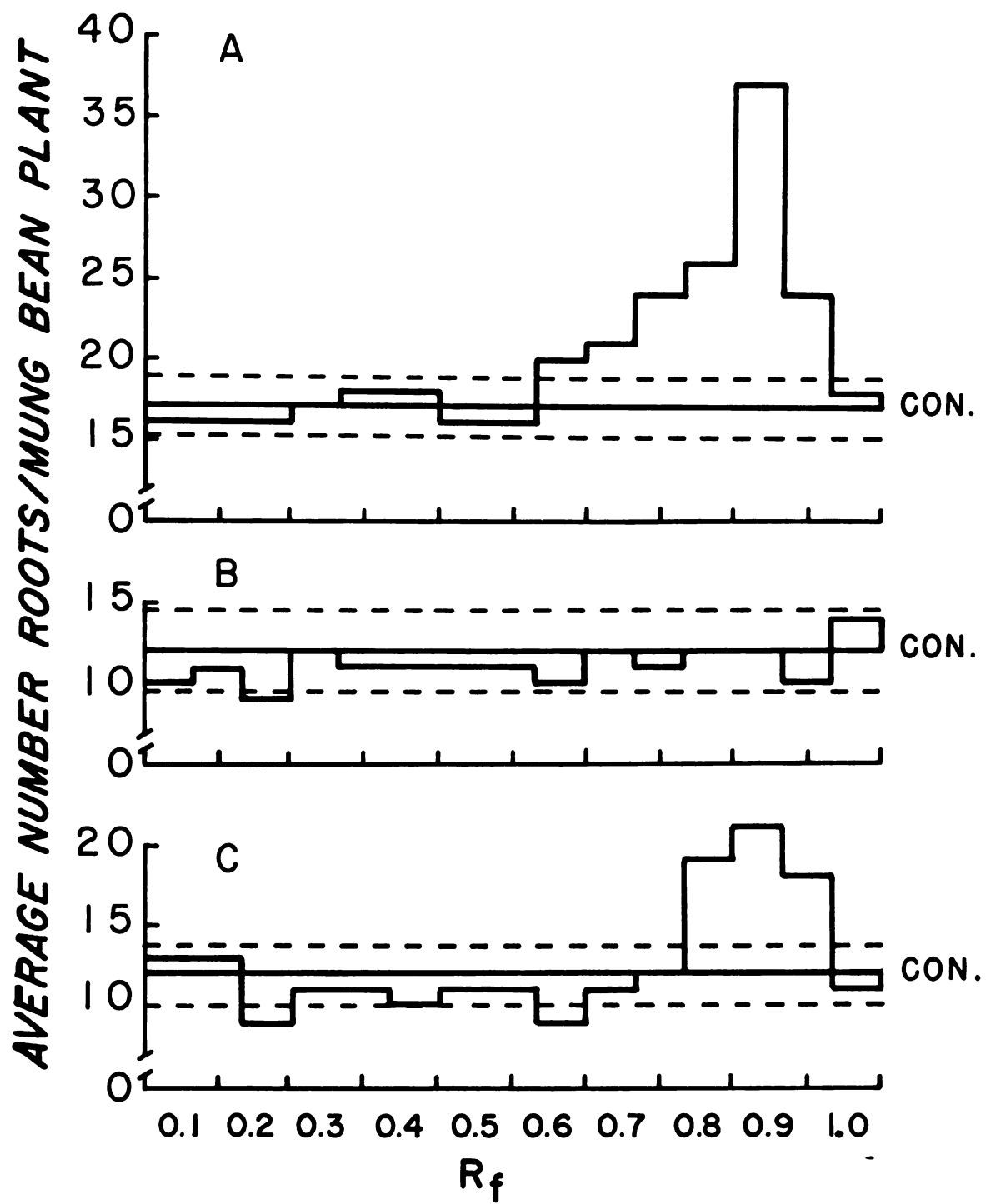


Figure 25.--Activity from the mung bean bioassay test of chromatograms of the acidic, (A); basic, (B); and neutral, (C); ether fractions of methanol extracts from the shoots of Juniperus chinensis 'Pfitzer'. Shoots collected from an outdoor plant in August. The plant material for the methanol extract was obtained from 0.3 grams dry material. Control (Con.) values are an average number roots per twelve mung bean plants. The dashed lines indicate twice the standard deviation of the mean for the control.



R_f 0.0-0.80 region, indicating the major peak at R_f 0.80-0.93 was the only fraction present in the partitioning experiments (Figures 23-25).

DISCUSSION

General

In addition to genetic and phenotypic differences of Andorra, Hetzi, and Pfitzer, subtle physiological differences exist between the clones which may play important roles in determining graft success potential. Such differences as shoot growth, root tip activity, and relative levels of growth promoters in response to chilling and day length will be discussed in relation to the results of preliminary grafting studies.

Physiological Changes in Juniper Clones as Affected by Varying Chilling and Photoperiod Conditions

Pinney (1970) and Hill (1953) stated that optimum grafting time is indicated by the presence of white root tips.

The time of year in which nurserymen move understocks from outdoors to a 18°C greenhouse varies from year to year depending on the nurseryman's work schedule and existing weather conditions. Because of this variability in working conditions the amount of natural chilling understocks receive also varies from year to year. Assuming the

nurseryman follows the same basic grafting procedures and subsequent handling of grafted plants each year the primary difference in the grafting practice would be the amount of chilling the understocks receive prior to being placed into the greenhouse.

Following the above line of reasoning the effects of chilling and photoperiod were related to root activity and graft-take. Chilling and greenhouse conditions were the same for both studies. The mung bean bioassay test was performed on the same plants from which the root activity data were taken, so that rooting cofactor level and root activity could be interrelated. For further comparison of data, shoots from outdoor individual clonal shrubs were collected and analyzed for cofactor level.

Assuming graft success is related to plant growth activity the practice of grafting after new root growth is initiated is valid. If junipers respond similarly to other woody conifers, one would expect root activity to decline during cold storage or outdoor conditions and then rise again after being moved to a more favorable environment.

Root activity for greenhouse plants declined from October through December for all clones and then plateaued at a constant rate (Figure 3). These results were expected since natural day length was decreasing until early February (Table 2) and daylight intensity was lower than during summer months.

The level of activity for greenhouse plants differed by clone, an indicant of genetic differences. Root activity level of Andorra plants after four weeks of chilling was the same as plants grown in the greenhouse; however, for Hetzi and Pfitzer root activity was much lower (Figures 3 and 4). One month after being moved into the greenhouse all clones demonstrated a higher level of root activity than plants growing in the greenhouse.

For all clones the root activity was lower after nine weeks of chilling than for plants grown in the greenhouse (Figures 3 and 4), but again one month after being moved into the greenhouse, root activity in all chilled plants was higher.

In general, by the second month following the transfer of both four- and nine-week chilled plants into the greenhouse activity had again declined. The decline in root activity in the second month may have occurred because of: 1) shorter day length in December and January; 2) a change in growth promoter and inhibitor balance between the shoots and roots forcing the roots to decline in activity; 3) increased shoot growth (change in slope of curve, Figures 7-8); or 4) some combination of the above possibilities.

Another factor that may have been playing an important role in decreased root activity during chilling was the dark storage. The longer the chilling and dark period continued the more food reserves were depleted; however, if the plant

was dormant the metabolism of the juniper would be at such a low level that photosynthesis would not be playing an important role even if light was not limiting.

Besides root activity patterns other growth responses may be related to graft success. Researchers (Lanphear and Meahl, 1966; Fadl and Hartmann, 1967; Vietez and Pena, 1968) showed that rooting potential for cuttings of some woody plants is cyclic. Specific chilling and/or day length treatments are beneficial in improving rooting.

The purpose of the first experiment was to measure shoot growth response as affected by day length. In general, shoot elongation ceased under short days, was intermediate under natural day length, and was continuous under long days. This response contradicted the reports by Waxman (1957) and Nitsch (1957b) who described Andorra juniper growth as being continuous under long and short days. Possible explanations for the difference in growth may be: 1) greenhouse environments differed; and 2) age of plant material differed.

The concept of a juniper growth cycle was suggested by Lanphear and Meahl (1966) in their discussion of seasonal response to rooting. In Andorra, they found that rooting potential declined beginning in February through May. They hypothesized that cofactors may reveal the rooting potential of a particular species but would not necessarily assure their availability at the site of root initiation. The

initial factor would be whether the cofactors were mobilized to the site of root initiation. Actively growing tissue might prevent the translocation of cofactors to the base of the cutting.

Under natural day length, Andorra shoot growth was nearly constant even though a small decline in growth was noted in November and December. Growth in Hetzi and Pfitzer ceased from November through the middle of January. Natural day length was very close to being a short day in terms of day length in December and January (Table 2). The shoot elongation data, however, indicated that the plants were responding more nearly like the short-day treatment. The increase in shoot elongation may have resulted from the nutrients and growth substances which accumulated during the longer and more intense daylight during early fall. The accumulated nutrients allowed the shoots to continue growing later into the fall.

The second experiment was designed to measure the effect of chilling on shoot growth. One complicating factor not accounted for in this study was the changing day length in relation to when the plants were moved from the dark cold storage room to the greenhouse. In general, growth rates differed between lengths of chilling treatment and between clones. Although Hetzi and Pfitzer responded differently, they did respond more nearly alike than when they were compared to Andorra.

Four weeks of cold storage did not significantly change the total growth in Andorra (Figure 8) when compared to natural day length. Nine or twelve weeks of cold storage resulted in reduced total growth for Andorra. The interpretation is made that extended dark cold storage period altered the growth cycle for Andorra to such an extent that the plants were not capable of overcoming the physiological changes resulting from the extended storage treatment.

The growth patterns for Hetzi and Pfitzer following four, nine and twelve weeks of cold storage were similar (Figures 9 and 10). These clones were in a quiescent state and once the environment (temperature, moisture and day length) was optimum for plant growth these clones resumed growth. Andorra did not respond similarly and thus was not in a quiescent state. Nienstaedt (1966) reported that in white spruce the quiescent state of growth begins during the first three weeks of December. High temperature, long photoperiod or a combination of both (Mergen, 1954; Olmsted, 1971) will result in resumption of growth.

Thus far, root activity and shoot growth have been discussed separately. A comparison of the two phenomenon shows that in the root activity studies Andorra was more active than Hetzi or Pfitzer while in shoot growth (following chilling) Hetzi and Pfitzer were more active than Andorra.

To further describe physiological changes occurring in outdoor, greenhouse, and cold storage treated plants the

mung bean bioassay was used. In both shoots and roots the major region of activity occurred at R_f 0.80-0.93 which has been described as cofactor 4 by Hess (1962b). This cofactor has been shown to correlate with rooting in distinguishing easy to root and difficult to root forms of Hedera helix L. and a cultivar of Hibiscus rosa-sinensis L. (1962a). Lanphear and Meahl (1966) also verified this region as active in their rooting studies with Andorra juniper.

A purification experiment was conducted to further characterize cofactor 4. The results indicated that cofactor 4 possessed similar characteristics in all clones and was acidic except for the clone Hetzi where the neutral fraction was equally active. No further interpretation is made of this study. Further studies will be required for identification of cofactor 4 in junipers.

Vietez and Pena (1967), correlated the periodicity in rooting potential of Salix atrocinera to relative changes in concentration of growth promoter. Lanphear and Meahl (1966) using the mung bean bioassay attempted to correlate rooting potential in Andorra to relative concentrations of total promotive activity in Andorra juniper. In this study they combined all promotive activity from the whole chromatogram, and were unable to show periodic changes.

In line with the above reasoning data from the bioassay in this study were analyzed differently. First, the daily fluctuation in the average number of roots of the

control was accounted for by calculating a ratio between each chromatogram section and the control. Second, the ratios were analyzed for the specific regions of the chromatogram. The R_f 0.80-0.93 region corresponded to the major peak and agreed with Hess' region that he labeled cofactor 4. The area of the chromatogram R_f 0.26-0.40 did not correspond to any of Hess' cofactors, but corresponded to an active region in Lanphear and Meahl's bioassayed (1963) chromatogram.

Relative concentrations of cofactor 4 in Andorra depicted cyclic differences between greenhouse and outdoor plants. When the two curves were superimposed the two treatments matched closely when the data for the greenhouse plants shifted by six months in relation to the data for the outdoor plants. That is, the relative levels of cofactor 4 in greenhouse plants during the winter were increasing at a rate similar to Andorra plants growing in the summer outdoors. Shoot growth in Andorra under natural day length corresponded closely to shoot growth of long day plants. In the summer the day length in July was approximately 15 hours which was very close to the 16 hours day length treatment for the greenhouse grown plants during the winter. The conclusion is drawn that Andorra plants in the winter whether on true long days or natural days with additional low light intensity respond physiologically the same as outdoor plants in the summer.

The shift of six months for Andorra greenhouse to outdoor for cofactor 4 did not occur in Hetzi and Pfitzer. In Hetzi a one month shift was observed while a two month shift was noted for Pfitzer. The differences in the degree of shifting is explained in the genetic differences among the clones. In addition, the degree of shift coincided to the differences in rooting potential and graft potential between the clones.

The original hypothesis for this experiment suggested that different parts of the chromatogram, representing different growth substances, would change at varying rates. The R_f 0.26-0.40 for Andorra (Figure 17) showed a shift in greenhouse to outdoor plants of six months--the same as for R_f 0.80-0.93. Also, when R_f 0.26-0.40 curves for greenhouse and outdoors treated Pfitzer were superimposed onto each other a shift of six months accounted for the best matching of points, which was different from the one month shift noted for R_f 0.80-0.93. The data in this experiment supports the hypothesis that growth substances are changing and these changes are noted by shift in activity of the various cofactors. This hypothesis explains why Lanphear and Meahl (1963) were unable to show cofactor differences throughout the year. Using selected regions of the chromatogram to quantify periodicity is often used in other specific assay systems (Vietez and Pena, 1968; Kawase, 1961).

After four weeks of chilling the relative concentration of cofactor 4 was higher when plants were first moved out of cold storage than after one month in the greenhouse environment. After nine weeks of storage the initial concentration of cofactor 4 was lower in all clones than after four weeks, and, in contrast increased one month later.

Combining the interpretation of four and nine week data the conclusion is drawn that for all clones, four weeks of cold was not sufficient to positively increase the cofactor 4 level one month after storage. After nine weeks however, the clones had completed their rest requirement or were in another physiological development stage and responded similarly to the root activity study by increasing after one month of cold. Cofactor 4 was in higher concentration in Hetzi and Pfitzer than in Andorra.

The only peak present in the roots of Andorra and Pfitzer was cofactor 4 (R_f 0.80-0.93). Some inhibitors may have been present in the R_f 0.0-0.80 region for both clones.

The greenhouse roots for Andorra and Pfitzer did not show any change or periodicity in cofactor 4. Apparently root activity was not related to the presence of cofactor 4 in the roots. Lack of periodicity in the root system would have indicated that the cofactor was made in the shoots and was transported to the roots at a constant rate; or it may be correlated to rooting potential which must be involved in

some mechanism controlling meristematic activity which results in rooting and perhaps to wound healing potential.

Graft Success as Affected by
Environmental Conditions

As previously mentioned grafting studies were performed using experimental design similar to that used for root activity, growth promoter, and shoot growth studies. In the first experiment the following information was most significant: 1) Highest successful graft-take occurred for Andorra, followed by Hetzi, and then Pfitzer. This order of success was also reported by Evans (1969). 2) Nine weeks of chilling prior to grafting resulted in highest graft survival followed by four and twelve weeks of chilling; and 3) Whenever greenhouse grown plant material was involved in the graft as either scion, stock or both the graft success was better than cold treated material. This study suggested the possible relationship between the amount of chilling the stock and/or scion receives and the potential for graft success. In contrast to the first year, when the previous experiment was repeated both four and nine weeks of chilling resulted in nearly equal graft survival percentage. The lower percent graft-take in the second year illustrates the inconsistency that propagators experience even under controlled conditions. Nine weeks of chilling was equal to or better than four and twelve weeks of chilling indicating

that the amount of chilling a plant receives may affect graft success. The conclusion is made that the stage of growth in relation to a growth cycle more directly affects graft-take and this stage is reached when the plants have been in cold storage between four and nine weeks. Once a plant is in a particular phase of its growth cycle then controlled environment may alter the plant's growth cycle by changing the time required for the plant to move from one phase of the growth cycle to another.

To illustrate the above hypothesis consider the first year's grafting data (Table 3). The plants were moved into cold storage in late October when day length and night temperatures were decreasing. The stock had already begun to enter a new growth phase before being moved into cold dark storage. After nine weeks of cold the grafts were moved into the greenhouse and new growth was initiated. Grafting at this time resulted in a very high percent. For the four weeks of chilling period graft success was moderately high.

In the second year, the plants were moved into cold storage in late September. At the time of moving, the plants were still actively growing. The plants may have been in a different physiological growth period than in the first year and thus the new environment was a shock to the plants. In other words, the plants were in a growth phase different than in the first year and the plants were not physiologically prepared for the cold dark storage. Graft

survival was nearly 50 percent for both four and nine weeks cold storage.

The lower graft survival in the second year may be explained as follows: For greenhouse scion and stock grafts the time of year grafting takes place is important. As the day length becomes shorter the rate of growth of all clones declines. In the first year the four and nine week grafts were made on November 29 and January 5. Shoot growth data (Figures 5-7) on and following these dates indicate that plants had a low growth rate from November 29 until mid-January, at which time they enter a high growth rate period. The plants grafted January 5 enter the high growth phase almost immediately. In the second year, grafts were made November 11 and December 17, eighteen days before grafts were made the first year. The longer delay between grafting and the new period of growth activity to occur in mid-January apparently decreased graft success.

Root activity of greenhouse plants declined from October through December (Figure 3) and plateaued beginning in late December or early January. It is hypothesized that the root activity pattern is a result of change in growth promoter and/or inhibitor synthesis rates within the roots; or a change in direction of movement of the growth promoters and/or inhibitors from the roots to the shoots or from the shoots to the roots. The hypothesis is made that the growth promoters were mobilized from the roots to the

shoots. Data show that root activity remained constant from January through May while shoot growth rate increased. At the same time cofactor 4 remained constant in both roots and shoots.

In the scion storage experiment the results indicated that length of time scions were stored affected graft survival. Scion wood should be used as soon as possible after scions are collected.

In relation to the scion storage study observations were recorded from dead grafts to determine if callusing had begun on unsuccessful grafts. Callusing had begun on the stock at the wound area, and between 50 and 100 percent of the surface area of the stock was covered by callus. However, in only a few instances was callus material forming on the scion. The fact that callus had not initiated on the scion indicates the probable reason for the unsuccessful grafts. A whole field of research in the physiological changes occurring in the scion wood has not been explored.

Assuming that graft success is the result of the presence or absence of some growth substance at the wounded surface region, then if the growth substance lacking could be applied to the wound area, the graft union would possess the potential for callusing and wound healing. Evans (1969) conducted a preliminary study following the above assumption. He applied extracts to each of three self-grafted juniper clones. His results indicated that the Andorra extract

significantly improved graft success in the Pfitzer self-graft. The Pfitzer extract on the other hand did not affect Andorra self-graft success but did affect subsequent scion vigor once the graft union healed.

A similar plant extract study was conducted for three years. Graft success was found to be poorer in all cases compared to the control. Plant extracts however, were shown to be less detrimental to graft success as compared to the application of synthetic growth regulator solution. The auxin:gibberellin:kinetin solution contained 10:20:10 mg/l and 10:10:2 mg/l for the four and twelve week application and resulted in near zero graft success. Application of 1:2:1 mg/l at nine weeks of cold did not result in higher percent survival than control but was greater than the previous concentrations. The suggestion is made that perhaps the concentrations were too high and lower concentrations may be more beneficial.

SUMMARY AND CONCLUSIONS

The primary objective of this study was to relate graft survival of three Juniperus L. clones subjected to different environmental conditions prior to grafting to physiological events occurring in the grafted understock. The genetically stable clones of Juniperus horizontalis 'Plumosa' (Andorra), J. chinensis 'Hetzi', and J. chinensis 'Pfitzer' were used since they represent two species within the genus and two clones within one species.

Three studies were conducted to investigate the relationship of plant growth, determined by root activity, shoot elongation and changes in level of growth promoters, to different environments.

Shoot elongation for all clones was dependent primarily upon day length. Short days caused a cessation of growth from October through mid-February. Shoot elongation commenced again around the first of March.

Natural days caused a sigmoidal pattern of growth in all clones. Shoot elongation continued from September to late October and discontinued in Hetzi and Pfitzer and was reduced in Andorra until mid-January.

Long days for all clones caused continuous growth through the winter with only small decreases in the growth rate.

Plants which had received low temperature (2°C) chilling for four, nine or twelve weeks prior to being moved into the greenhouse initiated new growth soon after entering the warmer environment.

Clonal differences in growth rate was evident in this experiment. Hetzi and Pfitzer demonstrated a greater positive response toward the cold rest period than did Andorra by growing at a greatly increased growth rate upon exposure to the 18°C greenhouse. The increased growth rate for Hetzi and Pfitzer was apparent for all chilling periods.

Root activity, determined by estimating the percent white roots on a root system, declined for all clones from October through December and then plateaued. The time interval during the plateau period corresponded to the period of new shoot elongation. The correlation of the two events suggested that growth promoter activity which had been directed toward the roots was redirected toward the shoot tips. The apparent decrease in activity in the shoots was caused by the increase in mass of the shoots as a result of shoot elongation. The level of the promoter measured by the mung bean bioassay in the roots remained constant throughout the experiment even though root activity had declined.

The identity of the above growth promoter is not known. Preliminary studies were conducted by partitioning a methanolic extract from the juniper roots and shoots into acidic, basic and neutral fractions and testing for promoter activity by means of the mung bean bioassay. The growth promoter was found to be acidic except for Hetzi in which a high level of activity was found in both the acidic and neutral fractions. The active zone on the paper chromatogram was R_f 0.80-0.93, which corresponds to a growth substance identified by other researchers as cofactor 4.

Additional experiments involving cofactor 4 were conducted to determine changes in level of cofactor of outdoor plants and greenhouse plants.

The pattern of change in level of cofactor 4 for both greenhouse and outdoor plants were cyclic. When the two curves were superimposed on each other the rate and level of change of cofactor matched closely when the summer portion of the outdoor cycle was compared to the winter phase of the greenhouse cycle. The shift of six months between cycles was explained by the observation that greenhouse plants in the winter were actively growing as determined by the shoot elongation study as were plants in the summer outdoors.

Similar comparisons were made for Hetzi and Pfitzer. In these clones the shift in the promoter cycle was one and two months, respectively. The small shift in these cycles

coincides with the great response noted in the shoot elongation study following the cold storage period.

To complete the primary objective of this study, three grafting experiments were conducted. Understocks and scions grown in the greenhouse and grafted in late December resulted in highest graft survival. This study was repeated for the second year with similar trends in survival from specific treatments although the total percent survival was lower.

The second grafting experiment determined the percent survival of grafts in which the scion had been cut several weeks prior to grafting. The data indicated that the longer the nurseryman waits between cutting scion wood and grafting the lower the percent survival he may expect.

The third experiment was designed to test the effect of applying leaf-stem extracts of one juniper or growth regulator solution to the graft union of another self-grafted juniper clone. In all cases, graft survival was less for the treated grafts than the control.

Under the conditions of this investigation the final conclusions are made:

1. Root activity determination used by the propagator to determine the time to begin grafting is valid.
2. Contrary to earlier reports, shoot elongation is affected by the length of the photoperiod.

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3. Hetzi and Pfitzer respond more positively to cold storage as determined by shoot elongation following storage than does Andorra.
4. Cofactor 4 as determined by the mung bean bioassay, is present in all clones and changes in level of activity is cyclic.
5. The change in the pattern of activity for cofactor 4 may be altered by artificial conditions, such as greenhouse growing of plants in the winter.
6. The clonal order of graft survival potential in decreasing potential is Andorra, Hetzi, Pfitzer.
7. Highest graft success for all clones was obtained from the 18°C/18°C (scion/stock) treatment.
8. Cold storage prior to grafting may be important; however, day length would appear to be more important.
9. Scion storage prior to grafting is not beneficial if scions are stored for longer than a few weeks.
10. Applications of plant extracts or growth regulators are detrimental to graft survival.

Suggestions for Future Research

The following suggestions for future research include:

1. Characterize the variation in graft success potential at monthly intervals for one year.
2. Compare graft survival of actively growing or dormant scion wood grafted to actively growing or dormant stock.

3. Determine graft survival as affected by dimension of scion wood, age of scion wood and position on tree from which scion is collected.
4. Determine graft survival when grafted plant is subjected to different soil and greenhouse temperatures and to different day lengths.
5. Girdle a shoot prior to cutting for scion material to possibly cause an accumulation of growth promoters at the base of the scion.
6. Determine rate of callus formation on wounded stock and scion when not grafted.
7. Using tissue culture methods study callus formation affected by addition of growth regulators.
8. Characterize physiological changes which occur in a scion during storage.
9. Investigate possible interactions of day length and temperature to shoot growth and root activity.

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