

AN ABSTRACT OF THE THESIS OF

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Title: THE NATURE OF FLOWER BUD INFLUENCE ON ROOT
REGENERATION IN THE RHODODENDRON SHOOT

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Flower bud influence on rootability of Rhododendron shoots was assayed by rooting leaf-petiole cuttings. Monthly samples of an easy- and an intermediate-to-root cultivar showed periods of poor rooting in the latter during flower development, although both rooted similarly during bud dormancy. The decrease in rooting-potential following flower initiation and development was avoided by preventing flowering with heavy shading (90 percent). The decreased rooting accompanying flowering was attributed to specific stages of flower development that were intensely competitive for growth substances needed in root regeneration.

Leaves from the lower portions of flowering shoots were larger and rooted less readily than those from similar positions on vegetative shoots. Shading experiments revealed that the association of large leaves with poor rooting is valid only in flowering shoots.

Responses to early terminal bud excision suggested that the increased leaf size and decreased rootability associated with flowering commences at time of initiation. Later excision of the terminal flower bud had less effect on leaf size, but enhanced rootability possibly as a result of eliminating this strong growth center from competing for growth substances needed in rooting.

Defoliation experiments revealed a quantitative aspect to the flowering hormone stimulus from leaves, while establishing a minimum leaf complement for continued flower development. Partial defoliation demonstrated the importance of leaves in flowering and rooting relationships; for example, removal of terminal leaves inhibited flowering, but enhanced rootability. Labelled auxin transport studies revealed a positive correlation between transport in leaf-petiole tissue and lamina rootability. However, there was an inverse relationship between the amount of auxin transported and that absorbed by petiole tissue. It was concluded that developing flowers mobilize auxins from subtending leaves, thereby depleting these tissues of such materials needed in root regeneration. The extent of the flower's influence on rooting of shoots in rhododendron depends on the cultivar and the stage of flower bud development at the time of sampling.

THE NATURE OF FLOWER BUD
INFLUENCE ON ROOT REGENERATION IN
THE RHODODENDRON SHOOT

by

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DEDICATION

This thesis is dedicated to my wife and family.

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THE NATURE OF FLOWER BUD INFLUENCE ON ROOT REGENERATION IN THE RHODODENDRON SHOOT

INTRODUCTION

Flowering significantly alters both qualitatively and quantitatively the synthesis of many important biochemical components in plants. Such changes affect physiological processes, such as rooting, and morphological features, such as leaf size. It is well recognized that rooting of cuttings is generally depressed by the presence of flower buds. However, the time and mode of this inhibition is not clear. Harada and Nitsch (1959) reported changes in various growth substances with flower initiation and development. Selim (1956), in a review, proposed that developing flower buds mobilize auxin leaving little for root formation. Thus, it appeared that rooting might be inhibited by both the initiatory and developmental phases of flowering.

The Rhododendron was selected for studying some of the above relationships because it was found (Adams, 1967) that many of its cultivars were profoundly influenced by flowering, and as in certain other species, rooting of leaves could be used to assay shoot rootability. Leaf-petiole cuttings were used throughout the study since leaves are considered sources of substances needed in flowering and rooting processes.

The object of this series of experiments was to study in detail root regeneration in the detached rhododendron shoot for the purpose of determining (1) the effects of flowering on size and rootability of leaves from different positions on the shoot, (2) the time at which flower inhibition of rooting occurs, and (3) changes in absorption and movement of labelled auxins in leaf tissues associated with flowering. From this study a concept of flower inhibition of rooting based on a competition for growth substances¹ is developed.

¹Compounds such as carbohydrates, auxins, and numerous other hormones needed in growth processes.

REVIEW OF LITERATURE

Influence of Endogenous Nutrients and Hormones on Rooting

McCallum (1905) regarded the capacity for plant organ regeneration to be controlled only by environmental conditions. Many investigations since that time have revealed that tissue dedifferentiation and initiation of new organs also requires the presence and balance of numerous internal constituents. Root regeneration, for example, has been shown to depend on constituents such as carbohydrates, auxins, vitamins, unidentified rooting cofactors, and nitrogenous compounds. These constituents, in addition to others, are formed in leaves and, in some cases, in buds.

It is well known that leaves supply important substances needed in root formation. Loeb (1915) proposed that a substance was transported basipetally from leaves to form roots at the base of stem cuttings and Kögl, Haagen-Smit and Erxleben (1934) termed this material as "heteroauxin" (indoleacetic acid--IAA). Bouillenne and Went (1933) concluded that non-auxins or rhizocalines were also formed in leaves and transported to the cutting base for control of root formation. In more recent work, Kawase (1964) obtained diffusates similar to rhizocalines following centrifugation of Salix alba shoots oriented in an upright position. However, these

diffusates were not found at the shoot tips following centrifugation of inverted shoots. Cooper (1936) proposed that exogenous auxin application facilitated the basipetal movement of rhizocalines; however, Stuart (1938) found only an accumulation of nutritive factors as a result of auxin application. The presence of several other endogenous substances, such as auxin-protecting phenolic derivatives (Hess, 1965), and many types of auxins (Luckwell, 1957) in bound and unbound forms (Hemberg, 1951), reveal the possibility that root formation may involve complex hormonal interactions.

The importance of nutritive substances, such as sugars and nitrogenous substances, in root formation was determined by defoliation experiments with Hibiscus (Van Overbeek, Gordon and Gregory, 1946). The nutrient supplying function of the leaf in rooting was replaced by the addition of sucrose and an organic form of nitrogen. Gregory and Samantarai (1950) concluded that the level of sugar was the major factor controlling rooting in old and juvenile leaves of Hedera helix. Humphries and Thorne (1964) demonstrated the apparent need for photosynthates in rooting by correlating the increased assimilation rates in isolated leaves with root initiation. Carbon dioxide uptake increased shortly before roots were visible, presumably when root initiation was occurring in the pericycle of the petiole. Literature on the subject clearly indicates that leaves contain all the constituents needed for rooting, a concept

demonstrated by Adams (1967) in using individual leaf-petioles of Rhododendron as a bioassay of shoot rootability.

The importance of leaves in rooting has been emphasized; however, both lateral and terminal buds also contribute considerably to root regeneration. Although buds apparently contribute no nutritive substances (Bouillenne and Went, 1933), they are thought to produce auxins or other auxin-like materials (Fadl and Hartmann, 1967) necessary in rooting.

Seasonal Fluctuations in Rooting

The time a shoot cutting is taken for propagation greatly affects its rootability. Wells (1953, 1954) has shown large differences in rooting of Rhododendron cultivars with respect to timing. The studies of Lanphear and Meahl (1963, 1966) with Juniperous, Vieitez and Peña (1965) with Salix, and Lee, McGuire and Kitchen (1969) with Rhododendron have shown concurrent seasonal trends in co-factor content with shoot and leaf rootability. More specifically, Spiegel (1957) found seasonal fluctuations in rooting of Vitus canes to be closely associated with endogenous auxin contents. Dore (1953) observed seasonal fluctuations in rooting of Armoracia cuttings, and that rooting was particularly low during flowering. He interpreted this as evidence of an antagonism between flowering and rooting. In his review Dore (1965) cited several such cases of

interactions. Adams and Roberts (1967) used flower development as a morphological index to determine the time for taking Rhododendron cuttings for propagation. This method proved more reliable than calendar-date in predicting shoot rootability, and emphasized the important influence of flower development on rooting.

Influence of Flowering on Rooting

It has been almost universally accepted that cuttings terminated by flower buds are less rootable than those with vegetative ones. This reduction in rooting has been noted, for example, by Grace (1939) with Picea, Hieke (1961) with Camellia, and Woycicki (1938) with Chrysanthemum, Dahlia, Dianthus, Fuchsia, Hydrangea and Pelargonium cuttings. Kemp (1948) suggested that flower buds inhibited the rooting of Rhododendron shoots, and DeBoer (1953) demonstrated slight increases in rooting this species by removing such buds, as did O'Rourke (1940) in Vaccinium.

Early work by Turezkaya, as reviewed by Selim (1956), revealed that root formation in cuttings of Perilla nankinensis and Soja hispida decreased with flower initiation and disappeared completely during anthesis. She concluded that developing flowers and fruits mobilized auxins needed in root formation. Leopold and Guernsey (1953) showed a change in direction of auxin flow in coleus stems after flower initiation, thus supporting the flower

bud mobilization concept. Cooke (1954) with soybean and Selim (1956) with Perilla found low auxin levels in the leaves of plants under flower inducing short day conditions. In contrast to only a single auxin, Harada and Nitsch (1959) found changes in the levels of three such growth substances following flower inducing treatments for short-day, long-day and cold-requiring type plants. Similarly, Jorgensen (1966) showed changes in six different auxins in the shoot tips of Rhododendron simsii after flower initiation. The levels of these auxins decreased approximately with flower initiation but increased again with further bud development.

Changes in leaf size and shape often occur in conjunction with the flowering condition. Ashby (1949) found that leaf shape of Kalanchoe was dependent on night length and subsequent flower formation. Thomas (1961) showed that Chenopodium leaves unfolding during flower initiation were larger than normal, and postulated that this phenomenon occurred in several other plant species. Adams and Roberts (1968) observed floral initiation in Rhododendron 'Roseum Elegans' when the central leaves were about half expanded, and that these leaves were of larger size than similarly positioned ones on vegetative shoots. It is clear that flowering causes changes in plant metabolism which alter regenerative capacity and leaf development.

Influence of Leaf Excision and
Shading on Plant Growth, Flowering and Rooting

Since flowers influence plant metabolism, their reduction or absence should readily affect plant growth and rooting processes. Organ excision and shading are two means of reducing or inhibiting flowering in plants.

Garner and Allard (1920) established that day length as perceived by leaves was important in flower induction in tobacco. Zeevaart (1958) demonstrated the importance of leaves in flowering by inducing vegetative plants of Perilla under non-inductive conditions to flower by grafting on induced donor leaves. Roberts (1923) demonstrated with plum and Fulford (1954) with apple that defoliation could prevent flower formation. There was a marked relationship between the removal of fractional parts of leaf blades and the number of blossoms formed.

In addition to influencing flowering, partial defoliation of shoots influences other facets of growth and development. However, Dormer (1965) cautioned against the use of defoliation for correlative growth studies because stimulation of remaining leaf growth could be due to only wounding effects. Hartt, Kortschak and Burr (1964) concluded from defoliation and translocation studies in sugar cane that leaves compete with one another for growth materials, particularly with the ones in higher shoot positions. In similar

studies with apple shoots, Quinlan (1966) concluded that vascular connections cannot be the only factor determining the distribution of assimilates, but a balance of photosynthates from all the leaves influence the distribution pattern. Roberts and Blaney (1968), working with Lilium, found that in addition to altering normal flower development, defoliation at critical times and positions on the flowering axis influenced the distribution of weight in the old and new portions of the bulb.

Changes in light intensity alter the physiology and morphological patterns of plant growth and can ultimately influence rooting. The most important function of light is as an energy source for photosynthesis. Rosenberg (1965) pointed out that the rate of photosynthesis was directly proportional to light intensity up to a saturation level. Similarly, Blackman and Rutter (1948) demonstrated with Scilla non-scripta an exact linear relationship between net assimilation rate and the logarithm of light intensity. Blackman and Wilson (1951a) showed such linear relationships with ten different plant species in shading experiments.

Perhaps the most important influence of light intensity on rooting is brought about through changes in endogenous auxin levels. Early work by Tang and Bonner (1947) and later by Galston and Baker (1951) demonstrated the ability of light to activate the oxidation of IAA. Although the nature of this auxin destroying enzyme

(IAA-oxidase) has not been fully elucidated, light has been shown to be an important factor in its activation (Fang and Butts, 1957). In addition to intensity, other workers have shown that ultra-violet (Klein, 1967) and far-red (Fletcher and Zalik, 1964) portions of the spectrum reduce auxin levels. Klein (1967) also noted that auxin destroying light treatments increased flowering. In shading experiments, Larson (1962) with Poinsettia and Ishida (1966) with Aster observed greater flowering under conditions of highest light intensity.

Light intensity has been shown to influence leaf size (Blackman and Wilson, 1951b; Davidson, 1966) and stem elongation (Sachs, 1964). In addition, fiber content (Yanagisawa, 1967) and lignin content (Kausch and Haas, 1966) have been reduced in plants grown under heavy shade.

Relationship of Polar Auxin Transport to Plant Growth, Flowering, and Rooting

An important feature of auxin control of plant growth is its polar transport. Van der Weij, as reviewed by Goldsmith (1968), very early showed auxin transport to occur predominately in a basipetal direction. Basipetal transport has been shown to occur actively in living tissue, and is specific for IAA and a few synthetic auxins (Leopold and Lam, 1961). From a review of the literature, McCready (1966) concluded that basipetal auxin transport was

ubiquitous to seed plants and occurred in most plant parts, although it was found to be erratic in roots (Bonnette and Torrey, 1965). Jacobs (1950) found auxin transport to be most active near shoot tips, declining with distance down the stem. McCready and Jacobs (1963) showed a similar decline in petioles of leaves taken from different positions on the shoot, and attributed this decrease in transport to tissue aging.

Some workers (Jacobs, 1950; Brown and Wetmore, 1959) observed changes in auxin transport at different stages of plant development, and concluded that physiological events accompany such changes. One such event which alters transport is flowering. Leopold and Guernsey (1953) and more recently Naqvi and Gordon (1965) demonstrated a loss of strict basipetal auxin transport following flower initiation in Coleus blumei. More specifically related to rooting, Zaerr and Mitchell (1967) observed a direct relationship between indoleacetic acid transport and root formation in bean hypocotyls.

The concept of basipetal auxin transport has provided one basic theory for this dissertation concerning the relationship between flowering and rooting. That is, with the occurrence of flowering, the transport of auxin and probably other hormonal substances becomes acropetally oriented, and thereafter a reduction in rooting is observed.

This review has indicated a few of the many morphological and physiological factors affecting rooting. Limited reference has been made to literature involved with rooting of rhododendron because of its general nature and lack of applicability. Flowering has been shown to decrease rooting, although the nature of this inhibition was unclear. Leaves are apparently the main source of hormonal and nutritional factors needed in flowering and rooting. Moreover, treatments influencing normal leaf function also alter flowering and other facets of plant growth.

GENERAL METHODS AND MATERIALS

Only general methods and materials are presented in this section. Specific techniques and plant materials used in individual experiments are considered in the first part of each results section.

Definition of Terms

(a) Origin and Type of Shoots: (Figures 1, 2, and 3)

Origin

1. Terminal shoot: grows from a terminal bud.
2. Lateral shoot: grows from a lateral bud.

Type

1. Vegetative shoot: a shoot terminated by a leaf bud.
2. Flowering shoot: a shoot terminated by a flower bud.

(b) Leaf-petiole cutting: (Figure 3)

unit used to assay shoot rootability, consisting of an individual leaf and its petiole.

(c) Root-ball:

the mass of fibrous roots and attached medium measured in centimeters of greatest diameter.



Figure 1. Terminal shoot of *Rhododendron* 'Roseum Elegans'



Figure 2. Lateral shoot of *Rhododendron* 'Roseum Elegans'



Figure 3. Leaf-petiole cuttings from a vegetative shoot (top) and from a flowering shoot (bottom) of *Rhododendron* 'Roseum Elegans'.

Sources and Handling of Cuttings

Plant materials for seasonal rooting studies were obtained from 10-15-year-old stock plants located at a single nursery in Portland, Oregon. Materials used in the remaining experiments were obtained from six-year-old plants growing in apple boxes at the OSU research greenhouses in Corvallis, Oregon. The plants at both sources were of flowering age and, depending on the experiment, from one of the following cultivars of Rhododendron catawbiense:

| <u>Cultivar</u> | <u>Ease of Rooting</u> |
|------------------|------------------------------|
| 'Cynthia' | easy (80-100 percent) |
| 'Pink Pearl' | intermediate (40-80 percent) |
| 'Roseum Elegans' | easy (80-100 percent) |

Uniform terminal or lateral shoots were obtained by tagging them according to size and development shortly after bud break in the spring. Flowering shoots were used in all the experiments unless otherwise stated. Leaves and attached petioles were removed from the terminal or lateral shoots to assay shoot rootability at a given position. Such leaf petiole cuttings have been shown to accurately assay shoot rootability (Adams, 1967). When used, the standard hormone treatment consisted of a five-second dip in 5,000 ppm "Jiffy Grow" (3-indolebutyric acid 0.05 percent, 2-naphthalene-acetic acid 0.05 percent, boron 0.0175 percent, phenyl mercuric

acetate 0.01 percent) prior to placing the cuttings in the rooting bench.

Propagation Techniques and Facilities

The leaf-petiole cuttings were placed in the rooting bench for 90 and 120 days for easy- and intermediate-to-root cultivars, respectively. Spacing in the bench was about one and one-half inches in the row and two inches between rows. The rooting benches were located in a greenhouse equipped with intermittent mist. Air temperature was maintained at 50-55°F during the night and 60-65°F during the day. The rooting medium was a mixture of one-half number 16 mesh Del Monte White sand and one-half Canadian peat moss, and was maintained at 70-75°F with heating cables.

Plant Growth Analysis

Time and Measurement of Flowering

The time of flower initiation was determined using a method similar to the one devised by Adams and Roberts (1968); therefore, only the general steps involved will be outlined: (Figure 4)

1. The number of bud scales encompassing floral primordia was counted in mature buds.

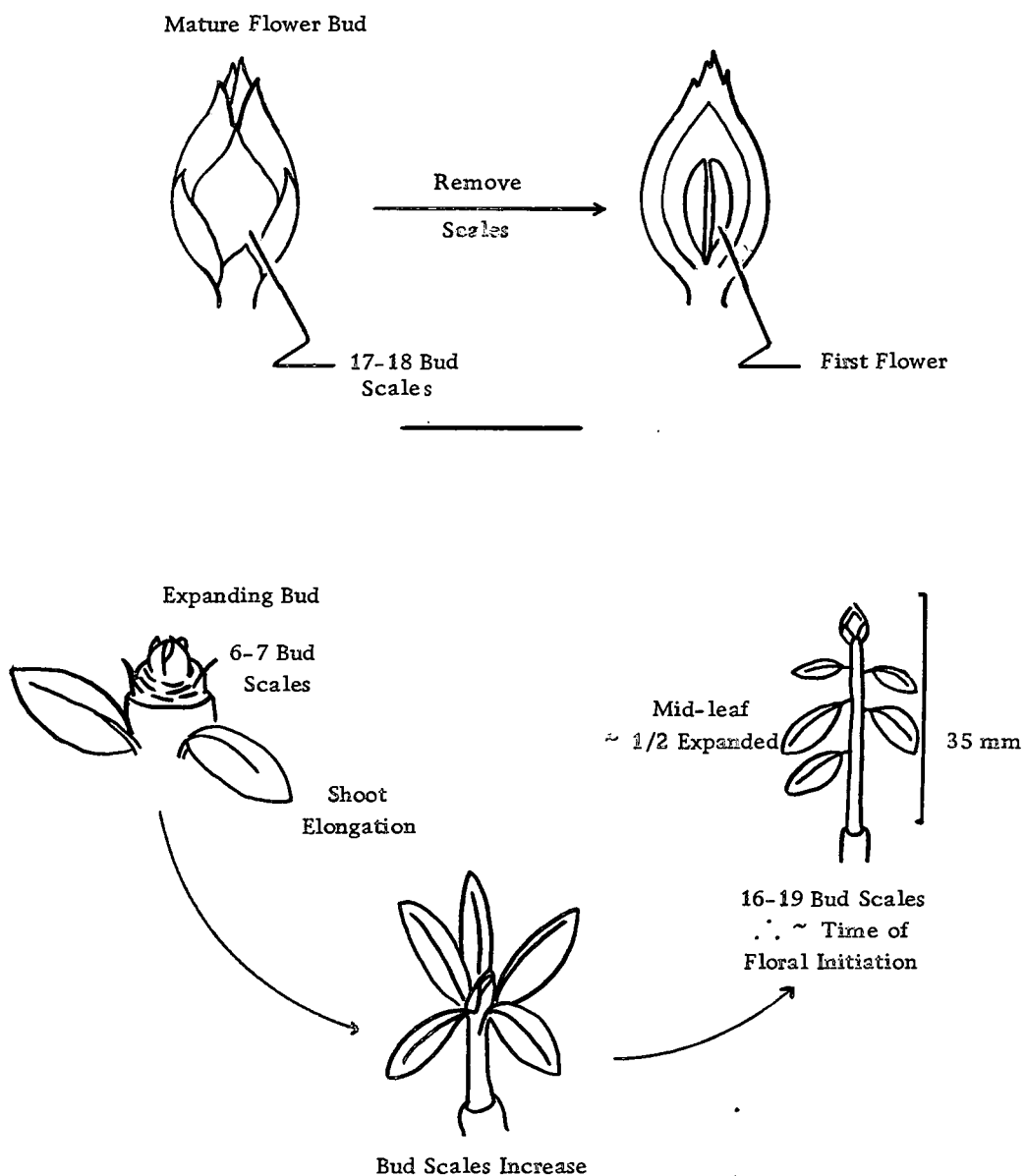


Figure 4. Diagram of the method used to determine time of flower initiation.

2. The number of bud scales in young shoots were counted prior to their elongation, and the increase in bud scales thereafter was followed by weekly sampling until the shoot was fully elongated.
3. Stage of shoot elongation was measured on the basis of shoot length from base to tip (cm).
4. Time of flower initiation was considered as that time when the number of primordia in the new terminal bud equalled the number of scales found later in mature flower buds.

Stage of flower bud development, used as a morphological time scale in estimating physiological age, was determined by measuring the diameter of the largest flower to the nearest tenth of a mm.

Development of Leaves

Leaf-petiole cuttings were used to determine leaf positional effects on rooting and to increase the amount of plant material available for a given experiment. For this purpose the shoot was arbitrarily divided into four zones, each containing approximately 25 percent of the leaf complement (Figure 5). The zones were numbered successively with leaf unfolding, the lower leaves being in zone 1 and the upper ones in zone 4.

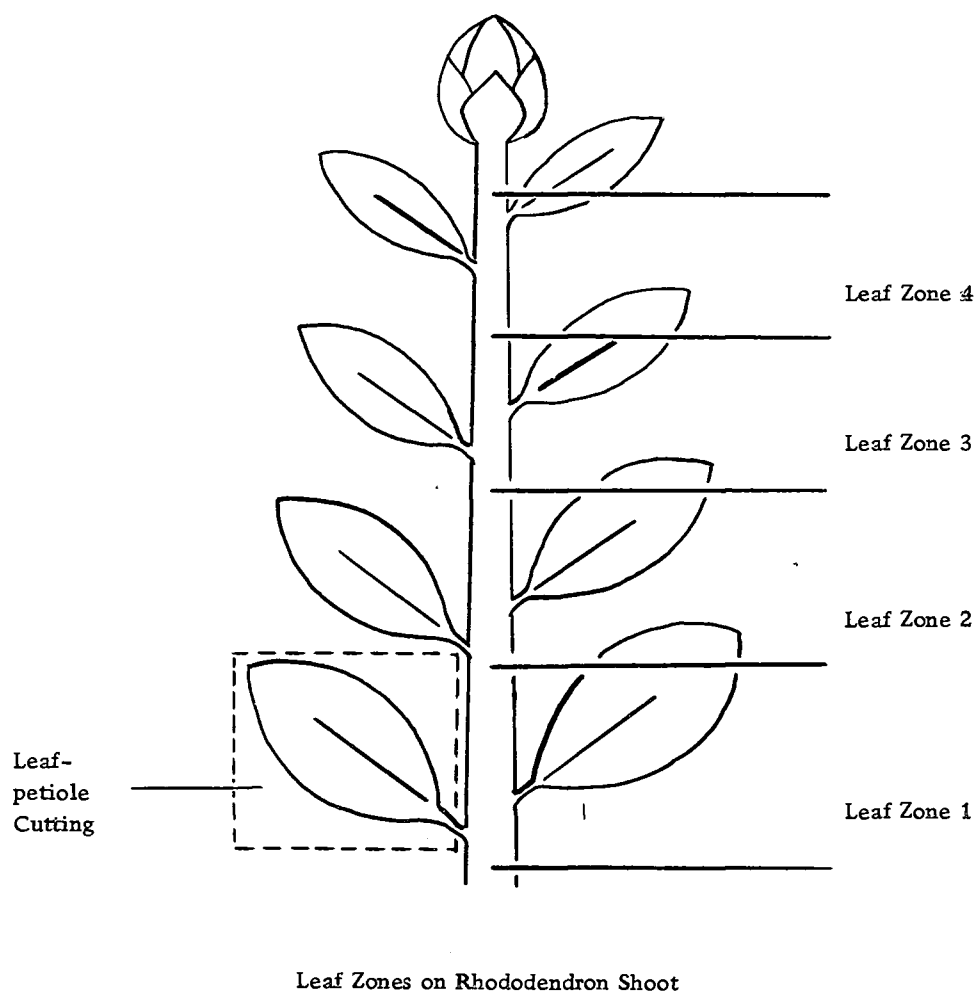


Figure 5. Diagrammatic representation of a leaf-petiole cutting and the arbitrary division of the shoot into four zones.

Leaf areas were determined by referring blade lengths to a standard curve constructed from a computed sample regression of leaf areas and blade lengths. Blade lengths (mm) were recorded prior to placing the cuttings in the rooting bench.

Rooting Response

Rooting responses were measured quantitatively as rooting-potential. Rooting-potential, calculated as root-ball diameter per 10 cm^2 of leaf area, was used to express the efficiency or physiological capacity of the leaf to develop a root system. Even the least rooting was recorded and measured to the nearest 0.5 cm.

Auxin Transport

Basic Rationale

The ability of petiole sections to transport auxin was used to indicate the impact of flowering on normal transport patterns. The basic rationale was that flowers presumably mobilize auxins, therefore the endogenous levels in tissues would be low and applied IAA-2- ^{14}C would be absorbed rather than transported. Therefore, definitive proof of auxins actually moving into the flower buds from leaves was not performed in this study.

Plant Materials and Procedure

Auxin transport in leaf petiole sections from flowering, vegetative, and specially treated rhododendron shoots was studied concurrently with rootability of remaining lamina-petiole stubs. The 6 mm petiole sections were placed upright on 0.2 ml circular receptor blocks of 1.5 percent agar (Figure 6). Since the rhododendron shoot was divided into four zones, transport was studied in four sets of petiole sections simultaneously by placing a larger donor block (2 ml) on the "leaf blade end" of the sections. This donor block contained a concentration of 10^{-5} M unlabelled and labelled indoleacetic acid (approximately 60,000 dpm IAA-2- 14 C with a specific activity of 47.2 mc/mM). The IAA from the donor block was allowed to move through the sections for three hours in the dark under conditions of fairly high humidity.

After the transport period, the agar blocks were placed in vials and fluor solution added for liquid scintillation counting on a Packard 3375 counter. The scintillation solution consisted of toluene: Triton X - 100 (2:1) and "Ominifluor" (98 percent PPO, 2 percent MSB) fluor compound. The fresh weight of the petiole sections was determined immediately after the transport period. Therefore, transport, calculated as the percent of labelled auxin in the receptor block (out of the total of 60,000 dpm in the donor)

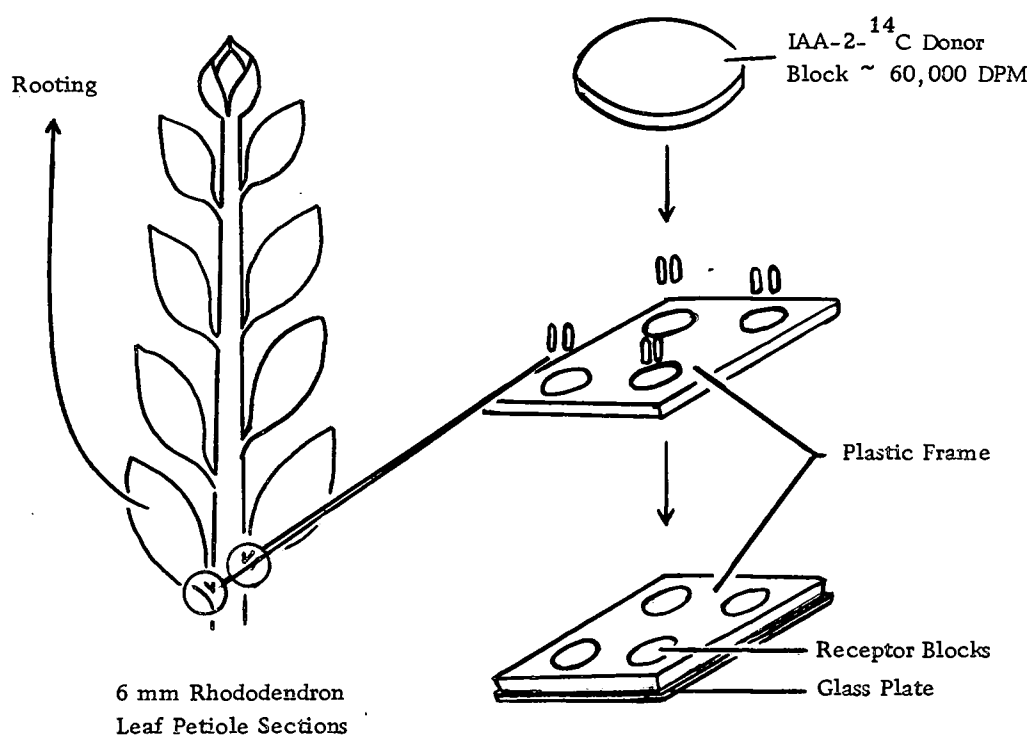


Figure 6. Diagram of the method used to measure transport of IAA-2-¹⁴C through petiole sections.

per 100 mg. fresh weight, was used to express the amount of labelled auxin moving through petiole sections.

The petioles from each zone were sectioned and placed in separate test tubes for a five-hour extraction period with 2 ml of 95 percent alcohol in a 50°C water bath. Similarly, a few of the receptor blocks were extracted with 2 ml of 95 percent alcohol for five hours. The petiole extracts were prepared for counting using the same techniques and fluor solutions mentioned above. Absorption, calculated as the percent of labelled auxin absorbed by the petiole tissue (out of the total of 60,000 dpm in the donor) per 100 mg fresh weight, was used to express the amount of labelled auxin absorbed by the petiole tissue.

Isolation and Identity of Presumptive IAA-2-¹⁴C

Chromatographic techniques were used to isolate the radioactivity of some of the extracts mentioned above. The extracts (0.2 ml) were applied to Whatmann Number 1 filter paper and developed in one of three solvent systems: (1) Isopropanol: Ammonium: Water (8:1:1), (2) N-Butanol: Ethanol: Water (7:1:2), (3) Chloroform: Acetic Acid (96:1). Following development, the chromatograms were scanned on a Packard Radiochromatogram Scanner Model 7201 to locate the positions of radioactive substances. Comparisons of R_f values were made with the authentic

labelled IAA. Further identification involved spraying with Ehrlich's reagent and examination of the strips under ultra-violet light.

Statistical Interpretation

The statistical methods used in interpreting the data are described in Steel and Torrie (1960). Depending on the experiment, the significance of differences in rooting performance, leaf area and auxin transport were determined using a randomized block or split plot arrangement of treatments and least significant difference tests. The shoot was considered a replication, since each was uniform and represented a given treatment unit.

RESULTS

Influence of Flower Bud Development on Rootability (I)

The object of this experiment was to measure the inhibitory effects of flower development on rooting in an easy-to-root cultivar ('Cynthia') and one that is moderately difficult to root ('Pink Pearl'). An additional purpose was to verify an earlier observation of intense rooting inhibition by flowers at certain stages of development.

Hormone treatments were used to determine if the effects of flower competition with rooting could be overcome.

Plant materials of 'Pink Pearl' and 'Cynthia' were obtained from 10-15-year-old stock plants growing in full sun at a single location in Portland, Oregon. Twelve uniform terminal shoots of each cultivar were collected at approximately three-week intervals from June, 1966 to May, 1967. Leaf-petiole cuttings from these shoots were used to assay shoot rootability. One half of the cuttings on each sampling date were given hormone treatment.

The terminal bud from each shoot was dissected and the diameter (mm) of the largest flower recorded. The time at which the largest flower reached 1 mm diameter was considered a standard point in morphological development, or base date. Shoot age was then calculated as days beyond this point. Chronological or calendar age was used only to indicate the month of sampling.

The 'Pink Pearl' and 'Cynthia' cuttings were given a 120- and 90-day rooting period, respectively. Table 1 presents the dates on which the various samples were placed in and removed from the rooting bench.

Table 1. Dates when leaf-petiole cuttings of Rhododendron 'Pink Pearl' and 'Cynthia' were placed in and removed from the rooting bench.

| Sample Number | Sampling Date 'Pink Pearl', 'Cynthia' | Recording Date | |
|---------------|--|----------------|---------------|
| | | 'Pink Pearl' | 'Cynthia' |
| 1 | Jun. 11, 1967 | Oct. 9, 1967 | Sep. 9, 1967 |
| 2 | Jul. 25 | Nov. 22 | Oct. 13 |
| 3 | Aug. 23 | Dec. 21 | Nov. 21 |
| 4 | Sep. 11 | Jan. 9, 1968 | Dec. 10 |
| 5 | Oct. 14 | Feb. 11 | Jan. 12, 1968 |
| 6 | Nov. 14 | Mar. 13 | Feb. 12 |
| 7 | Dec. 8 | Apr. 7 | Mar. 8 |
| 8 | Jan. 13, 1968 | May 12 | Apr. 12 |
| 9 | Feb. 17 | Jun. 16 | May 17 |
| 10 | Mar. 20 | Jul. 18 | Jun. 18 |
| 11 | Apr. 17 | Aug. 15 | Jul. 16 |
| 12 | May 14 | Sep. 11 | Aug. 12 |

Rooting response, measured as rooting-potential, was plotted against time, based on flower development.

Effects of Flower Development on the Rooting of 'Cynthia' Cuttings

Flower bud diameter in expanding 'Cynthia' shoots showed sigmoidal development, increasing in size throughout the summer but leveling off in November and remaining constant until bud break the following spring (Figure 7). The period of rapid shoot expansion

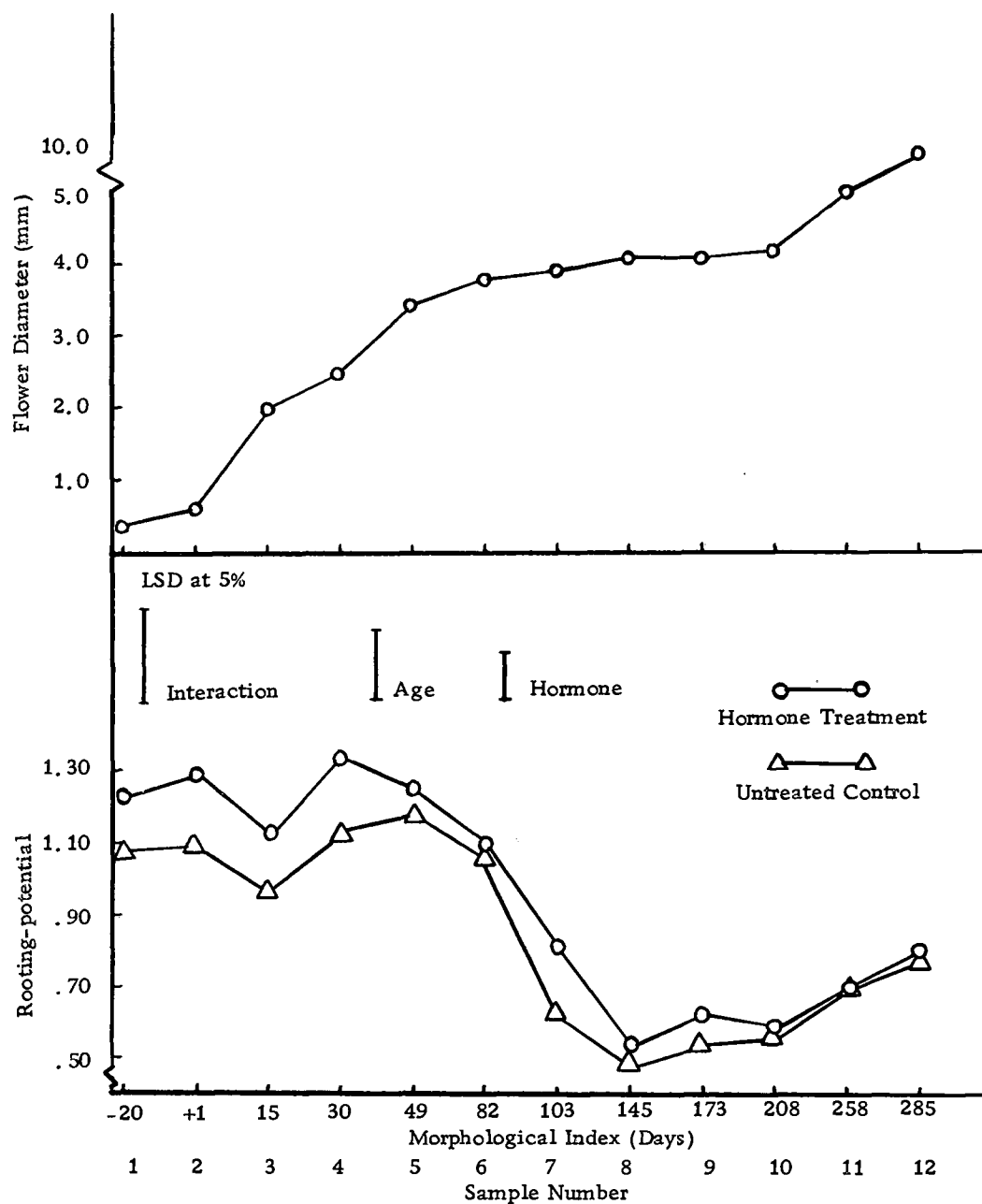


Figure 7. Rooting response of leaf-petiole cuttings from *Rhododendron* 'Cynthia' shoots as related to sampling date and flower development (physiological age).

began in early June, and the base date in morphological development was reached in early July.

The rooting-potential of 'Cynthia' cuttings showed no marked changes during the period (-20 to +82 days from base date) of flower development (Figure 7). There was a slight decline at +15 days but otherwise rooting-potential remained fairly constant during flower bud development, dropping with the onset of dormancy. There was a slight increase in rooting with the breaking of dormancy.

There was no significant enhancement of rooting with hormone treatment, although slight improvement was obtained during early stages of flower development.

Effects of Flower Development on the Rooting of 'Pink Pearl' Cuttings

Flower development in 'Pink Pearl' shoots was similar to that of 'Cynthia', except that a noticeable reduction in bud growth rate occurred at 57 days (sample 5) after the morphological base date (Figure 8). This base date in morphological development was reached in early August.

In contrast to 'Cynthia' there were significant changes in leaf rootability during flower development in 'Pink Pearl'. There was an initial drop in rooting-potential at -12 days, but followed by a peak in rooting at +37 days (Figure 8). This was followed in turn

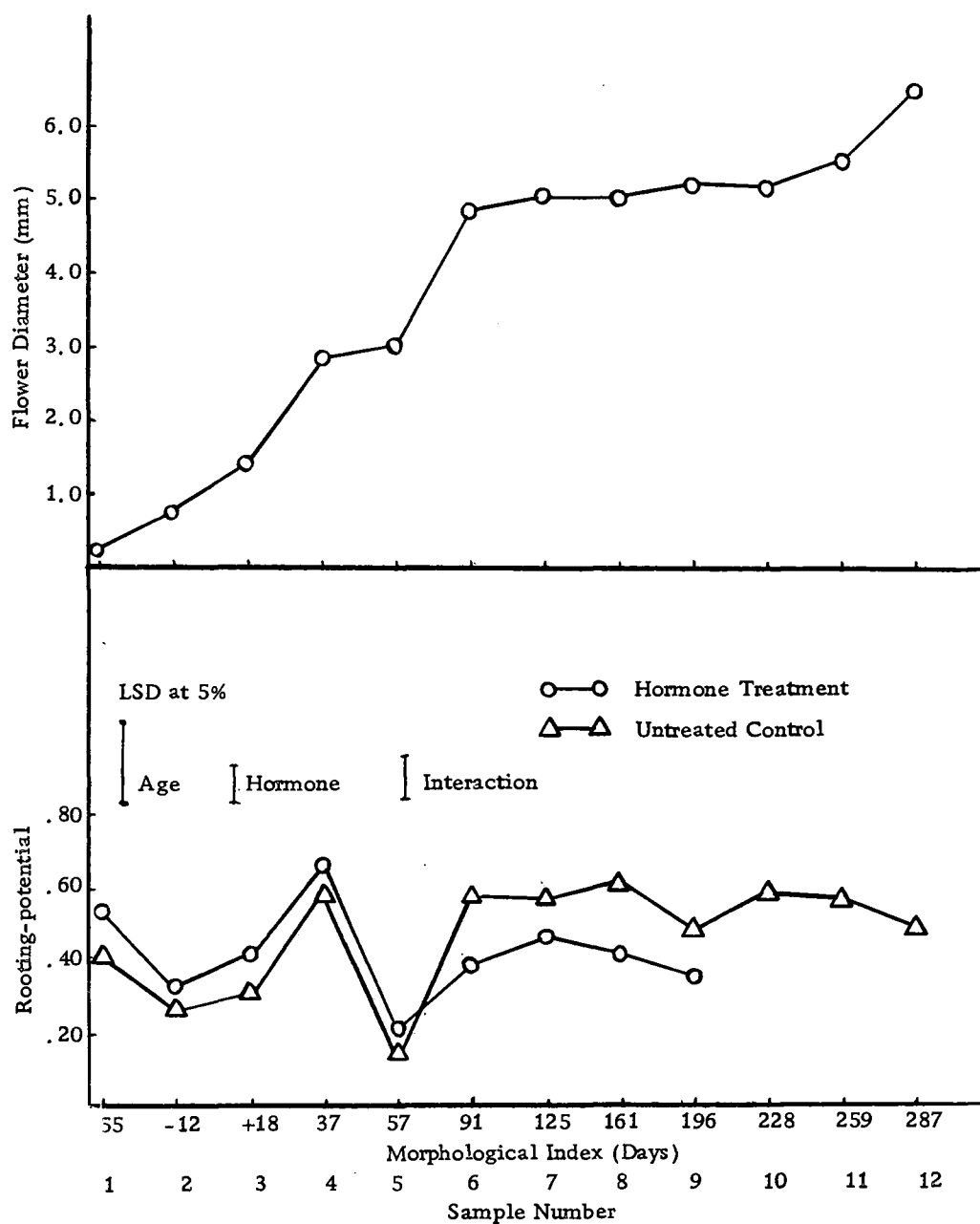


Figure 8. Rooting response of leaf-petiole cuttings from Rhododendron 'Pink Pearl' shoots as related to sampling date and flower development (physiological age).

by a significant reduction in rootability at +57 days. Adams (1967) observed a similar period of low rooting in this cultivar. After this low point, rootability again increased to a relatively high level at +91 days, particularly in untreated cuttings, and remained fairly constant.

Hormone treatment enhanced rooting slightly during the early stages of flower development, but at +91 days (sample 5), such treatment slightly inhibited rooting-potential. Phytotoxicity and consequently a loss of cuttings occurred with hormone treatment at +228 - +287 days.

The two cultivars showed very different rooting responses to the presence of flower-buds. Rooting in 'Cynthia' was virtually unaffected by flower development, but declined with the approach of dormancy. 'Pink Pearl' shoots, on the other hand, showed definite changes in rootability with certain stages of flower development. Response to hormone treatment was not great with either cultivar, except that rooting in 'Pink Pearl' was significantly inhibited by hormone treatment during March, April and May.

Leaf Size and Rooting-potential as Related to Their Position on Vegetative and Flowering Shoots (II)

Previous work has shown that leaves unfolding during flower initiation are subsequently larger than those expanded before or

after initiation (Thomas, 1961). Such leaves contain low auxin levels (Humphries and Wheeler, 1963), which may account for their poor rootability. It has not been clearly demonstrated that flowering decreases rootability of all the leaves on a shoot, or only those expanded during flower initiation.

The purpose of this study was (a) to determine the effects of position on size and rootability of leaves from flowering and vegetative shoots of 'Pink Pearl' or 'Roseum Elegans', and (b) to determine if hormone treatment enhances rooting equally in all leaf zones.

Uniform shoots of 'Pink Pearl' and 'Roseum Elegans' were obtained from six-year-old stock plants growing in apple boxes at the OSU research greenhouses. On August 20, 1966, shoot and leaf expansion completed, ten vegetative and ten flowering shoots were collected from each cultivar and leaf-petioles used to assay rootability. At the same time leaf areas were determined. After 90 days for 'Roseum Elegans' and 120 days for 'Pink Pearl', rooting evaluations were made. Differences in leaf area and rooting-potential were considered for each leaf zone on the shoot.

Leaf-petiole cuttings from twelve vegetative and twelve flowering 'Pink Pearl' shoots were sampled on August 20 from the same plants described above. All of the cuttings from six of each shoot type received hormone treatment. Rooting evaluations were made after 120 days in the rooting bench.

Effects of Flowering on the Size and Rooting-potential of Leaves

The leaves from flowering shoots of both cultivars were consistently larger than those from vegetative ones, with the largest leaves in zone 2 and decreasing in size to zone 4 (Figure 9). However, there was no significant difference between the size of zone 4 leaves on flowering and vegetative shoots. Differences in the size of leaves from the four zones on flowering and vegetative shoots were similar with both cultivars.

The rooting-potential of leaves from flowering shoots of 'Roseum Elegans' was greatest in the basal and terminal leaves (zones 1 and 4), and the lowest in zone 2 (Figure 9). The leaves from vegetative shoots showed significantly greater rooting than those from flowering shoots in zones 1 and 2; however, the magnitude of differences between the two types of shoots in this cultivar decreased toward the upper leaf zones.

The rooting-potential of leaves from flowering shoots of 'Pink Pearl' increased progressively from zone 2 to 3 with an even greater increase to zone 4 (Figure 9). The rooting-potential of leaves from vegetative shoots was significantly greater than those from flowering shoots in the two lower leaf zones; however, in contrast, zone 4 leaves from flowering shoots rooted better than those from this zone on vegetative shoots. Rooting-potential in general decreased with flowering, except the leaves from zone 4 of 'Pink Pearl' shoots.

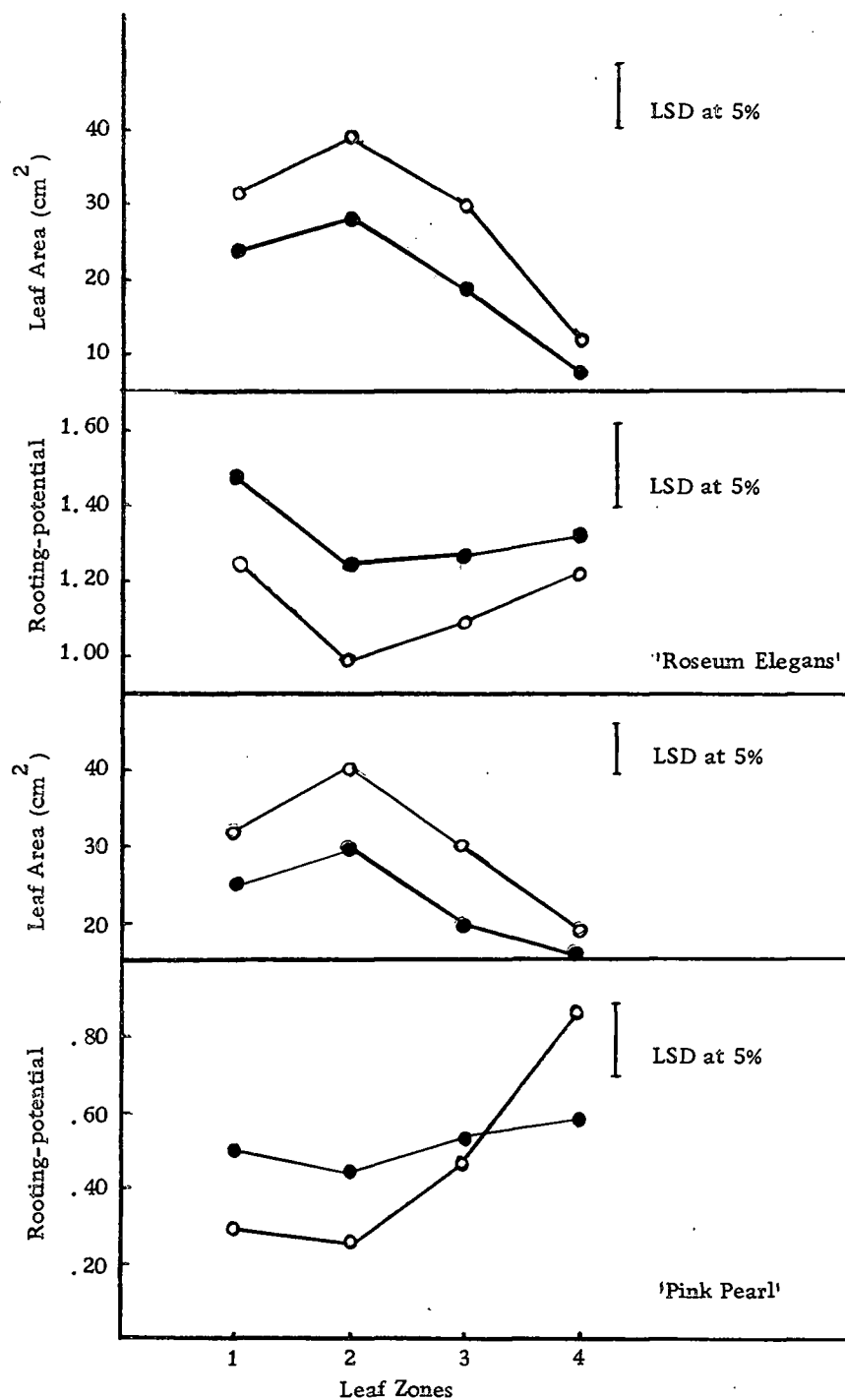


Figure 9. Leaf area and rooting-potential of leaf-petiole cuttings taken from flowering (○—○) and vegetative (●—●) *Rhododendron* 'Pink Pearl' and 'Roseum Elegans' shoots.

Effects of Hormone Treatment on Rooting-potential

The rooting-potential of untreated leaf-petiole cuttings from flowering 'Pink Pearl' shoots was greatest in the terminal leaves with a decrease in rooting towards the basal leaves, while those from vegetative shoots showed only small difference in rooting among zones (Figure 10). Hormone treatment of leaves from flowering shoots significantly increased rooting in the three lower zones, but such treatment actually decreased rooting in zone 4.

Hormone treatment had little effect on the rooting of leaves from the two lower zones of vegetative shoots. On the other hand, there was significant enhancement of rooting with treatment of leaves in the two upper zones. Thus, hormone treatment enhanced the rooting of the lower leaves of flowering shoots and the upper leaves of vegetative shoots.

Time of Root Inhibition by Flowering (III)

Previous work (experiment I) showed that certain stages of flower development affected rooting more than others. Also, it was found that the effect of flowering on rooting of leaves depended on their position on the shoot (experiment II). Turezkaya (Selim, 1956) found in cuttings of Perilla nankinenses that flowering decreased rooting near the time of flower initiation; however, there was

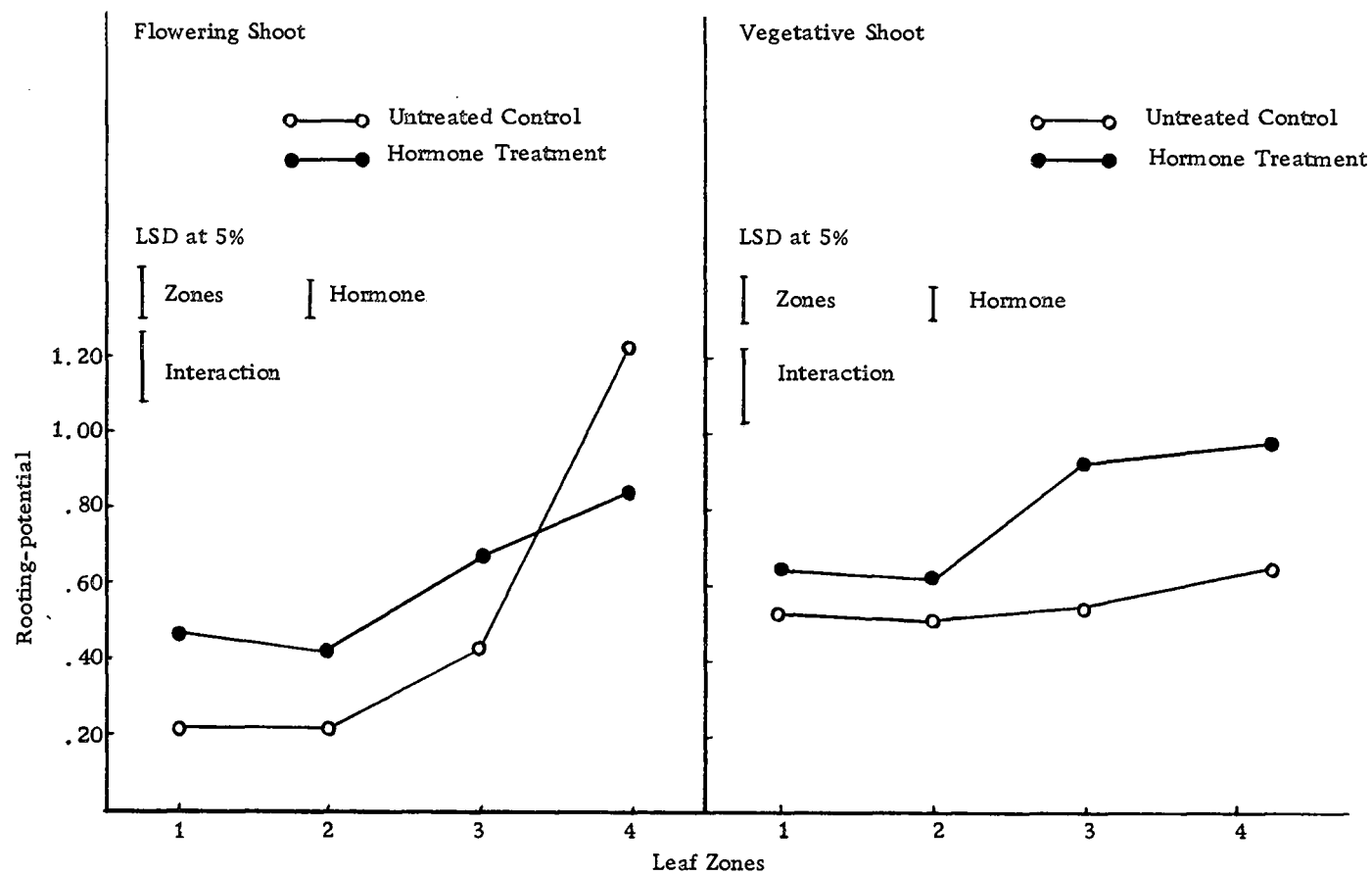


Figure 10. Effect of hormone treatment on the rooting-potential of leaf-petiole cuttings from various zones of flowering and vegetative *Rhododendron* 'Pink Pearl' shoots.

insufficient evidence as to whether inhibition occurred at time of flower initiation or at later stages of development. Since flower initiation in rhododendron has been shown to occur with approximately 35 mm shoot elongation, excision of the apex before this time should remove the effects of flowering. It also was observed that leaves of flowering shoots are larger than those of vegetative ones. Thus, the purpose of this study was to determine if the removal of flowering influence by terminal bud excision before, during, or after flower initiation could effectively reduce leaf size and enhance rootability of rhododendron leaves.

The stock plants used in the experiment were six years old and grown in apple boxes at the OSU research greenhouses. Lateral buds in the axils of the second or third leaf below the current terminal flower buds were selected and tagged. Previous studies showed these buds to produce predominantly flowering shoots. During the first two weeks of June, the terminals of six or more expanding shoots were excised with a probe at each of six stages:

| <u>Stage of shoot development</u> | <u>Shoot length at the time of terminal bud excision</u> |
|-----------------------------------|--|
| 1 | 20-29 mm |
| 2 | 30-35 mm |
| 3 | 36-45 mm |
| 4 | 46-55 mm |
| 5 | 56-65 mm |
| 6 | 66-75 mm |

On September 1, 1966 six shoots from each stage of bud excision, and an equal number of flowering controls, were collected and their leaf-petioles used to assay rootability. The rooting-potential of the untreated leaf-petiole cuttings was determined after a 120-day rooting period.

Lateral buds subtending the excised terminals were dissected at time of sampling to determine the extent of their development.

Effects of Terminal Bud Excision on Leaf Size and Lateral Bud Development

Terminal bud excision of potentially flowering shoots at all stages of development generally decreased leaf size in the two lower leaf zones (Figure 11). Excision during the first three stages decreased leaf size in zone 3, but no treatment significantly altered leaf size in zone 4. Bud excision before flower initiation (stage 2) was the only treatment that consistently reduced leaf size in the first three zones.

Lateral buds on shoots that had terminals removed during stage 1 were inactive, that is, these buds contained the same number of scales as those on intact flowering shoots. However, the lateral buds on shoots whose terminal buds were excised at later stages of shoot development (2-6) showed active growth of new scales and differentiation of leaves.

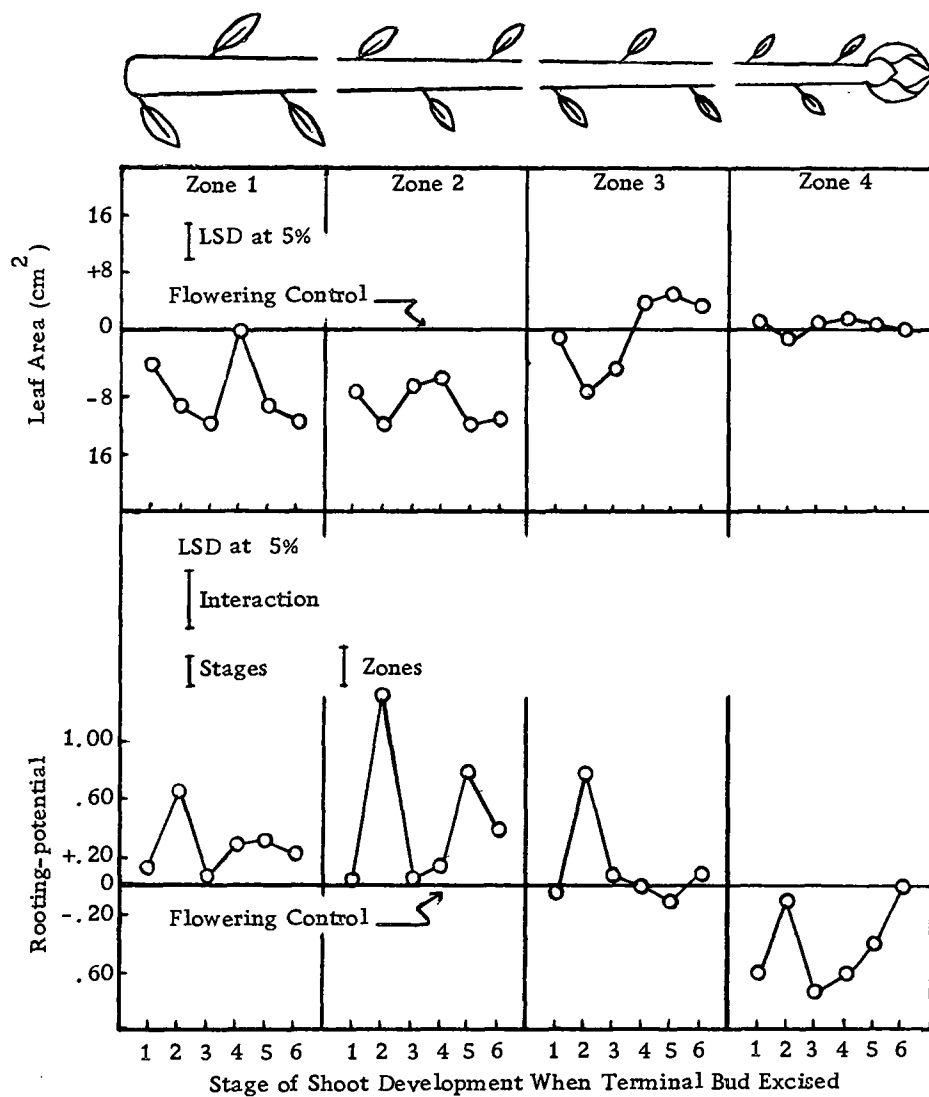


Figure 11. Leaf area and rooting-potential of leaf-petiole cuttings from various zones of *Rhododendron* 'Pink Pearl' shoots following terminal bud excision at 1-(20-29 mm), 2-(30-35 mm), 3-(36-45 mm), 4-(46-55 mm), 5-(56-65 mm) and 6-(66-75 mm) stages of shoot development.

Effects of Terminal Bud Excision on Rooting-potential

There was a tendency for rooting-potential to be inversely related to leaf size in the lower three zones (Figure 11). Table 2 summarizes the effects of terminal bud excision on rooting-potential.

Table 2. Effects of terminal bud excision at six stages of shoot development on rooting-potential of leaf-petiole cuttings from various leaf zones of *Rhododendron* 'Pink Pearl' shoots.

| Leaf Zone | Stage of Shoot Development When Terminal Bud Excised | | | | | |
|-----------|---|-------|------|------|------|------|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| 1 | +.17 ^a | + .60 | +.08 | +.30 | +.34 | +.23 |
| 2 | +.03 | +1.30 | +.05 | +.10 | +.80 | +.40 |
| 3 | -.05 | + .75 | +.08 | +.07 | -.10 | +.08 |
| 4 | -.63 | - .10 | -.70 | -.60 | -.40 | .00 |

LSD at 5% - Interaction = .38

^a Rooting-potential, (+) greater than flowering control, (-) less than flowering control.

This experiment indicates that the reduced rootability accompanying flowering probably begins at time of initiation. Excision of the apex prior to flower initiation (stage 2) reduced leaf size in the first three zones and significantly improved leaf rootability. Very early and later excision also improved rooting-potential slightly in the lower leaf zones. However, bud excision significantly reduced

the rooting-potential of zone 4 leaves except when done during stages 2 and 6.

Importance of Leaves in Flowering and Rooting (IV)

Leaves are thought to be the site of synthesis of materials required in flower initiation and development. Therefore, complete or partial defoliation should provide a means of inhibiting flower bud development and altering the function of remaining leaves. In addition, partial defoliation should be a useful means of studying the role of various leaves as exporters or users of materials needed in growth and development. An experiment using controlled defoliation treatments was designed to determine the importance of leaves in flowering, and the significance of their position on the shoot in supplying growth substances needed in flower development and root regeneration.

Eighty uniform terminal shoots on 'Pink Pearl' and 'Roseum Elegans' stock plants located at the OSU research greenhouses were tagged shortly after bud break in the spring of 1966. Three shoots of each cultivar were subsequently defoliated when they attained 20, 40, 60, 80, 100, 120, 140, 160, 180, and 200 mm in length. Field observation of the effects of defoliation on flowering were made in early October.

One hundred uniform terminal buds on similar 'Pink Pearl' and 'Roseum Elegans' stock plants were tagged in the spring of 1967. When the shoots reached 60 - 70 mm in length, various portions of the leaf complement were removed from different positions. Ten shoots each were given four partial defoliation treatments with intact shoots as controls for a total of fifty shoots of each cultivar. The young leaves were removed from either the lower or upper portions of the shoot leaving the following leaf complement:

| Quantity ² and Position of Leaves Retained on Shoot | | | | | |
|---|---|---|-------|---|---|
| 25 percent of lower leaves retained | | | | | |
| 50 | " | " | " | " | " |
| 25 | " | " | upper | " | " |
| 50 | " | " | " | " | " |
| control - all leaves retained | | | | | |

The shoots were collected for propagation on September 1, and leaf-petiole cuttings were used to assay shoot rootability. The effects of partial defoliation on leaf and flower development were determined at that time. The 'Roseum Elegans' and 'Pink Pearl' cuttings were in the rooting bench for a period of 90 and 120 days,

²Percentage of the total leaf complement retained after partial defoliation of shoots.

respectively. Rootability of leaves from treated shoots was compared only to those leaves from similar positions on control shoots.

Effects of Complete Defoliation on Flower Development

Since the two cultivars responded similarly to defoliation, the observations are pertinent to both. Response of the shoots to complete defoliation at various stages of development are shown in Table 3.

Table 3. Effects of complete defoliation at different stages of shoot development on type of growth response in *Rhododendron* 'Pink Pearl' and 'Roseum Elegans' terminal buds.

| Shoot Length When Defoliated mm | Growth Response |
|---------------------------------------|---|
| 20 | vegetative development |
| 40 | |
| 60 | |
| 80 | |
| 100 | |
| 120 | transition stage (teratological development) |
| 140 | |
| 160 | |
| 180 | flower development |
| 200 | |
| 220 | |
| 240 | |

Shoot defoliation prior to its reaching 100 mm in length produced a second flush of vegetative growth (Figure 12). Transitional



Figure 12. Secondary growth flush resulting from complete defoliation of *Rhododendron* 'Pink Pearl' shoots at 20-100 mm stage of development.

or teratological development³ occurred with defoliation at shoot lengths of 100 - 140 mm (Figure 13), and smaller than normal flowers developed when defoliation was delayed until after 140 mm of elongation.

Effects of Partial Defoliation on Flower Development

Vegetative reversion resulted in both cultivars when only 25 percent of the leaves (out of the total leaf complement) were retained in the lowest part of the shoot (Figure 14). However, when only 25 percent of the upper leaves were retained, the terminal bud aborted and lateral shoot growth resulted. Smaller than normal flower buds developed in 'Pink Pearl' when 50 percent of the lower leaves were removed, but vegetative reversion occurred in 'Roseum Elegans' shoots with such treatment. Flower buds that were smaller than normal (untreated controls) developed in both cultivars when only 50 percent of the upper leaves were retained. These results indicate that a certain amount of leaf surface is necessary for sustained flower development, and as in the case of 'Roseum Elegans', these leaves must be present in specific areas of the shoot.

³ Malformation in flower buds as a result of phylloidy of the bracts and petaloidy of the stamens.

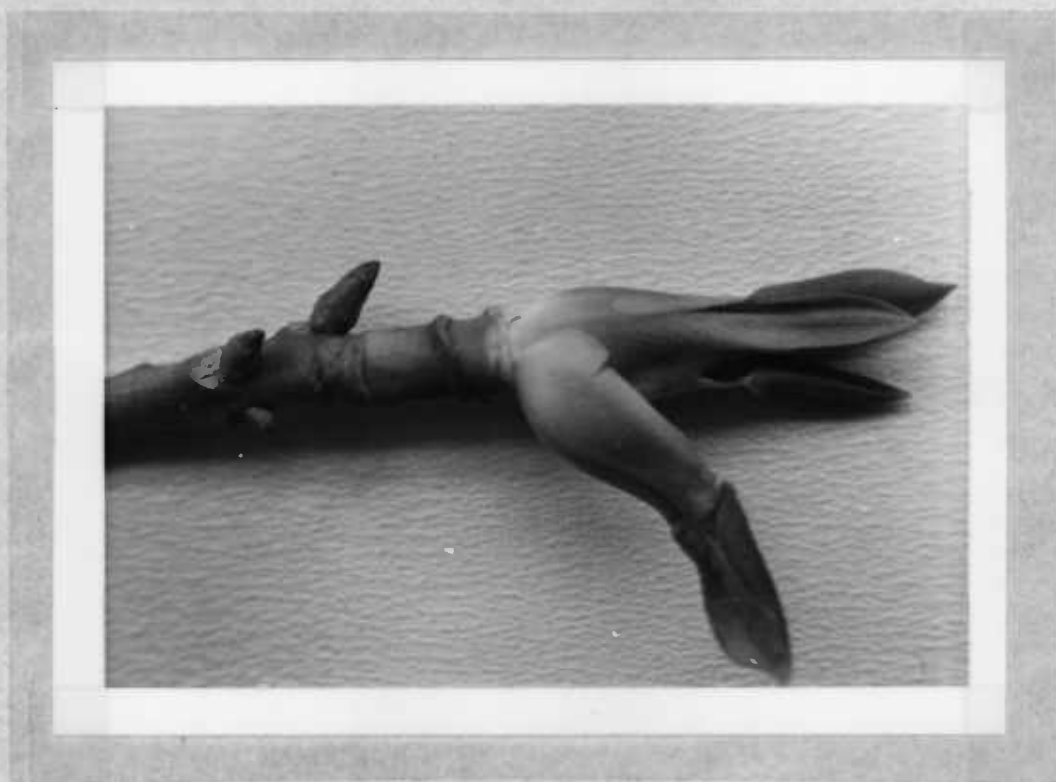


Figure 13. Teratological flower bud formation resulting from complete defoliation of *Rhododendron* 'Roseum Elegans' shoots at 100-140 mm stage of development.

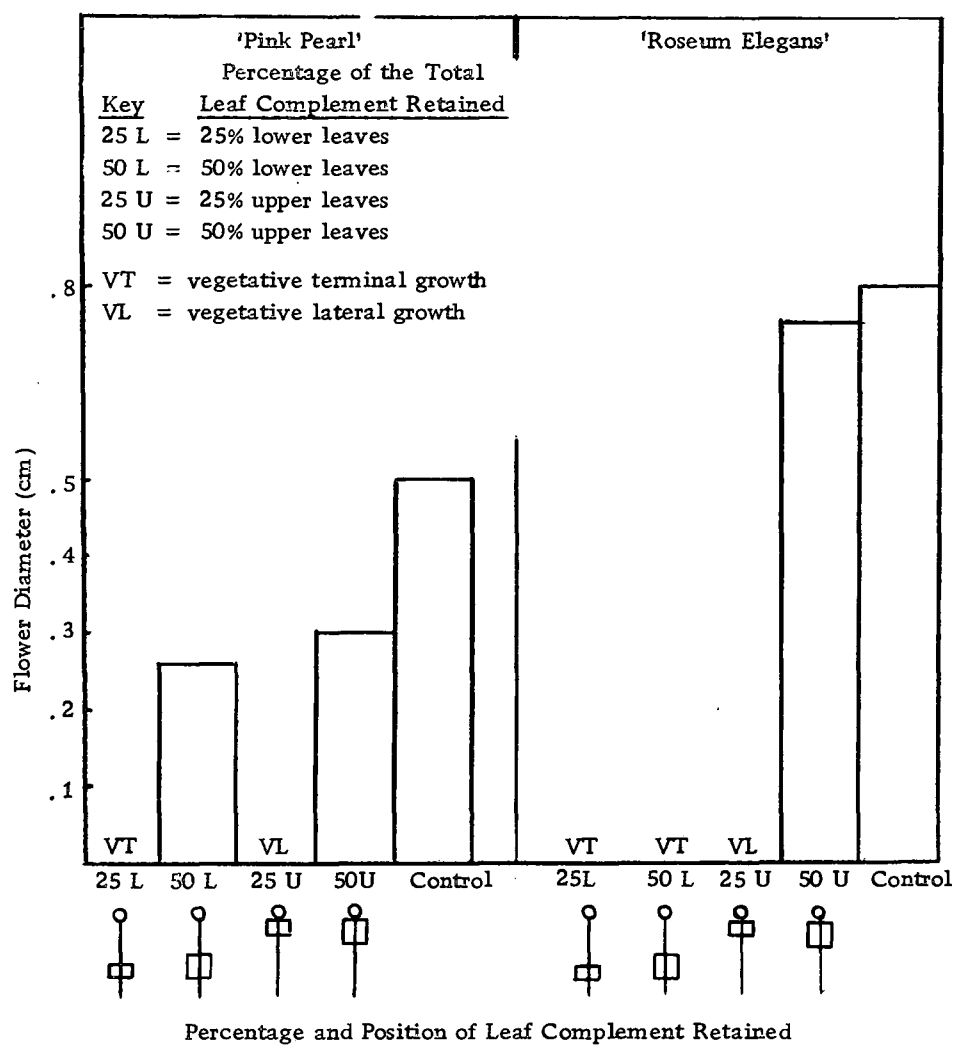


Figure 14. Effects of partial defoliation on flower bud development in *Rhododendron* 'Pink Pearl' and 'Roseum Elegans' shoots.

Effects of Partial Defoliation on Leaf Size and Rooting-potential

Defoliation of the upper portions of shoots of both cultivars produced no significant change in the size of the remaining lower leaves (Figures 15 and 16). However, removing lower leaves significantly increased the size of the normally small terminal leaves.

There was a general inverse relationship between leaf size and rooting-potential in partially defoliated 'Roseum Elegans' shoots (Figure 15). Removing the upper leaves significantly increased the rootability of the remaining lower leaves. This increase was greatest with 75 percent removal of the upper leaves (out of the total leaf complement), which dropped somewhat with the removal of only 50 percent of these leaves. The removal of 50 percent of the lower leaves significantly decreased rooting-potential of remaining upper leaves.

Removing 75 percent of the upper leaves on 'Pink Pearl' shoots significantly increased the rooting-potential of remaining lower leaves, while removing only 50 percent of the upper leaves had no significant effect (Figure 16). However, the removal of 50 percent of the lower leaves significantly decreased the rooting-potential of the remaining upper leaves.

Partial defoliation before 100 mm of shoot elongation greatly affected the development of the flower buds. Removing upper leaves

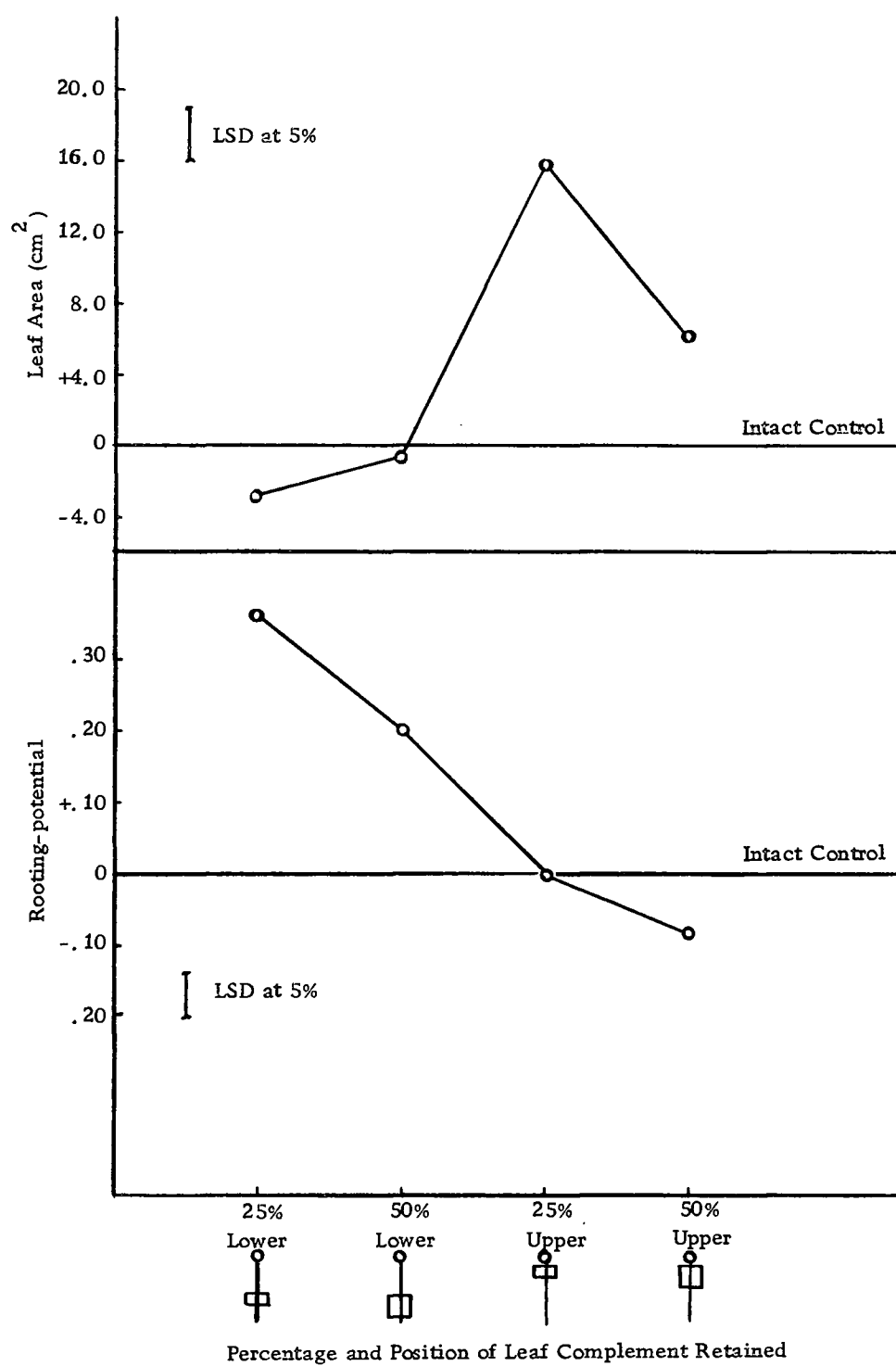


Figure 15. Effects of partial defoliation on the size and rooting-potential of leaves retained on *Rhododendron* 'Roseum Elegans' shoots.

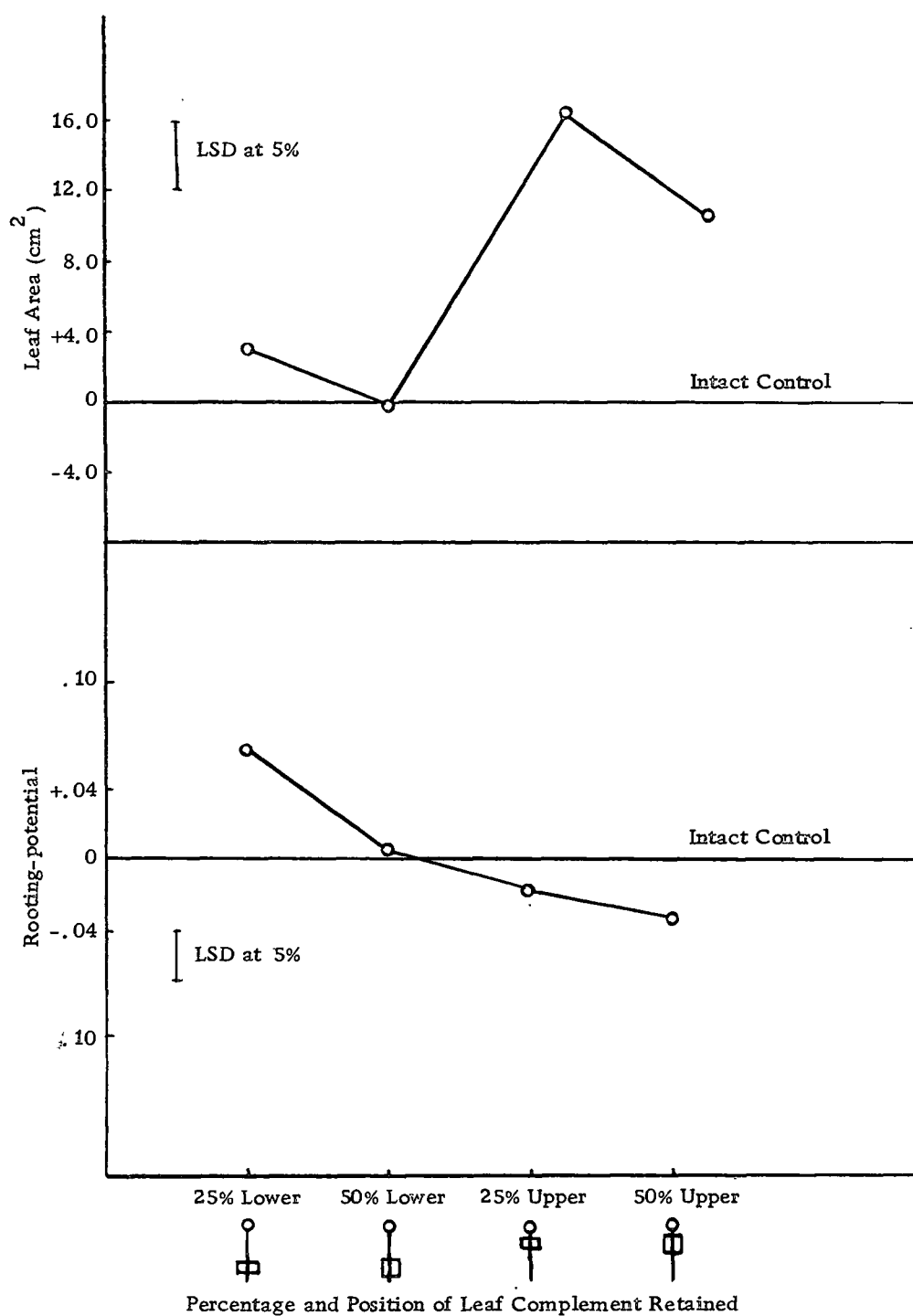


Figure 16. Effects of partial defoliation on the size and rooting-potential of leaves retained on *Rhododendron* 'Pink Pearl' shoots.

tended to increase rooting of the remaining lower leaves; however, removing the lower leaves reduced the rootability of the retained upper leaves.

Shading Treatments Designed to Study the Effects of Flowering and Leaf Size on Rootability (V)

The previous experiment (IV) suggested that leaves are important suppliers of factors needed in flower and root development. Therefore, changes in hormonal and nutritive substances in the leaves should be reflected in both flowering and rooting. It has been observed that leaves of flowering plants are larger than those of vegetative ones, and that shading increases leaf size. Also, plants grown under low light intensity are known to have less enzymatic activity and greater auxin content than those in full sunlight.

The intent of this experiment was to study the effect of shade on flower development and leaf size, and if changes in growth substances occurring with heavy shade could mask the flowering influence on rooting.

Six-year-old stock plants of 'Pink Pearl' and 'Roseum Elegans', bearing approximately 100 terminal and lateral flower buds, were grown in apple boxes at the OSU research greenhouses. Prior to bud break in early May, two plants of each cultivar were placed outdoors under each of four wooden frames covered with saran

shade cloth giving 25, 50, 75, and 90 percent shade according to manufacturer's specifications.

Light intensity and temperature under these different shades were measured during the 1967 growing season. Spectral intensity measurements were taken between 11:00 am and 1:00 pm under these shades with an ISCO model SR spectroradiometer. Light intensity readings were expressed as microwatts/cm²/millimicron and approximate foot-candles in the visible light range. Temperatures were recorded with individual Tempscribe recorders placed at plant height under each shade frame. High and low daily temperatures were composited and averaged for expression of temperature on a monthly basis.

Forty uniform, terminal buds, or four per cultivar under each shade treatment, were tagged shortly after bud break. As these buds expanded and developed into shoots, the area of their mid-terminal leaves was determined at four day intervals until fully expanded.

The percentage of flower buds, or the relationship of flower buds to total buds on each stock plant, was determined in 1967 and 1968 for each of the shade treatments. Shoots from the various shade treatments were used in several rooting experiments, and the type of bud terminating the shoot was recorded at the time of sample collection. Also, the flowering and vegetative shoots remaining on the stock plants were counted at the end of the season for determining

the percentage of flower buds out of the total number of buds for a given light treatment.

Thirty terminal 'Roseum Elegans' shoots, or six from each shade treatment, were collected at regular monthly intervals from June (6/12/68) until September (9/12/68). The diameter of the terminal flower bud (mm) was recorded at time of collection. Leaf-petiole cuttings were used to assay shoot rootability after a 90-day rooting period. Shoot rooting-potential was determined by compositing those for individual leaf zones.

The effects of shade treatment on leaf size and rooting-potential in various leaf zones were also studied in cultivars 'Pink Pearl' and 'Roseum Elegans'. Six shoots per shade treatment and cultivar, or a total of sixty shoots, were collected on September 1, 1967. Leaf size was determined as outlined previously, and leaf-petiole cuttings were used to assay shoot rootability. After a standard rooting period, the rooting-potential for each leaf zone was determined.

The response of shaded cuttings to hormone treatment was studied in 'Roseum Elegans'. Twelve shoots from each shade treatment were collected on four monthly sampling dates beginning in early June (6/12/68). Leaf-petiole cuttings from six of the shoots on a given sampling date received the standard hormone treatment. Rooting-potential was determined after a 90-day rooting period.

Effects of Shading on Light Intensity and Quality

The spectral readings for the different shade treatments on a clear day in July are shown in Figure 17. The same general spectral and intensity relationships between treatments were observed on overcast days. The principal energy peaks occurred at approximately 525 and 610 mu. The characteristics of the spectra were not greatly changed by shading, except with 90 percent shade, where practically all the light was excluded. The amount of light excluded by the saran cloth roughly approximated the manufacturer's rating; that is, a 25 percent shade allowed approximately 75 percent of full sunlight to reach the plants, etc. Thus, the quality of light reaching the plants was not altered markedly, only the intensity was changed by the shade cloth.

Effects of Shading on Air Temperature

The highest air temperatures generally occurred during July and August, while September and October were coolest (Table 4). Day temperatures were slightly cooler under the lightest shade; night temperatures were unchanged. The air temperatures under intermediate shades (50 and 75 percent) were as much as 5-10 degrees cooler during the day; night temperatures were 5-9 degrees higher than the unshaded plots. Increases of 5-6 and 5-10 degrees

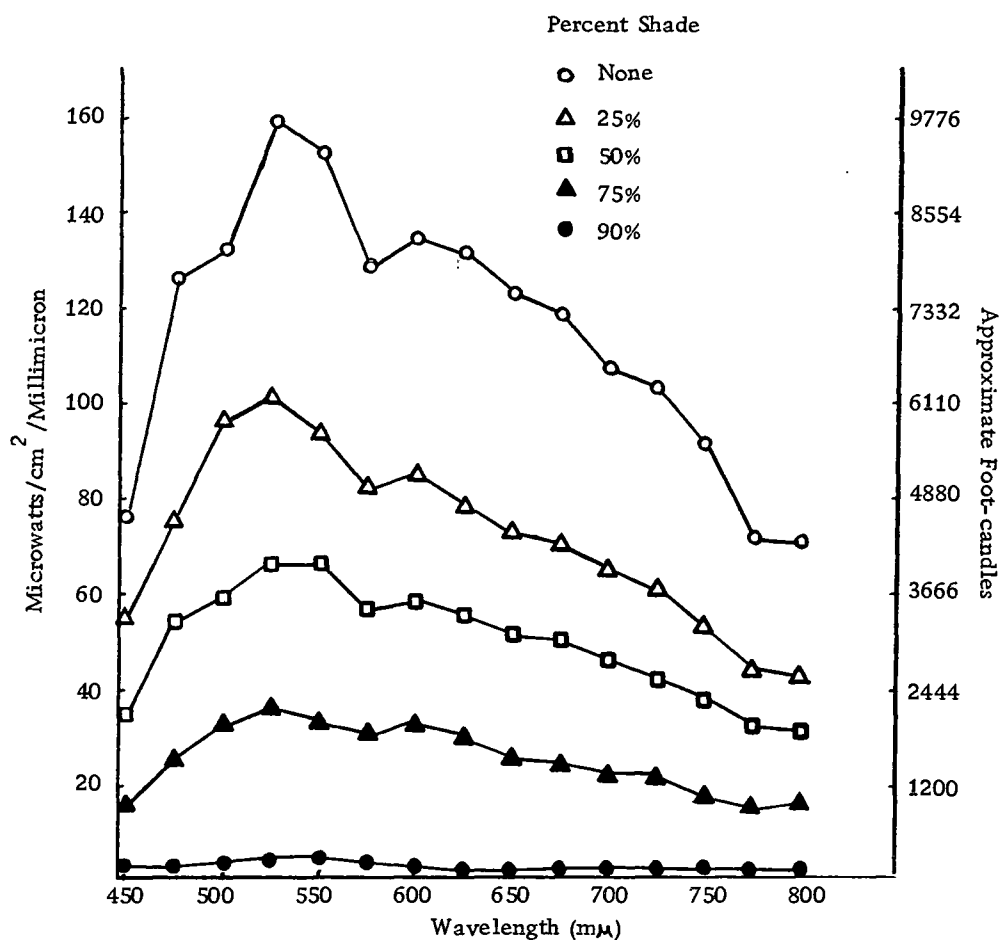


Figure 17. Effects of different amounts of shade on the intensity and quality of light measured on a clear day in July, 1967 at 11:00 am with an ISCO model SR spectroradiometer.

Table 4. Effects of different amounts of shade on the high, low and average air temperatures from May through October, 1967, at Corvallis, Oregon.

| Temp. ^a | | May | | | June | | | July | | | Aug. | | | Sept. | | | Oct. | | |
|--------------------|------|-----|----|------|------|----|------|------|----|------|------|----|------|-------|----|------|------|----|------|
| | | Hi | Lo | Ave. | Hi | Lo | Ave. | Hi | Lo | Ave. | Hi | Lo | Ave. | Hi | Lo | Ave. | Hi | Lo | Ave. |
| Percent Shade | | | | | | | | | | | | | | | | | | | |
| 1967 | None | 73 | 45 | 59 | 79 | 50 | 64 | 85 | 50 | 68 | 83 | 60 | 72 | 82 | 48 | 65 | 65 | 44 | 54 |
| | 25% | 72 | 49 | 60 | 75 | 51 | 63 | 81 | 52 | 66 | 80 | 53 | 66 | 76 | 50 | 63 | 67 | 49 | 58 |
| | 50% | 71 | 54 | 62 | 72 | 59 | 65 | 74 | 58 | 66 | 76 | 60 | 68 | 72 | 58 | 65 | 68 | 52 | 60 |
| | 75% | 72 | 53 | 62 | 74 | 59 | 66 | 75 | 59 | 67 | 76 | 60 | 68 | 74 | 58 | 66 | 68 | 56 | 62 |
| | 90% | 75 | 55 | 65 | 81 | 59 | 69 | 87 | 60 | 73 | 90 | 61 | 75 | 84 | 59 | 71 | 68 | 56 | 62 |
| 1968 | None | 66 | 43 | 55 | 73 | 46 | 60 | 83 | 49 | 66 | 78 | 53 | 65 | 73 | 48 | 61 | 62 | 41 | 52 |

a = degrees Fahrenheit

in the day and night temperatures, respectively, were observed under the heaviest saran cloth covering (90 percent shade), so average day-night temperatures were 5-10 degrees above unshaded conditions. The air temperatures were affected most by shading treatment during the fall months, as night temperatures progressively decreased. However, during the months of most active growth and flower induction (May, June), temperatures under saran were not strikingly different from ambient conditions.

Effects of Shading on Leaf Growth Rate and Size

Changes in light intensity and temperature did not modify the curvilinear pattern of leaf expansion (Figure 18). Leaf expansion was completed at approximately the same time under all shading treatments. The mid-terminal leaves of shoots under heavy shade were initially larger and remained so throughout the expansion period. Even though the size of leaves was altered, the rate and cessation of growth were constant under all shading treatments.

Effects of Shading on Flower Bud Number

Shading effectively reduced the frequency of flowering (Figure 19). The flowering percentage of plants under 25 percent shade was nearly identical with unshaded plants. However, with greater amounts of shade, the percentage of flower buds decreased

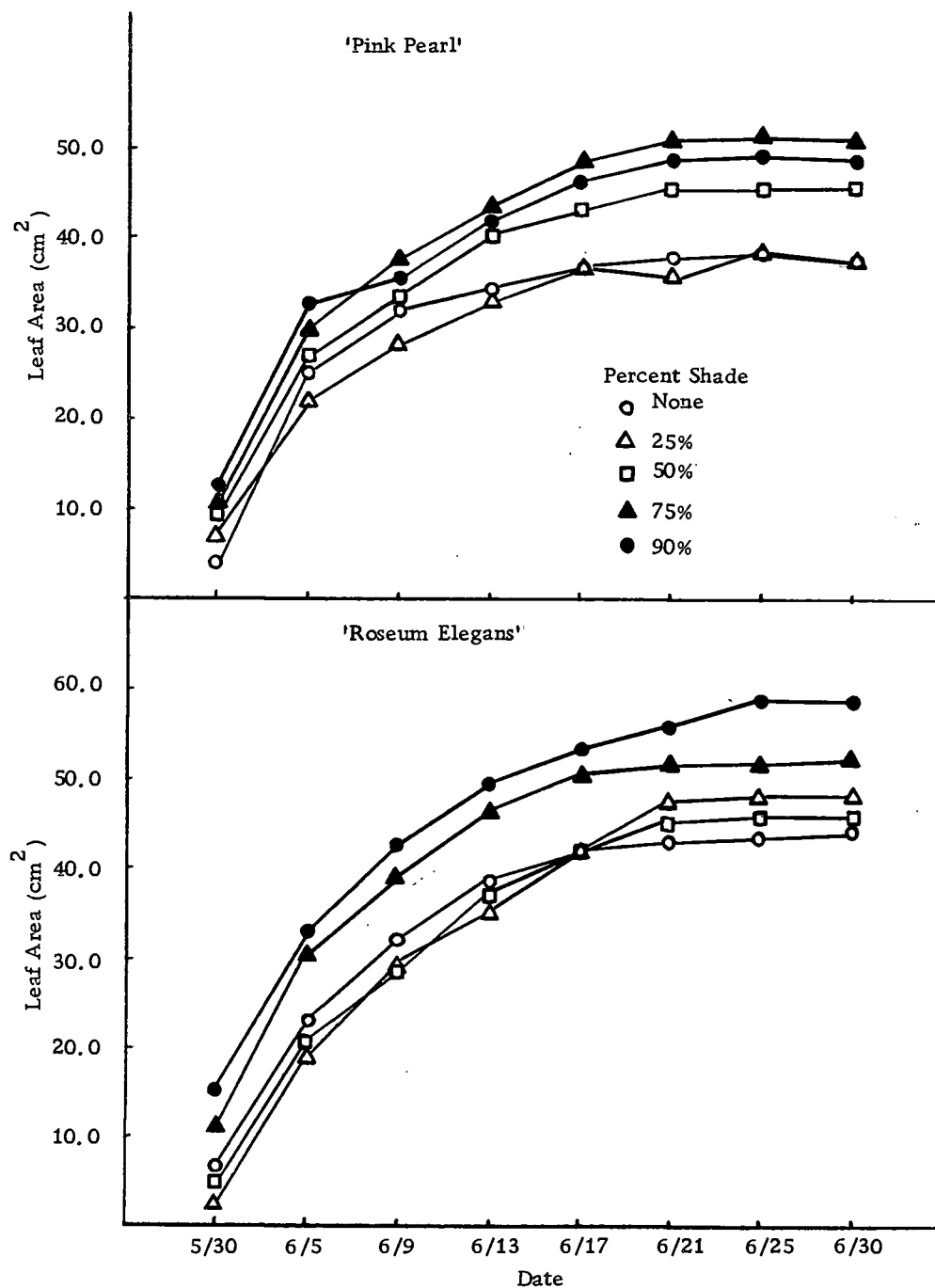


Figure 18. Effects of shading on the rate and extent of mid-terminal leaf expansion on *Rhododendron* 'Pink Pearl' and 'Roseum Elegans' shoots.

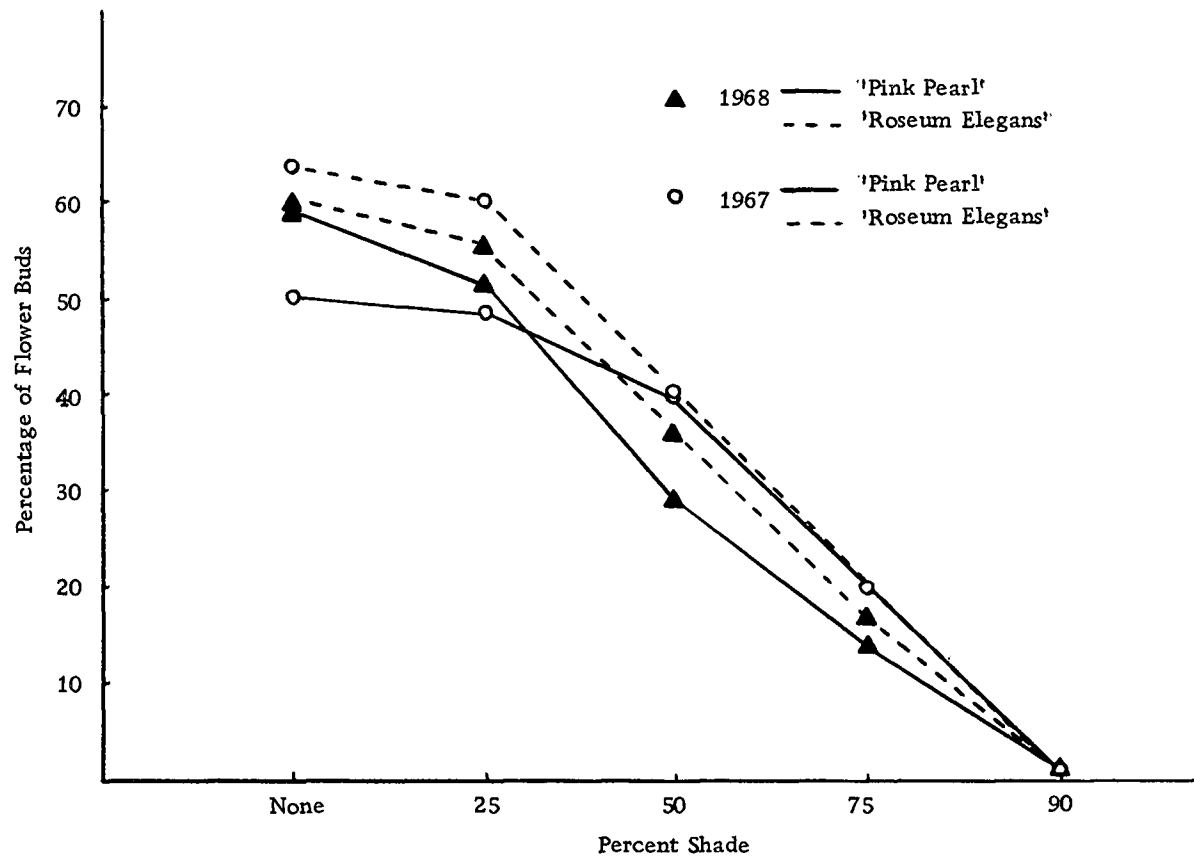


Figure 19. Effects of shading on flower bud numbers in Rhododendron 'Pink Pearl' and 'Roseum Elegans.'

linearly with virtually no flowers produced under 90 percent shade. Reductions in flowering with shading were nearly identical in 1967 and 1968 with different sets of stock plants.

Effects of Shading on Flower Development

Light shading (25 and 50 percent) did not appreciably change the normal growth rate and size of flower buds initiated (Figure 20), but 90 percent shade prevented initiation. The 75 percent shade altered growth rate slightly and produced flower buds that were smaller than normal in size. Flower size with other shading treatments was slightly less than those under full sunlight. Because growth rate and flower bud size under the various shades was not uniform, the morphological time scale, described in the general methods and materials section, was not used in this experiment.

Effects of Shading and Sampling Date on Rootability

Progressive aging reduced leaf rootability with all shade treatments (Figure 20). Leaves from the various treatments showed little difference in rooting on the first sampling, but those receiving heavy shade showed slight increase in rootability over the other treatments with the last two samplings. There was a striking reduction in rooting at the time of the second sampling in July. However, this reduction of rooting was not evident in leaves from shoots under

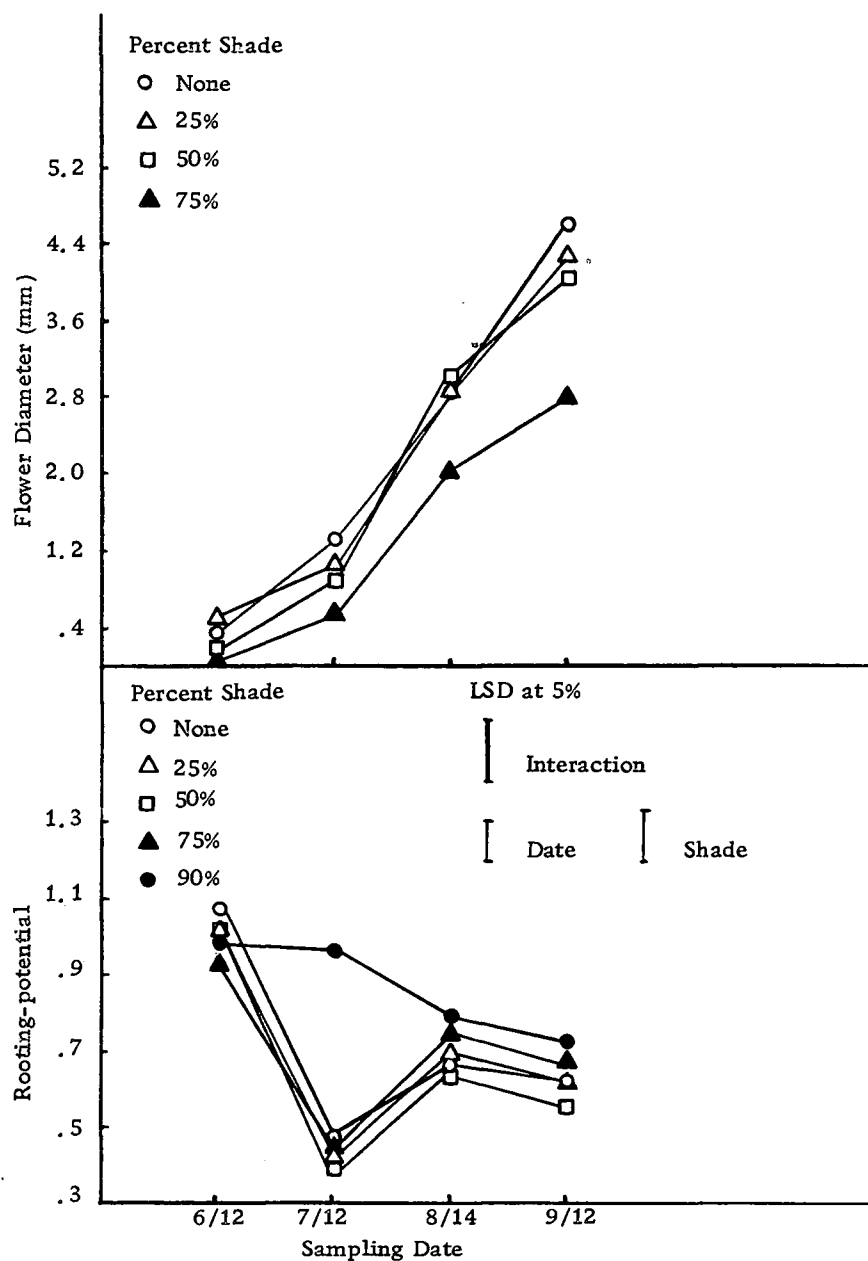


Figure 20. Effects of shading on the development of flower buds and the rooting-potential of leaf-petiole cuttings from *Rhododendron* 'Roseum Elegans' shoots.

heavy shade, where flowering was prevented, again confirming the effects of flower bud development on rooting.

Effects of Shading on Size and Rooting-potential of Leaves

Shading generally increased leaf size in both 'Roseum Elegans' and 'Pink Pearl' (Figures 21 and 22). Shading did not significantly alter leaf size in the basal zone of 'Roseum Elegans' shoots, but did increase leaf size in the upper three zones of the shoot.

Leaf sizes in two lower zones of 'Pink Pearl' shoots were significantly increased by shading treatment. Shading also produced larger leaves in zone 3, except in those shoots grown under 50 percent shade. Reducing light intensity by shading, except under heaviest shade, had little effect on the size of leaves developed in zone 4.

The rooting-potential of 'Roseum Elegans' leaves, except zone 4 leaves, from shoots grown under the heaviest shade was significantly greater than those grown under full sunlight (Figure 21). The rootability of zone 1 leaves from shoots grown under 25 percent shade was significantly reduced, and also those from zones 2 and 3 showed slightly reduced rootability. The other shade treatments had little or no effect on rooting-potential.

The rootability of 'Pink Pearl' leaves was similar to that of 'Roseum Elegans' (Figure 22). That is, the only significant

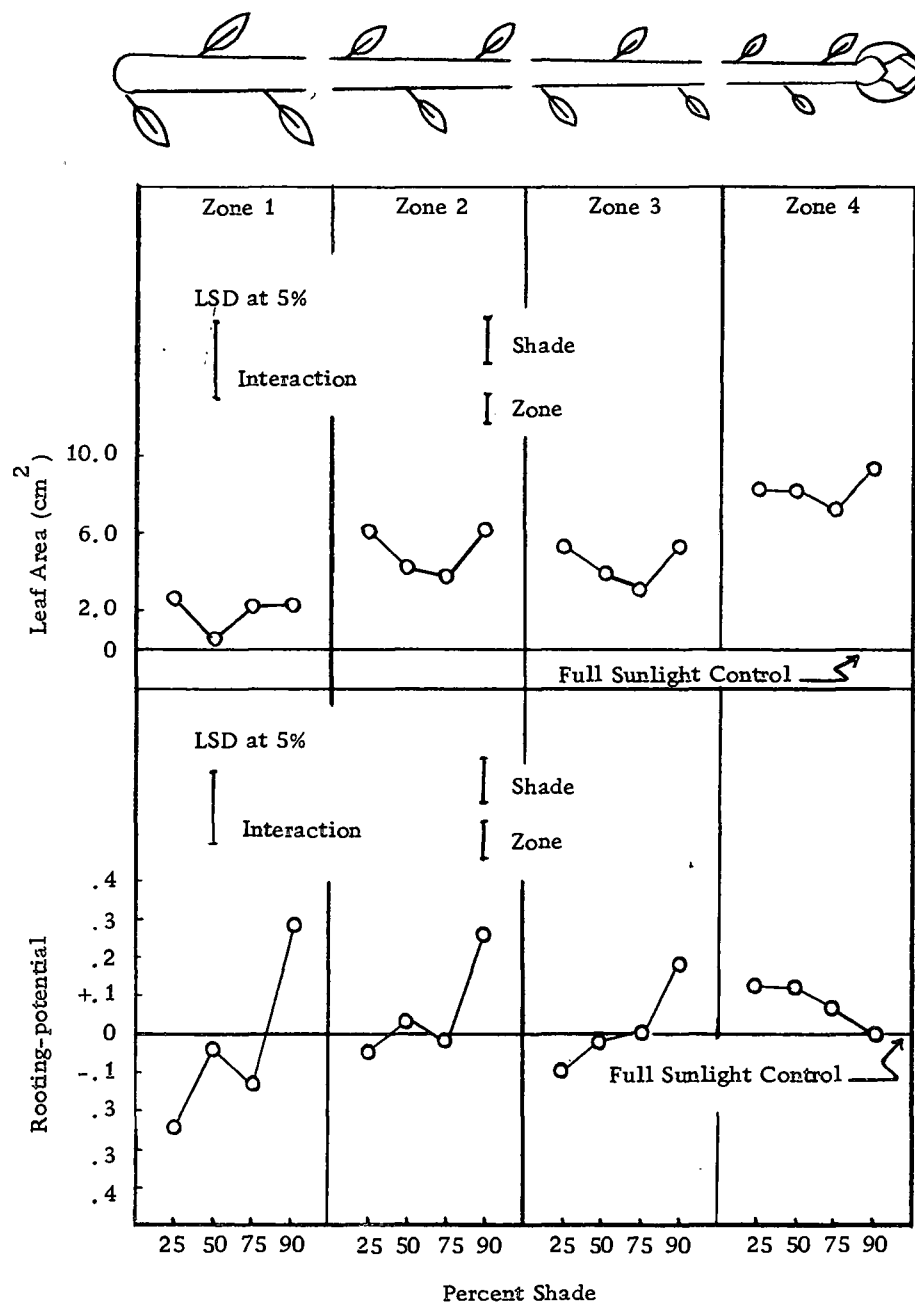


Figure 21. Effects of shading on leaf size and rooting-potential of leaf-petiole cuttings from various zones of *Rhododendron* 'Roseum Elegans' shoots.

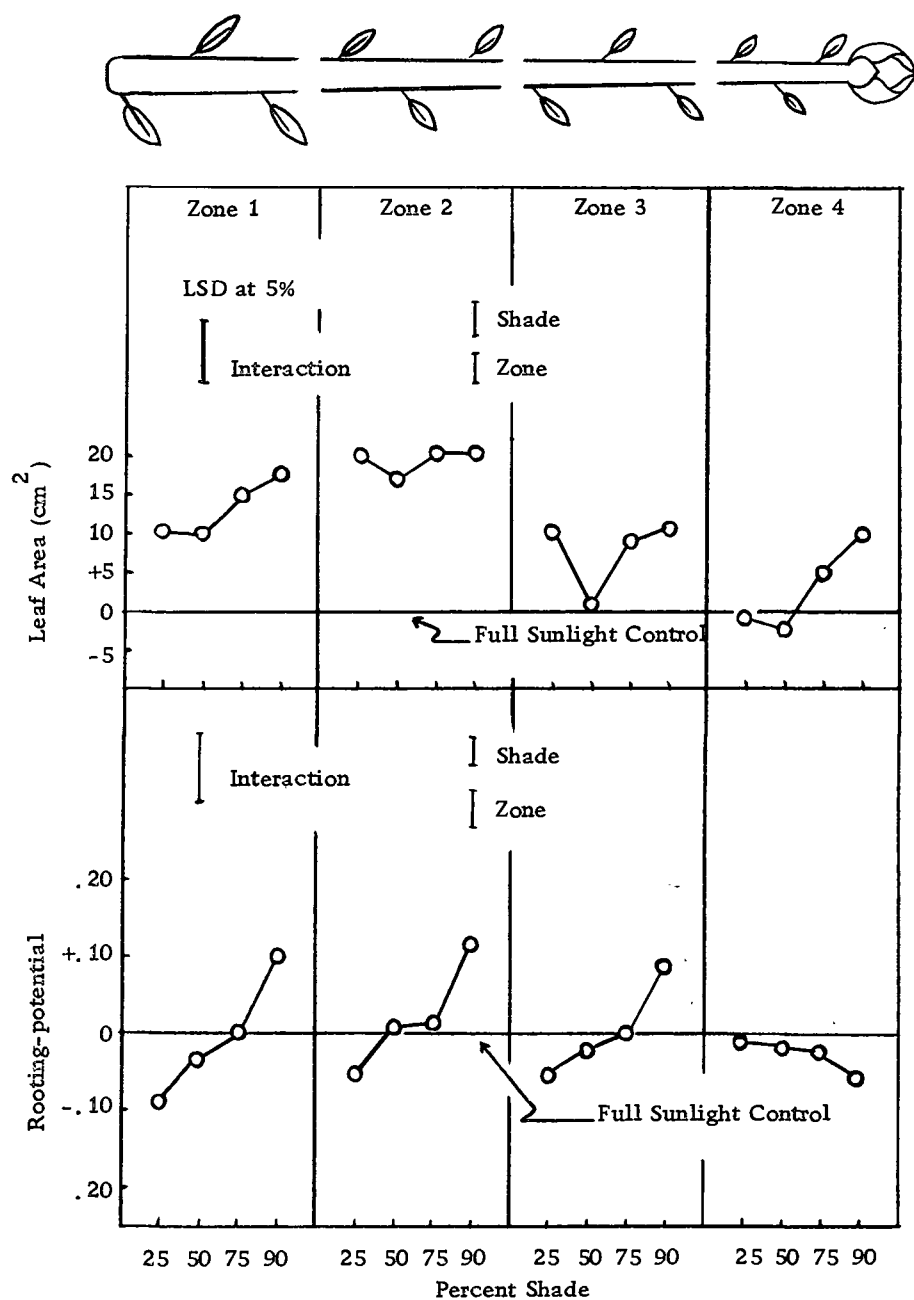


Figure 22. Effects of shading on leaf size and rooting-potential of leaf-petiole cuttings from various zones of *Rhododendron* 'Pink Pearl' shoots.

increases in rooting-potential from shading occurred in leaves from the lower three zones of heavily shaded shoots. The rooting of zone 1 leaves from shoots grown under 25 percent shade was significantly reduced, and there was also some reduction in those from the upper three zones. Thus, reducing light intensity did not greatly affect rooting until flowering was prevented by heavy shading (90 percent shade).

Effects of Shading and Hormone Treatment on Rooting-potential

It is clear that interactions occurred between hormone and shading treatments and date of sampling (Figure 23). Hormone treatment reduced the rootability of leaves sampled in early June from shaded and unshaded shoots. However, hormone treatment enhanced the rooting of leaves from unshaded and 25 percent shade treatments at later samplings. There was little rooting response to hormone treatment of leaves from shoots grown under intermediate shades (75 and 50 percent shade) with later sampling, except in September, when hormone treatment significantly improved rooting of leaves from 50 percent shade. In contrast to these responses, hormone treatment significantly inhibited the rooting of leaves from shoots grown under 90 percent shade on all but the last sampling date in September.

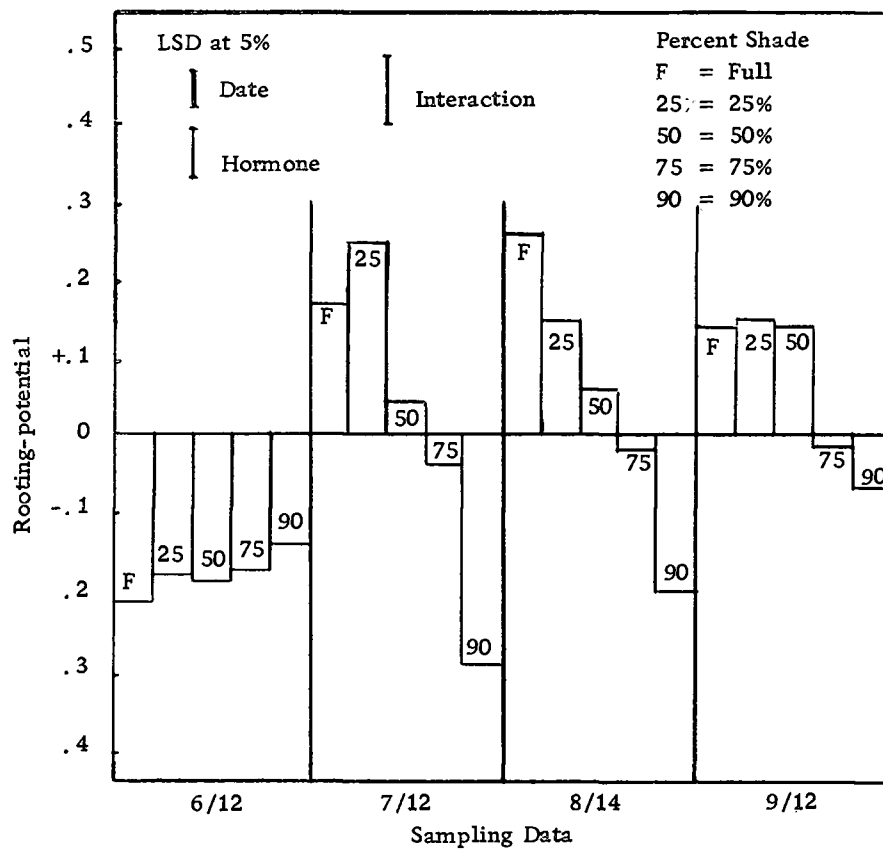


Figure 23. Effects of hormone treatment on rooting-potential of leaf-petiole cuttings taken from *Rhododendron* 'Roseum Elegans' shoots grown under different amounts of shade.

Hormone treatment was most effective in promoting the rooting of leaves from shoots grown under the least amounts of shade (unshaded and 25 percent shade), where flower bud development was normal. Rooting response to hormone treatment increased progressively with later samplings in leaves receiving 50 percent shade, and showed significant enhancement in September. The detrimental effects of hormone treatment on the rootability of leaves grown under heavy shade (75 and 90 percent) disappeared with later sampling. This decline in inhibition was especially evident in leaves from plants grown under 90 percent shade, where flowering was prevented.

Influence of Flowering on Auxin Transport (VI)

It was pointed out in Selim's (1956) review that decreased rooting associated with flowering may be the result of more effective mobilization of growth substances by developing flowers than by root initials. Leopold and Guernsey (1953) observed a loss of basipetal auxin movement in Coleus blumei stems when flowering occurred. This study was designed to determine if flowering affects the ability of leaf-petiole tissue to mobilize or transport IAA and the relationship of transport to rooting-potential.

Auxin transport was studied in petiole sections from flowering and vegetative 'Roseum Elegans' and 'Pink Pearl' shoots. Lateral

buds in the axils of the second and third leaf below the terminal buds of six-year-old stock plants were tagged, and six each of vegetative and flowering shoots were collected on September 20, 1968 for evaluating rootability and auxin transport. Radioactive auxin transport was measured in the lower six mm of leaf-petiole sections as described in the general methods, and an assay of rooting was made concurrently on the remaining lamina-petiole stubs. The amount of labelled auxin absorbed by the petiole tissue was determined shortly after transport and ethanol extraction. The leaf-petiole cuttings were given the standard 120- and 90-day rooting period for 'Pink Pearl' and 'Roseum Elegans', respectively. Rootability was expressed as rooting-potential, and the amount of labelled auxin movement through petiole sections was expressed as transport (see general methods). The relationship of rooting-potential to auxin transport in leaves from flowering and vegetative shoots was determined by regression analysis.

The effects of preventing flowering by bud excision on auxin transport was studied in leaves from lateral shoots of 'Pink Pearl' and 'Roseum Elegans'. The terminal buds of six expanding shoots of each cultivar were excised at each of three stages of development in the same manner as performed in experiment III:

| <u>Stage of shoot development</u> | <u>Shoot length at the time of terminal bud excision</u> |
|-----------------------------------|--|
| 1 | 20-29 mm |
| 2 | 30-35 mm |
| 6 | 60-75 mm |

On August 20, 1968, shoots from each treatment and an equal number of untreated flowering controls were removed for evaluation of auxin transport and rooting. These results were composited and averaged for the four leaf zones and expressed on the basis of the whole shoot.

The effects of preventing flowering by shading on auxin transport was studied in leaves from terminal shoots of 'Pink Pearl' grown under five shading treatments at the OSU research greenhouses. A total of forty cuttings, or eight per shade treatment, were sampled on September 10, 1968. The amount of auxin transported and absorbed by the petiole tissue was determined as outlined in the general methods section. The lamina-petiole stubs were placed in the rooting bench for 120 days to assay rootability. The rooting and transport results obtained for the four leaf zones were composited in evaluating shoot response.

Some of the alcohol extracts of the petiole tissue were used in confirming the authenticity of IAA. The extracts were spotted on filter paper strips and developed in one of the three solvent systems

described in the main methods section. The strips were scanned to determine the Rf of the radioactive spots, and further identification involved spraying the strips with Erlich's solution and observing under ultra-violet light.

Effects of Flowering on Auxin Transport and Rooting-potential

A significant positive correlation was found between rooting-potential and auxin transport in flowering and vegetative shoots (Figure 24). The clustering of points, depending on whether the shoots were vegetative or flowering, was observed with both 'Pink Pearl' and 'Roseum Elegans'. Thus, the increased rootability of the vegetative shoots was accompanied by increased auxin transport.

The relationship between rooting-potential and auxin transport in the individual leaf zones of flowering and vegetative shoots was not as distinct as the composite of values for these shoots (Figures 25 and 26). The rooting-potential of leaves from vegetative 'Roseum Elegans' shoots was significantly higher than those from flowering shoots in the two basal leaf zones (Figure 25). However, there was little difference in rootability between leaves of the two shoot types in the two upper zones. The amount of labelled auxin transported by petiole sections from vegetative shoots was generally greater than that of flowering ones; however, these differences were significant only with zone 2 leaves. It was found that leaf

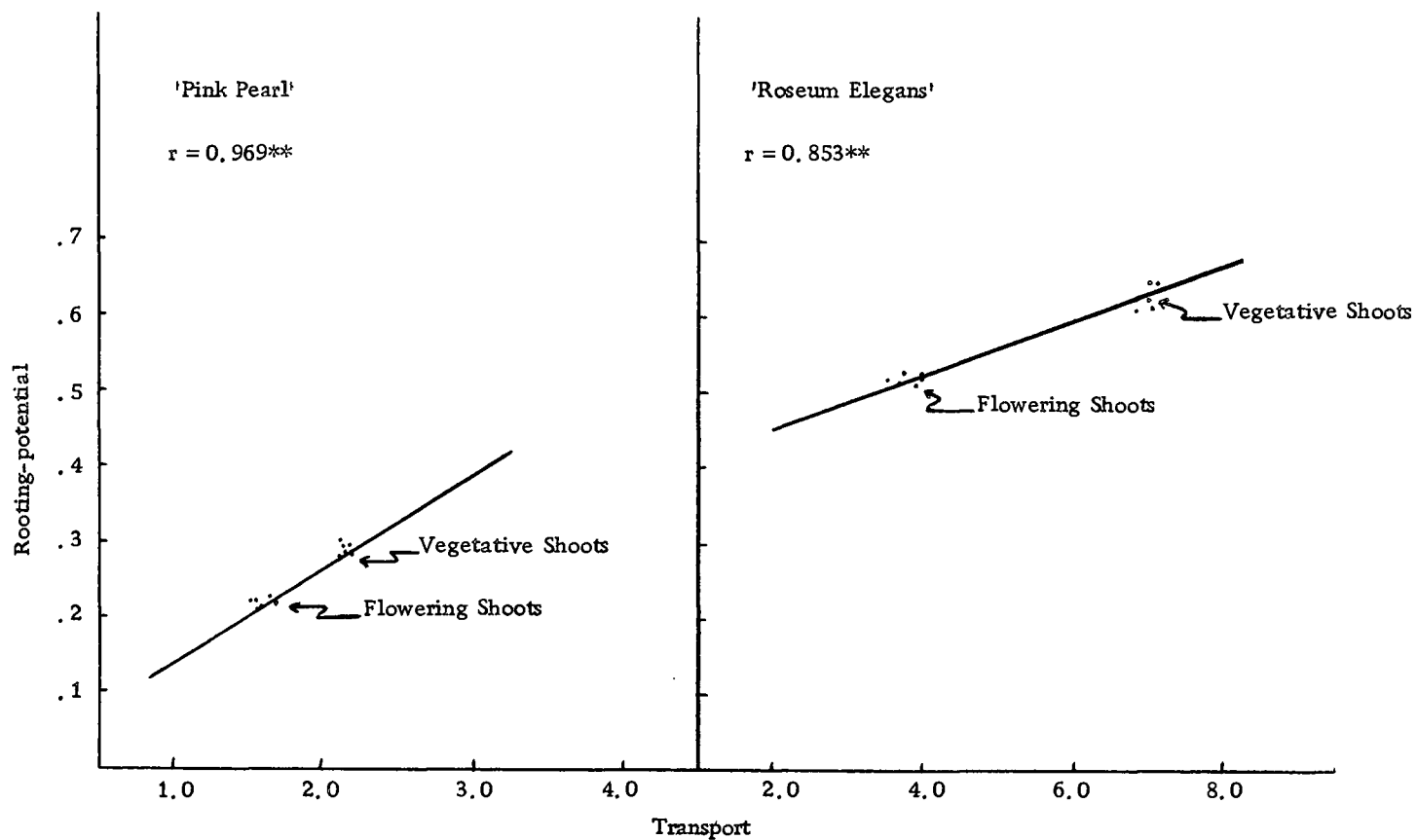


Figure 24. Relationship between rooting-potential and IAA-2-¹⁴C transport in leaf-petiole cuttings taken from Rhododendron 'Pink Pearl' and 'Roseum Elegans' vegetative and flowering shoots.

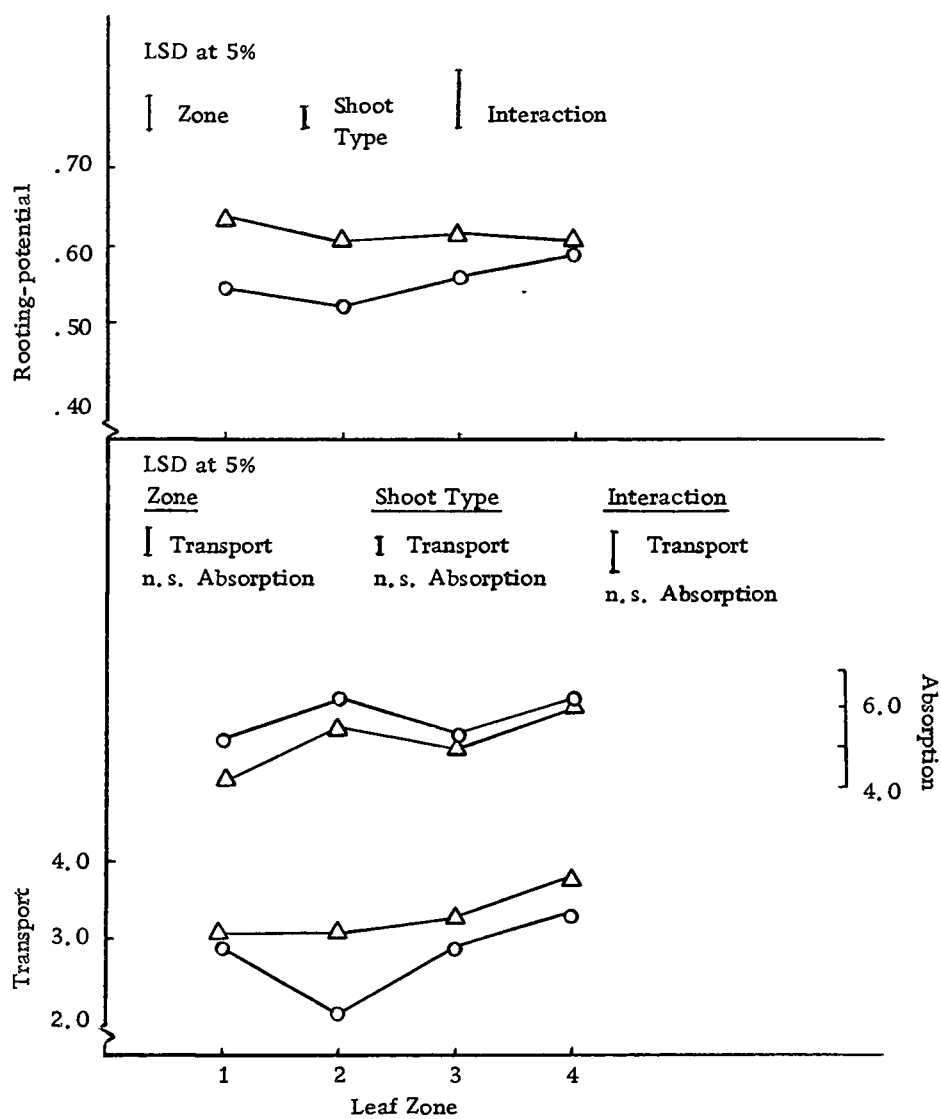


Figure 25. Rooting-potential, transport and absorption of IAA-2-¹⁴C in tissues of leaf petiole cuttings from different leaf zones of vegetative (Δ—Δ) and flowering (o—o) *Rhododendron* 'Roseum Elegans' shoots.

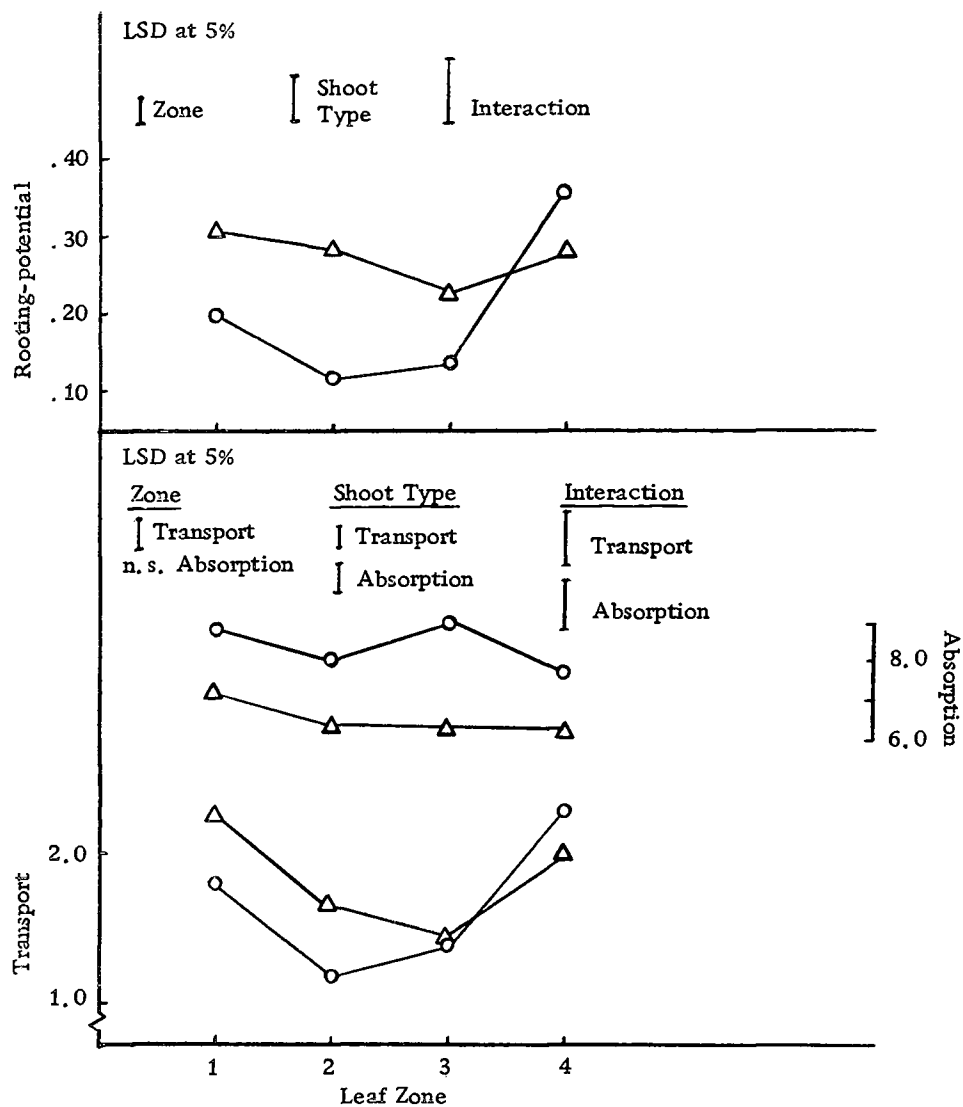


Figure 26. Rooting-potential, transport and absorption of IAA-2-¹⁴C in tissues of leaf zones of vegetative (Δ—Δ) and flowering (○—○) *Rhododendron* 'Pink Pearl' shoots.

petiole tissue from all the zones of flowering shoots absorbed slightly more labelled auxin than those from vegetative ones.

The relationship between leaf-petiole rootability and auxin transport in the individual leaf zones was slightly more distinct in 'Pink Pearl' (Figure 26). The rooting-potential of leaves from flowering shoots was typically low in the three basal zones, but increased greatly in the terminal leaves (zone 4). The leaves from the three basal zones of vegetative shoots had greater rootability than the same leaves from flowering shoots, but the opposite was true of the terminal leaves (zone 4), where those from flowering shoots rooted best. Auxin transport in petiole sections of basal leaves (zones 1 and 2) from vegetative shoots was significantly greater than that in petiole sections from flowering shoots. Differences in transport in leaf zones 3 and 4 were not significant. Even so, the leaf-petioles from zone 4 of flowering shoots showed slightly greater ability for transport than those from the same zone of vegetative shoots. Leaf-petiole tissue from flowering shoots absorbed significantly greater amounts of labelled auxin than comparable tissue from vegetative shoots. It thus appeared that the labelled auxin was being absorbed by the petiole tissue of flowering plants rather than being transported.

Effects of Bud Excision on Rooting-potential and Transport

Excision of the shoot apex before or after flower initiation had occurred significantly enhanced rooting in both cultivars over that of intact flowering controls (Figures 27 and 28). The rooting-potential of leaves from 'Pink Pearl' shoots was highest with terminal bud excision during stage 2 of shoot development, with less enhancement if done before or after this time. The rooting-potential of leaves from 'Roseum Elegans' shoots on the other hand was greatest with terminal bud excision during stage 1, and declined with excision at later stages.

Terminal bud excision at all stages of shoot development significantly increased the ability for auxin transport in petiole sections of leaves from 'Roseum Elegans' shoots (Figure 27). This increase in auxin transport was fairly consistent with excision treatment before and after flower initiation. The amount of labelled auxin retained by the petiole tissue was not significantly influenced by bud excision treatment.

Auxin transport in petiole sections from 'Pink Pearl' shoots was also significantly increased by terminal bud excision (Figure 28). This enhancement of auxin transport with bud excision was greatest when done prior to flower initiation. The amount of labelled auxin absorbed by the petiole tissue from treated shoots

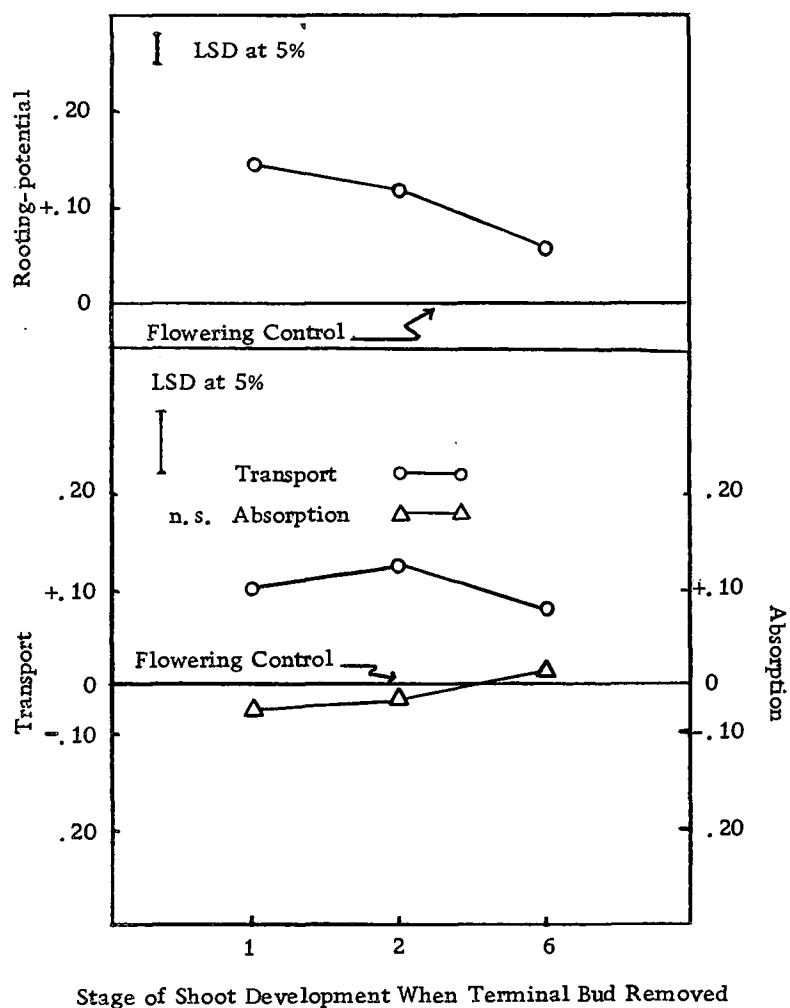


Figure 27. Rooting-potential, transport and absorption of IAA-2-¹⁴C in tissues of leaf-petiole cuttings from *Rhododendron* 'Roseum Elegans' shoots following terminal bud excision at 1-(20-29 mm), 2-(30-35 mm), and 6-(66-75 mm) stages of shoot development.

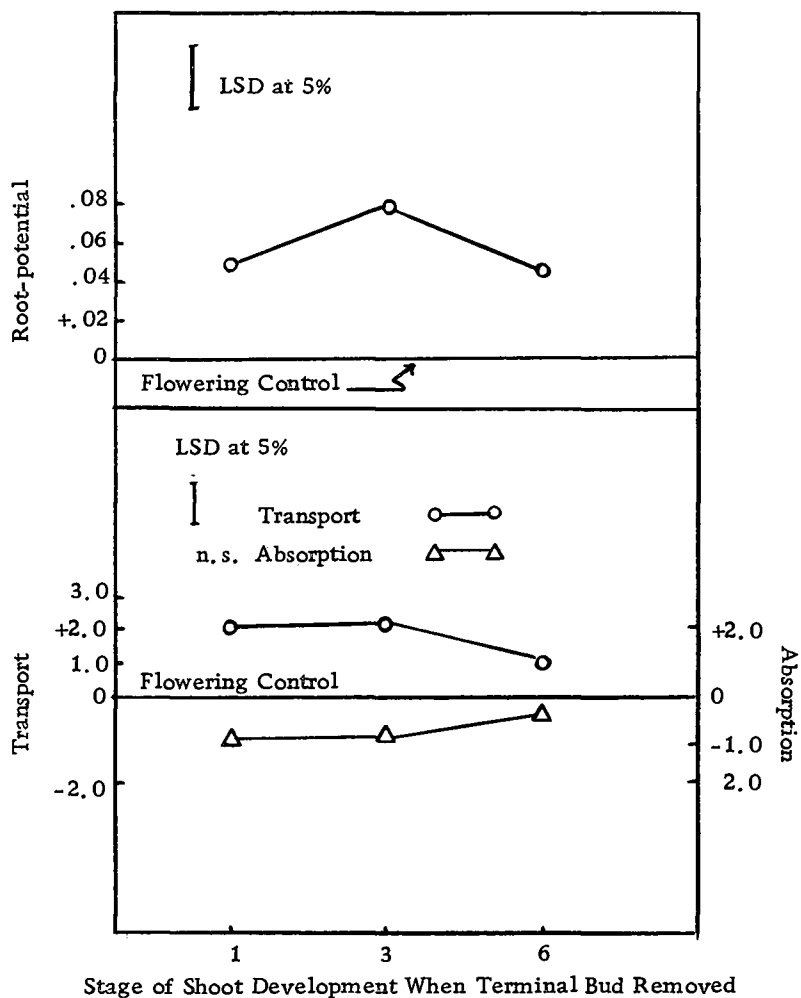


Figure 28. Rooting-potential, transport and absorption of IAA-2-¹⁴C in tissues of leaf-petiole cuttings from *Rhododendron* 'Pink Pearl' shoots following terminal bud excision at 1-(20-29 mm), 2-(30-35 mm), and 6-(66-75 mm) stages of shoot development.

was somewhat less than that of the intact flowering controls.

Effects of Shading on Rooting-potential and Transport

The only significant difference in leaf-petiole rooting-potential, as a result of differential shading, was the increased rooting associated with the heaviest shade (90 percent), which prevented flowering (Figure 29). Similarly, there was a significant increase in auxin movement through petioles from shoots that were grown under 90 percent shade. Petiole sections from the other treatments showed little difference in their ability to transport auxin. The amount of labelled auxin absorbed by petiole tissue was significantly less with the heavy shading than the other shade treatments.

Authenticity of IAA-2-¹⁴C in the Tissue Extractions

Strip chart scans of chromatograms of alcohol extracts of petiole tissue from some of the shading experiments are presented in Figure 30. The radioactivity in extracts of the petiole tissue from the various shade treatments closely paralleled the Rf values of the authentic IAA-2-¹⁴C at approximately 0.52. The same Rf values were obtained from the agar block extracts. Since the level of radioactivity from the extracts was relatively low for strip chart counting, the strips were also cut into cm sections and placed in vials for counting by liquid scintillation. The Rf values obtained

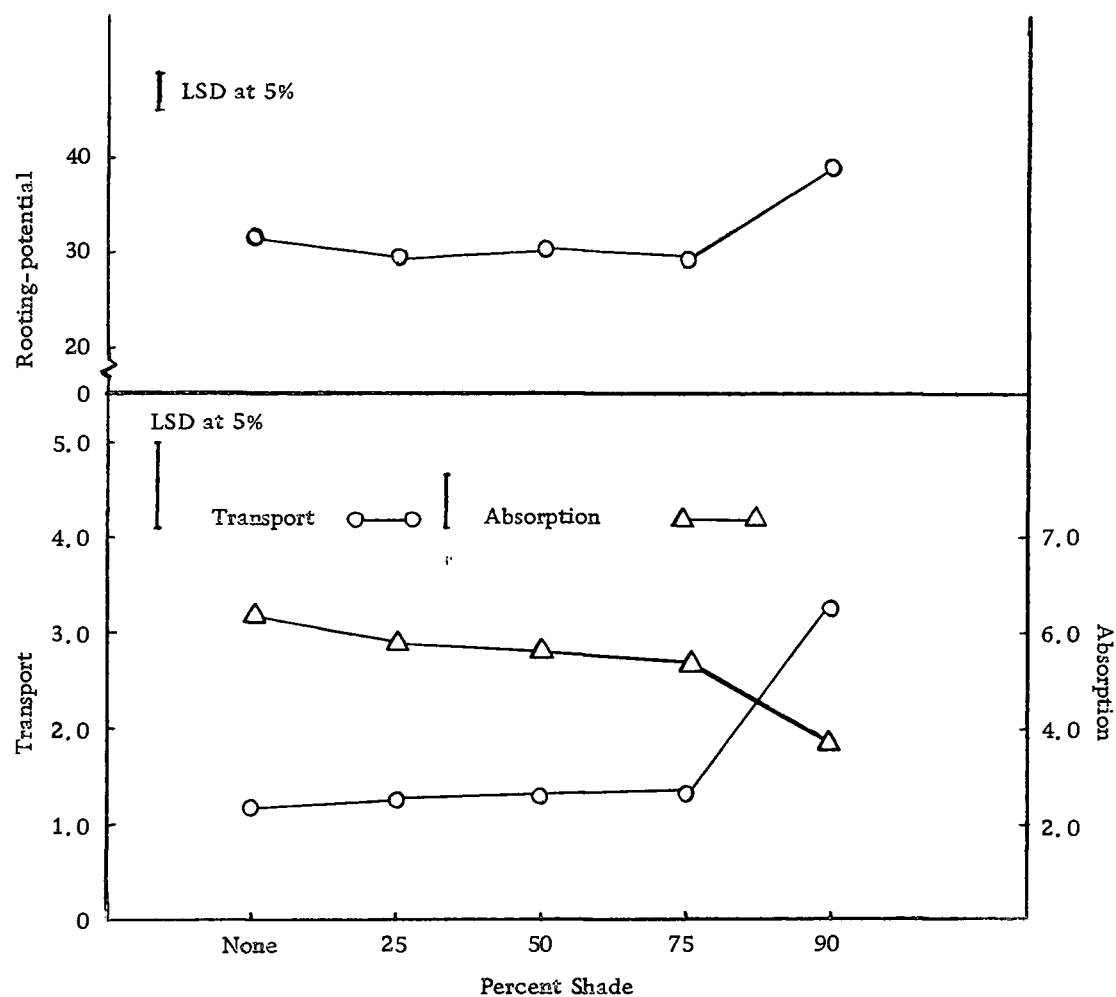


Figure 29. Effects of shading on rooting-potential, transport and absorption of IAA-2-¹⁴C in tissues of leaf-petiole cuttings from Rhododendron 'Pink Pearl' shoots.

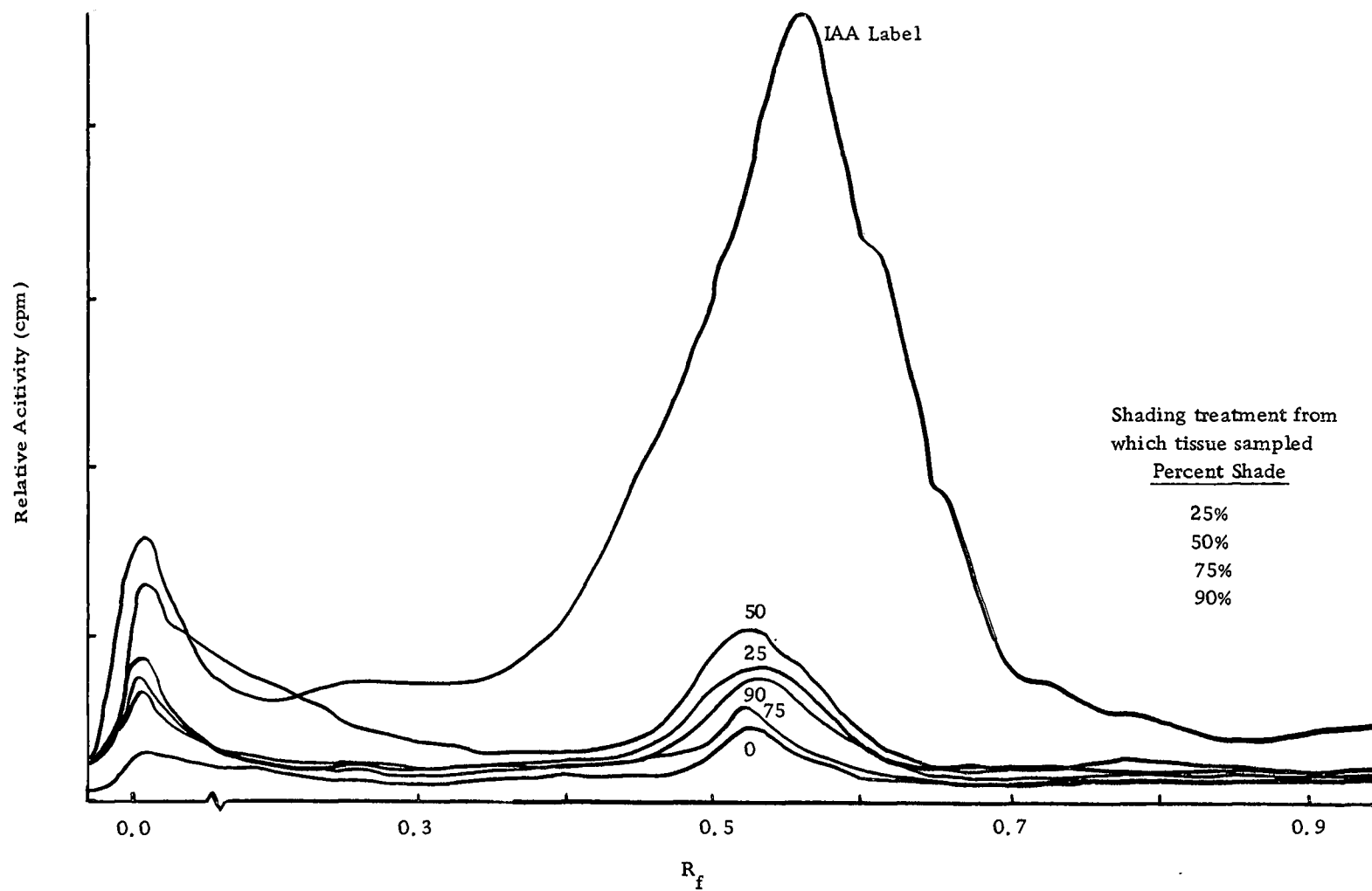


Figure 30. Tracing of strip chart scans of chromatographs of alcohol extracts from Rhododendron 'Pink Pearl' leaf-petiole sections after development in isopropanol:ammonium:water (8:1:1) solvent.

with this technique corresponded closely to those obtained with the strip chart scanner. Development in two other solvent systems produced similar results (Table 5). The Rf values from the various treatments corresponded closely with that of pure IAA, all of which were lower in the chloroform:acetic acid solvent system.

Table 5. Rf values of chromatograms of alcohol extracts from Rhododendron 'Pink Pearl' leaf-petiole sections after development in three different solvent systems.

| Solvent System | Rf values for petiole sections from shoots grown under different amounts of shade | | | | | |
|--|---|-----|-----|-----|-----|----------|
| | None | 25 | 50 | 75 | 90 | Pure IAA |
| Isopropanol:Ammonium: Water (8:1:1) | .54 | .52 | .54 | .51 | .51 | .52 |
| N-Butanol: Ethanol: Water (7:1:2) | .50 | .50 | .52 | .50 | .50 | .50 |
| Chloroform: Acetic Acid (95:1) | .44 | .45 | .44 | .44 | .44 | .46 |

The locations of the spots from the extracts were discernable and were comparable with the Rf values of pure IAA by means of spraying with Ehrlich's reagent and examination under ultraviolet light.

DISCUSSION

Numerous workers have established that shoots with terminal leaf buds regenerate roots better than those with terminal flower buds. This research was done not merely to reiterate this fact but to gain insight as to the nature of this influence.

Developmental Effects of Flowering on Rooting

Adams and Roberts (1968), in a study of timing the taking of Rhododendron cuttings for maximum rooting, found chronological age to be unreliable. A morphological time scale, based on flower development, proved more reliable, and in a two-year study a period of low rooting was found to correspond to a certain stage of development. A similar period of decreased rooting at the same stage of morphological development was observed with the cultivar 'Pink Pearl' in these studies (Figure 8). Adams proposed that this period of decreased rooting was associated with a specific stage of flower development, namely mega- or microsporogenesis. In the present study, a period of retarded rooting and also retarded flower development appeared to be associated with such a specific stage of development.

The decreased rootability of 'Roseum Elegans' leaves in July may also be associated with a specific stage of flower development

(Figure 20). This supposition is supported by the fact that there was no such decrease in rooting when flowering was prevented by heavy (90 percent) shading. It is also important to consider that the decreased rooting occurred on the same date even though the intensities of growth rate and size of the flowers was less with intermediate shading (75, 50 and 25 percent) than with full sunlight controls.

This suggests that the developmental stage of flowering (mega- or microsporogenesis) which competes with rooting is associated with but independent of flower size. It could probably be a biological timing mechanism, thought to be responsible for controlling numerous plant processes (Salisbury, 1963), that is triggered at flower initiation. Flower initiation in *rhododendron* has been found to occur during rapid shoot elongation and leaf expansion in early spring (Adams and Roberts, 1968; Johnson and Roberts, 1968). Since shading did not affect the rate of leaf expansion in 'Roseum Elegans', it is conceivable that time of flower initiation was also unchanged by shading treatment, except that heavy shading prevented flowering (Figure 18). Thus, perhaps the stage of flower development, competitive with rooting, was unchanged since flower initiation probably occurred at the same time in all plants with the exception of those under the heaviest shade. Therefore, a timing index based on flower initiation, rather than morphological development (flower size) as proposed by Adams and Roberts (1967), might be

more accurate for predicting shoot rootability in rhododendron.

Rhododendron cultivars show great differences in ease of rooting (Wells, 1953 and 1954; Adams, 1967). Part of this variability may be related to a cultivar's sensitivity to flowering. In 'Cynthia', for example, rooting was high, and there were no significant decreases in rooting during flower development as contrasted to 'Pink Pearl' (Figures 7 and 8). However, during bud dormancy (November to April) the two cultivars showed approximately the same rooting-potential. It is evident that the degree of correlative relationship and competition between flowering and root regeneration is controlled genetically.

Flowering Effects on Leaf Size and Rootability as Related to Their Position

Changes in leaf size and shape often occur with onset of flowering. Thomas (1961) showed increased leaf expansion accompanying flower initiation in Chenopodium, and Humphries and Wheeler (1963) found leaf expansion in Phaseolus vulgaris increased as auxin levels decreased. Thus, the lower leaves of 'Pink Pearl' and 'Roseum Elegans' shoots were larger perhaps as a result of being unfolded during flower initiation and having reduced auxin levels (Figure 9). Terminal leaves may always be small, regardless of flowering, because they are expanded after flower initiation.

The larger leaves from flowering 'Pink Pearl' and 'Roseum Elegans' shoots generally showed low rooting capacity, perhaps reflecting their naturally low auxin levels. However, leaf rootability at different positions on the shoot clearly indicates that flowering brings about changes other than a general decrease in auxin levels. For example, the upper leaves of flowering 'Pink Pearl' shoots have a higher rooting-potential than those from vegetative ones, possibly because the flower bud mobilizes growth substances from the subtending leaves into this part of the plant. On the other hand, the rooting-potential of all leaves in 'Roseum Elegans' shoots was reduced by flowering, indicating that the ability of flower buds to mobilize growth substances in this cultivar may be weak.

Leaf size was also increased in 'Roseum Elegans' and 'Pink Pearl' shoots by shading (Figures 21 and 22). Similar increases in leaf size with shading were observed by Davidson (1966) in six different species. Leaves from shoots grown under heavy shade or low light intensity would be expected to have higher auxin levels because high light intensity is known to reduce auxin levels by increasing IAA-oxidase activity (Fang and Butts, 1957). However, the only significant increase in rooting-potential with either cultivar was found in leaves from shoots under the heaviest shade treatment (Figures 21 and 22). Thus, the only increases in rooting from shade treatment were obtained with the prevention of flowering. The

decreased rootability of terminal leaves from heavily shaded 'Pink Pearl' shoots may have been the result of preventing the development of metabolic "sink". That is, by preventing flowering, the mobilization of growth substances into these terminal leaves decreased and leaf rootability was therefore less than the flowering controls. The reduction in rooting-potential of basal leaves in both cultivars associated with 25 percent shading may have been the result of a combination of three factors: (1) flower development unchanged, (2) IAA-oxidase activity unchanged, and (3) reduced carbohydrate and nutrient element levels.

These experiments substantiate that flowering causes significant changes in the size and rootability of leaves from different positions on rhododendron shoots. Adams (1967) concluded that smaller leaves were always superior in rooting to larger ones because they retained the ability for cell division and synthesis of growth substances. However, the present study leads one to conclude that the association of poor rooting with large leaves is valid only in flowering shoots.

Time of Flower Influence on Leaf Size and Rooting-potential

It has been established that flowering increases the size, but decreases the rooting-potential of leaves from certain positions on the shoot. Consequently, if flowering is prevented or flower buds

are removed during development, leaf rootability should be improved. However, DeBoer (1953), working with Rhododendron and O'Rourke (1940) with Vaccinium, found that excision of well developed flower buds did not significantly improve the rooting of shoots. These results suggest that the inhibition of rooting by flowering occurs at an early stage of development. Substances, such as auxins, steroids, lipids and numerous other compounds, are known to be altered by flower induction (Searle, 1965). Therefore, excision of the meristem prior to flower induction should eliminate biochemical reactions responsible for increased leaf size and decreased rootability.

Excision of terminal meristems or buds before, and in most cases after, flower initiation reduced leaf size in the lower three zones, but had little effect on those in zone 4 (Figure 11). The insensitivity of terminal leaves to bud excision support the earlier conclusion that flowering does not affect leaves near the terminal bud, and therefore leaf size in this zone is not changed. Conceivably leaf size was reduced in the lower zones because flower initiation was prevented, as well as later stages of development and their accompanying biochemical changes that affect leaf growth.

Leopold (1964) pointed out that auxin production is usually associated with actively growing tissues, such as expanding buds. Thus, the leaves from shoots whose terminal meristems were

excised at stage 1 rooted poorly possibly because of less auxin and/or other rooting factors associated with the retarded lateral buds (Figure 11). The increased rooting of leaves in the first three zones with excision during stage 2 may be attributable to preventing initiation and concomitant biochemical changes that decrease rooting. The decreased rootability of terminal leaves associated with bud excision may be due to the removal of the "sink" that would have mobilized growth substances to this part of the plant. Any enhancement in rooting with later excision might be interpreted as the removal of a competing growth center that would have the potential for mobilizing substances needed in rooting. The results obtained in these experiments clearly show that the correlated inhibition of rooting by flowering commences at the time of initiation when growth substances, such as auxins, have been found to decrease (Cooper, 1954 and Jorgensen, 1966). Then a mobilization center or "sink" is established by the developing flower bud, and rooting decreases further as a result of the flower buds continued mobilization of growth substances.

Hormone Treatment and Rooting

Since endogenous substances, such as auxins, decrease with flower initiation, hormone treatment would presumably correct this situation. However, hormone treatment only slightly increased

rooting of leaf-petiole cuttings taken from 'Cynthia' and 'Pink Pearl' after flower buds had started to develop (Figures 7 and 8). When dormant, hormone treatment had no effect on rooting of 'Cynthia', and actually inhibited rooting of 'Pink Pearl'. These responses to hormone treatment during the winter months may reflect high levels of endogenous auxins, such that added hormone treatment resulted in auxin levels that were inhibitive and even phytotoxic to rooting. Perhaps a more feasible explanation could be made on the basis of interactions between natural growth inhibitors, such as abscisic acid, and the hormone to produce the inhibitive and phytotoxic effects.

The lower leaves of flowering shoots might be expected to have low levels of endogenous auxin, because of its acropetal mobilization by developing flower buds (Figure 10). Therefore, perhaps these leaves responded to hormone treatment because of an auxin deficiency. However, hormone treatment depressed the rooting-potential of the small terminal leaves, possibly as a result of hyperauxin levels. In addition, leaves nearest the flower buds are known to possess many hormone-like compounds that may interact with applied hormone to depress rooting. On the other hand, the rootability of leaves from the upper portions of vegetative shoots was significantly enhanced by hormone treatment. This response possibly reflects low levels of endogenous rooting substances in

these upper leaves because of little or no acropetal mobilization by terminal vegetative buds. There was a striking similarity in the pattern of rooting response to hormone treatment of leaves from different zones on flowering and vegetative shoots, although the rootability of leaves from vegetative shoots was higher. It is apparent that hormone treatment masks the usual effects of flowering on rooting of leaves from different zones.

The rooting response of cuttings from shaded shoots to hormone treatment was used as a means of estimating internal auxin levels (Figure 23). Hormone treatment inhibited the rooting of cuttings taken in early June. It is conceivable that rooting was high in all untreated cuttings at this time because flower buds had not begun to mobilize auxins. Therefore, hormone treatment perhaps caused hyperauxin levels in the sensitive young tissue that inhibited rooting. Hormone treatment enhanced the rooting of later leaf samples from shoots grown under high light conditions, which may be indicative of reduced endogenous auxin levels because of high IAA-oxidase activity and auxin mobilization by developing flower buds. Cuttings from shoots grown under moderate shading (50 and 75 percent) showed no significant response to hormone treatment. Auxin mobilization by the flower buds under these shade treatments may have been small because of their reduced size, leaving the content of the leaves relatively high. With the prevention of flowering

under heavy shade (90 percent), hormone treatment inhibited rooting, which may be indicative of high endogenous auxin levels. The decreasing tendency of hormone treatment to inhibit rooting of cuttings from the heavy shade with later sampling suggests that the level of free auxin decreases with tissue aging and/or an increase of natural growth inhibitors with onset of dormancy.

These experiments confirm the findings of other workers, that reduced auxin levels may be one of the changes occurring with flower initiation. However, definitive proof of such a change is complex and was not attempted in this study. The effects of flowering on shoot rootability after initiation are conceived as mobilization of growth substances, such as auxins, by developing flower buds.

Correlative Relationship of Leaves to Flowering and Rooting

The fact that complete defoliation at an early stage of shoot elongation caused a second flush of growth and that flowers developed only at later stages of elongation (140 mm), is indicative of a quantitative aspect of the flower hormone stimulus. More than 25 percent of the total leaf complement was necessary for continued flower development (Figure 14). The terminal leaves of 'Roseum Elegans' appeared to be more important than basal ones in flower development, since at least 50 percent of the leaves retained had to be in the terminal portions of the shoots for flower development.

This may indicate that these leaves export specific flowering substances not found in other leaves. On the other hand, all the leaves of 'Pink Pearl' seemed to contribute equally to flower development, since bud development was nearly equal with 50 percent defoliation whether in the upper or lower portions of the shoot. The necessity for certain amounts of leaf surface is probably related to a carbohydrate requirement of flower developmental processes.

The improvement of rooting with the removal of 75 percent of the total leaf complement in the upper portions of shoots suggests that the remaining lower ones retained certain growth substances that would have been mobilized by developing flower buds (Figures 15 and 16). However, with removal of only 50 percent of the upper leaves of 'Pink Pearl', flower buds developed and apparently mobilized substances as normal, so that the rooting was not different from controls. The removal of lower leaves resulted in reduced rootability in the remaining terminal leaves, particularly when flowering occurred. This may indicate: (1) that lower leaves export certain substances, important in rooting, to the terminal leaves and flower buds, and (2) that the remaining terminal leaves become predominantly exporters of growth substances important in rooting but also needed in flower development. The leaves in both the upper and lower positions on the shoot apparently contribute growth substances important in flower and root development.

Mobilization of Growth Substances by Flower Buds

Leopold and Guernsey (1953) and Naqvi and Gordon (1965) showed a loss of strict basipetal auxin transport in Coleus blumei with flowering. This change in direction of auxin movement has been suggested as an important factor in the correlative inhibition of rooting by flowering.

There was a significant correlation between transport ability and flowering, with flowering significantly reducing leaf rooting-potential and capacity for auxin transport (Figure 24). The relationship between the capacity for auxin transport and rooting-potential of leaves from various zones on flowering and vegetative shoots was more pronounced with 'Pink Pearl' than 'Roseum Elegans', possibly because of the stronger auxin mobilizing ability of the 'Pink Pearl' flower buds (Figures 25 and 26). As a result of strong mobilization by the flower, tissue from the different leaf zones might contain different levels of auxins, thus altering the capacity for transport.

The amount of labelled auxin absorbed by petiole tissue from vegetative shoots was less than from flowering shoots. Although these differences were not significant in the case of 'Roseum Elegans', they were in 'Pink Pearl', and possibly reflect the relative mobilizing capacity of the flower buds. These results with 'Pink

Pearl¹ indicate that the labelled auxin was more readily absorbed than transported by the tissues of flowering shoots.

The prevention of flowering by terminal bud excision or heavy shading significantly improved rooting and the capacity for auxin transport (Figures 27, 28, and 29). There was an inverse relationship between auxin absorption and transport in flowering shoots, supporting the hypothesis that flowers mobilize growth substances, such as auxins, and reduce the amounts available for rooting.

Conclusions

The nature of flower bud influence on root regeneration is a complex phenomenon. A schema showing some of the effects of flowering on rooting-potential, and their interaction, is presented in Figure 31. Although incomplete and possibly over-simplified, it serves to illustrate certain facets of flower bud influence on root regeneration covered in this research.

It was found that the correlative inhibition of rooting by flowering differed with cultivars. Thus, experimental results must be qualified as to the cultivar used for valid interpretation of flower effects on rooting.

This research confirms that leaves are necessary for both flowering and rooting. After flowering, morphological and biochemical changes occur in leaves that affect rootability. Leaves from

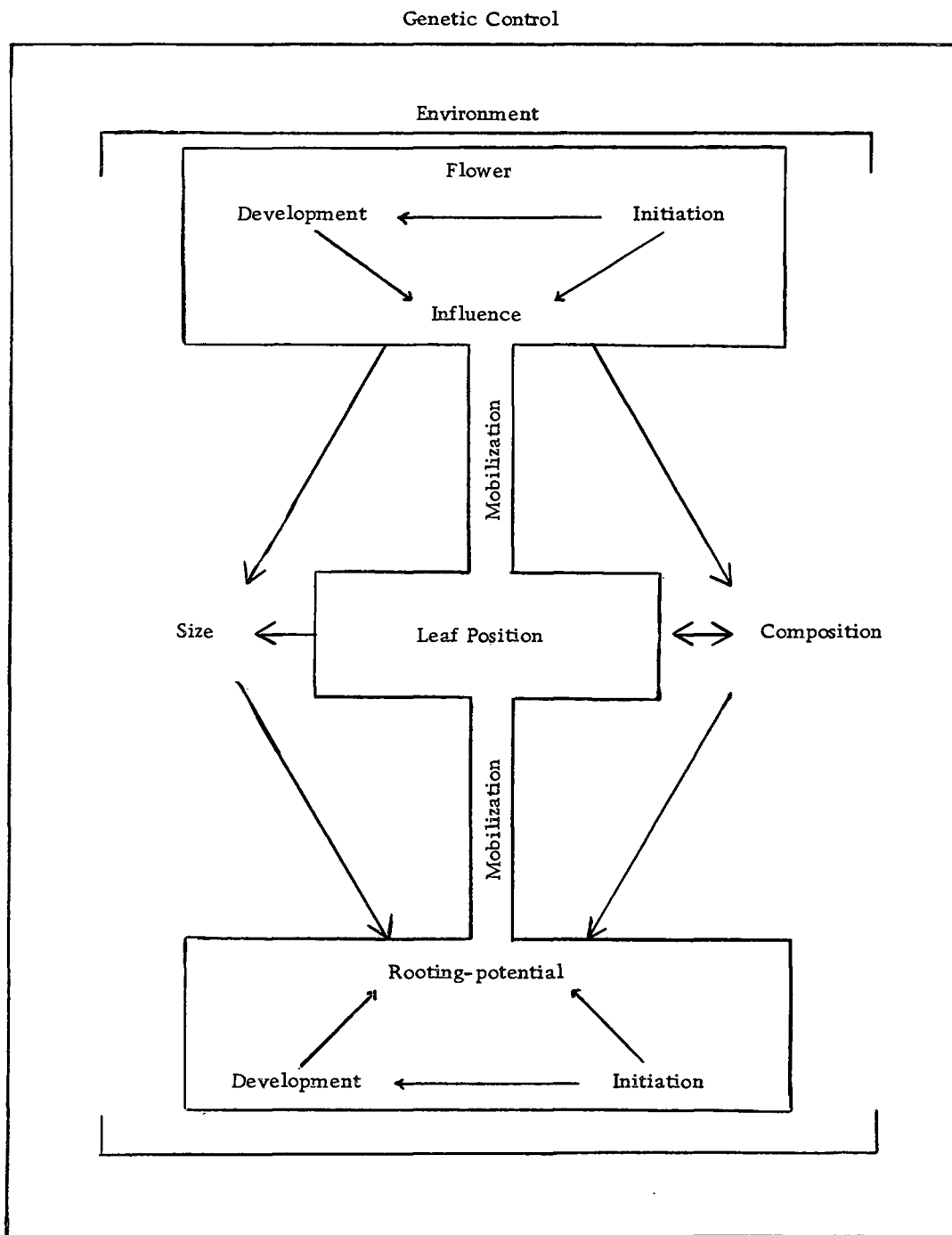


Figure 31. A schema representing the correlative effects of flowering on rooting-potential.

lower portions of shoots are enlarged by and lose rooting-potential with flowering. Flowering also probably changes the ability of leaves to export or import growth substances. Moreover, the strict basipetal movement of auxin is apparently altered by flowering. This research establishes that the capacity for basipetal auxin transport and root regeneration in leaves is reduced by flowering. Based on these evidences a proposal similar to Selims' (1956) is suggested: the correlated inhibition of rooting by developing flower buds occurs as a result of acropetal mobilization of growth substances needed in rooting. It is clear that the extent of flower influence on rooting of rhododendron shoots depends on the cultivar and stage of flower bud development at the time of sampling.

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