INFLUENCE OF PLANT EXTRACTS AND PHOTOPERIOD ON ROOTING AND CARBOHYDRATE PHYSIOLOGY OF CUTTINGS

by

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Short title

ROOT INDUCTION BY PLANT EXTRACTS AND PHOTOPERIOD

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ABSTRACT

M.Sc. CLAUDE RICHER LECLERC Plant Science

INFLUENCE OF PLANT EXTRACTS AND PHOTOPERIOD ON ROOTING AND CARBOHYDRATE PHYSIOLOGY OF CUTTINGS

The influence of Salix and Populus plant extracts as rooting promoters, the interaction of these plant extracts with indole butyric acid (IBA) on rooting of cuttings, and also the influence of photoperiod on rooting and carbohydrate physiology of cuttings during propagation were studied.

Salix and Populus plant extract treatments in combination with IBA influenced positively the rooting ability of cuttings of Thuja occidentalis and Juniperus sabina between November and January, and of Juniperus sabina between February and June. Salix extracts in combination with IBA gave the most consistent results. Seasonal plant extracts, derived from twigs collected at intervals over a one-year period, and applied to Cotoneaster acutifolia cuttings alone or in combination with different concentrations of IBA (5000 or 20000 ppm), inhibited rooting of this species. All treatments with IBA plus plant extracts caused significant injury to the cuttings. Seasonal Salix extracts promoted rooting of cuttings of Philadelphus coronarius and Ribes alpinum but showed little influence on rooting of Cornus elegantissima.

Between November and January, cuttings of Thuja and Juniperus rooted under extended (16-hour) and natural photoperiods showed increased rooting accompanied generally with lower contents of total soluble sugars and starch under extended photoperiod. Between February and June cuttings showed no difference in rooting due to photoperiod.

A negative correlation was obtained between rooting percentage of Philadelphus and amounts of total phenols in seasonal Salix extracts. There was a corresponding positive correlation for Ribes. Correlations also were found between rooting percentage of Philadelphus and soluble sugar content of seasonal Salix extracts, and between rooting percentage of Ribes and sugar/starch ratio of the seasonal extracts. No significant correlations were found between nine elements (N, P, K, Ca, Mg, Fe, Mn, Cu, and Zn) analyzed in seasonal Salix extracts and rooting responses of these three species.

RESUME

M.Sc. CLAUDE RICHER LECLERC Plant Science

INFLUENCE DES EXTRAITS DE PLANTES ET DE LA PHOTOPERIODE SUR L'ENRACINEMENT ET LA PHYSIOLOGIE DES BOUTURES

L'influence d'extraits de plantes telles que Salix et Populus comme agent promoteur de la formation des racines, l'intéraction de ces extraits avec l'acide indole butyrique (AIB) sur l'enracinement des boutures, ainsi que l'influence de la photopériode sur le comportement des hydrates de carbones dans les boutures lors de la propagation, ont été étudiés.

Les traitements préparés avec les extraits de Salix et de Populus combinés avec l'AIB influencent positivement la formation des racines chez les boutures de Thuja occidentalis et de Juniperus sabina enracinées entre le mois de novembre et de janvier, et celles de Juniperus sabina enracinées entre février et juin. De tous les traitements, ceux provenant de la combinaison des extraits de Salix et d'AIB donnent les meilleurs résultats. Les extraits saisoniers de plantes, provenant des rameaux prélevés à plusieurs intervalles pendant une période d'une année, appliqués sur des boutures de Cotoneaster acutifolia, seuls ou en combinaison avec différentes concentrations d'AIB, inhibent l'enracinement de cette espèce. Tous les traitements avec l'AIB combinés aux extraits occasionnent des dommages sur les boutures. Les extraits saisonniers de Salix appliqués sur les boutures de Philadelphus coronarius, Ribes alpinum et Cornus elegantissima, favorisent l'enracinement de Philadelphus et de Ribes mais ont moins d'influence sur l'enracinement de Cornus. Entre novembre et janvier, les boutures de Thuja et de Juniperus soumis à une longue photopériode (16 heures) et à une photopériode normale, montrent une augmentation de l'enracinement et une diminution des sucres solubles et de l'amidon sous la longue photopériode. Enracines entre février et juin, les boutures ne montrent aucune différence entre les deux photopériodes.

Une corrélation négative a été observée entre le pourcentage d'enracinement de Philadelphus et le contenu en phénols totals des extraits saisonniers de Salix. Une correlation correspondante, positive a été notée pour Ribes. Des corrélations ont également été trouvées entre le pourcentage d'enracinement de Philadelphus et le contenu en sucres des extraits de Salix et entre le pourcentage d'enracinement de Ribes et le ratio sucre/amidon. Aucune corrélation n'a été trouvée entre les 9 éléments (N, P, K, Ca, Mg, Fe, Mn, Cu, et Zn) analysés dans les extraits saissoniers de Salix et les réponses à l'enracinement des boutures.

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INTRODUCTION

Propagation by stem cuttings is one of the most important and basic areas of nursery culture (Girouard and Hess 1964; Hartmann and Kester 1975; Hess 1963; Whitcomb 1978). As a vegetative means of propagation, this technique preserves the ornamental characteristics of stock plants (Hartmann and Kester 1975).

Numerous techniques can be utilized to increase rooting of stem cuttings. For instance, exposure of cuttings or of stock plants to light may modify rooting behavior (Ellyard 1976). According to Stoutemeyer and Close (1946, 1947), exposure of cuttings to light of different spectral qualities influenced rooting ability. While some species produced more roots under long photoperiod (Baker and Link 1963; Snyder 1955; Waxman 1957) others rooted best under short photoperiod (Kamp and Van Dunen 1958) or were inhibited by light (Shapiro 1958).

The season in which cuttings are taken can have a dramatic influence on rooting (Hartmann and Kester 1975) but little is known about the controlling mechanism of seasonal rooting response. According to Alvim et al. (1976) and Nanda and Anand (1970), the seasonal changes in rootability of cuttings appears to be related to the level of endogenous hormones.

Although exogenous application of natural or synthetic auxins has had the greatest impact on root promotion of cuttings (Cooper 1935; Edmonds 1973; Went 1934), the rooting process is now recognized to be the result of interaction or balance between hormones (including growth promoters and growth inhibitors), rather than an apparent direct response to a single growth hormone (Bojarczuck 1978; Thimann 1977; Wareing 1973). Furthermore, other substances which are not clearly identified are also known to interact in the rooting process (Bonner 1937; Hess 1962; Thimann 1977).

The rooting potential of cuttings of different plant species varies considerably. While some species root easily, others root with difficulty and some do not root even with endogenous application of growth substances (Bojarczuck 1978; Edwards and Thomas 1980; Hess 1963; Okoro and Grace 1978; Schier and Campbell 1976; Shapiro 1958; Thimann and Delisle 1939).

Chmelar (1974) tested the rooting capacity of 107 willow taxa (Salix sp.) and found that most of them rooted easily. Willows and poplars (Populus sp.) have been shown to have preformed, lateral root primordia at their nodes (Bulloch 1973; Carlson 1938; Densmore and Zasada 1978; Haissing 1970; Libby 1974; Trecul 1846). Thus, cuttings of these species root easily. Alvim et al. (1976) showed variation in hormone content of willow and poplar cuttings and Nanda and Jain (1972) demonstrated that rooting of these species varied considerably with season. Kawase (1964, 1981) has shown that willow extract promoted

rooting of many plant species. Thus, it appears that willow and poplar extracts may contain the right balance of hormone and other unknown substances capable of improving rooting.

The purpose of this study was to investigate the influence of willow and poplar extracts as rooting promoters, the interaction of these plant extracts with indole butyric acid (IBA) on rooting of cuttings, and also the influence of photoperiod on rooting and carbohydrate physiology of cuttings during propagation.

REVIEW OF LITERATURE

1. Factors influencing rooting of cuttings

Numerous environmental and other factors influence rooting response of cuttings. Some of these factors can be controlled or modified. These include the source and the type of wood selected for cuttings (lateral vs terminal shoots, different parts of the shoot); the time of the year in which cuttings are taken; environmental conditions during rooting, such as humidity control by misting; the composition and temperature of the rooting medium; and light intensity and duration during rooting (Hackett 1969; Hartmann and Kester 1975; Stromquist and Eliasson 1979; Tustin 1977).

Other factors may be less amenable to control or modification. These include age and nutritional status of stock plants; growth habit and genetic origin of the stock plant; and physiological condition and endogenous composition of hormones and rooting cofactors of the cuttings.

2. Influence of light

Since 1686, daylength was observed to affect the growth of plants. Garner and Allard (1920) confirmed that long and short days affected growth of many herbaceous plants in different ways. This phenomenon, known as photoperiodism, influences flowering, vegetative growth, leaf

abcission, cambial activity, rooting of cuttings and many other processes (Nitsch 1957; Piringer 1961; Wareing 1956; Waxman 1955; Whalley and Cockshull 1976).

2.1 Quality and source

According to Stoutemeyer and Close (1946, 1947), the exposure of cuttings to light of different spectral qualities was found to cause large differences in the quantity of roots and in the speed of rooting of cuttings. In general, light having a high proportion of red radiation was found to be best, while blue light was usually the least favorable during rooting. Of five light sources, incandescent (INC), high pressure sodium (HPS), metal halide (MH), cool light fluorescent (F) and clear mercury (HG), Cathey and Campbell (1975) observed that the order of their effectiveness was: INC > HPS >> MH = F >> HG with regards to rooting of a wide range of woody trees, shrubs and herbaceous plants. In general, radiation in the red-orange end of the spectrum seems to favor rooting of cuttings but, in one test, Thimann (1977) reported that when stock plants were exposed for six weeks to light sources of different quality, cuttings from plants exposed to blue light rooted more easily.

2.2 Photoperiodism

2.2.1 During rooting

The rooting process in response to photoperiod has yielded variable and often conflicting results. Thus light requirements for rooting of woody ornamentals have never been critically defined in spite of the obvious practical importance (Loach and Whalley 1978).

Artificial light sources modified the behavior of cuttings (Ellyard 1976). Waxman (1955) observed that the rooting response of summer cuttings of Cornus florida under intermittent mist was greater under long (18-hour) days than under natural daylength or under short (9-hour) days. Extended photoperiod in some cases has increased rooting percentage (Ellyard 1976), size and number of roots (Waxman 1965), and speed and extent of rooting as measured by number and length of roots initiated (Fahali et al. 1979; Nitsch 1957). Baker and Link (1963), Lanphear and Meahl (1966), Piringer (1961), and Zimmerman and Hitchock (1929) reported that rooting quality of woody species was often superior when cuttings were rooted under long days. On the contrary, rooting has been shown to be inhibited by light (Shapiro 1958). Whaley (1977) reported that extended photoperiod caused a depressant effect on rooting.

Interestingly, Baker and Link (1963) examined the response of cuttings from 26 different species subjected to natural, 18-hour, and 24-hour daylengths and concluded that photoperiod had little effect during different seasons of year with regards to easily-rooted species.

However, extended daylengths improved initiation and number of roots in several difficult-to-root species; this response was relatively greater for dormant or hardwood cuttings.

Numerous studies on the influence of photoperiod on cuttings during rooting have shown that deciduous woody angiosperms tended to respond differently than do evergreen species; photoperiod generally favors rooting of angiosperm cuttings (Baker and Link 1963). The effect of photoperiod on rooting of evergreen cuttings seems to be more variable. For instance, in the case of Juniperus sp., rooting was not affected markedly by photoperiod treatments in experiments by Smith et al. (1971), but Lanphear and Meahl (1961) obtained a highly significant response under long photoperiod (18 to 24 hours) compared with natural photoperiod. On the other hand, Kamp and Van Dunen (1958) found that cuttings of some conifers rooted best under short days. Snyder (1955) reported that long photoperiod was detrimental to the rooting of Taxus cuspidata.

Lanphear and Meahl (1966) reported that rooting of evergreen cuttings was enhanced by factors that encouraged a dormant condition, and was inhibited by related factors which stimulated growth. Thus, according to Baker and Link (1963), Lanphear and Meahl (1966), and Waxman and Nitsch (1956), it is difficult to make any generalization about the influence of photoperiod on rooting of evergreen cuttings.

2.2.2 Stock plant

It appears that the photoperiodic regime or quality of light to which stock plants have been exposed exerts a marked effect on the ability of cuttings to root (Baker and Link 1963; Ellyard 1976; Nitsch 1957; Piringer 1961; Smith and Wareing 1972; Waxman 1957; Wright 1977).

Moshkov and Kocherzhenko (1939) noted that cuttings of Salix pierotti and Salix babylonica rooted better when taken from stock plants subjected to short day (SD) treatments, while cuttings of Salix undulata rooted better when taken from stock plants grown under long day (LD) treatments.

Barba and Pokorny (1975) demonstrated that cuttings from stock plants of Rhododendron obtusum japonicum cv. Hinodegiri and Snow, which had been grown for four months under 15-hour photoperiod, rooted better than those taken from stock plants grown for the same duration of time under 9-hour photoperiod. Steponkus and Hogan (1967) obtained a higher percentage of rooting for Abelia grandiflora cuttings from stock plants maintained under short days (9 to 12 hours) than cuttings from long day stock plants (18 to 24 hours). Piringer (1961) also showed that when stock plants of Weigela sp. were grown continuously under long days, softwood cuttings taken at any time rooted readily in the greenhouse; stock plants grown under short days of less than 12 hours became quiescent and their cuttings were more difficult to root.

3. Influence of season

The time of the year in which cuttings are taken in some cases can have a dramatic influence on rooting (Hartmann and Kester 1975), but little is known about the controlling mechanism of this seasonal response of rooting (Lanphear 1963).

Certain species, such as Ligustrum spp, root readily when cuttings are taken almost anytime of the year (Hartmann and Loreti 1965). Softwood cuttings of deciduous woody species taken during spring or summer usually tend to root more easily than hardwood cuttings produced in the winter (Hartmann and Kester 1975). For plants which are difficult to root, such as Rhododendron sp, it is often necessary to resort to the use of easier-to-root softwood cuttings (Olieman et al. 1971). Rooting of pecan cuttings has been attempted by a number of researchers but with limited success. Sparks and Pokorny (1966) reported that rootability of pecan was influenced by time of the year cuttings were taken. Tognoni et al. (1977) obtained very poor or almost no rooting of Picea glauca cuttings during winter months, although there was a sudden increase in rooting activity during spring. Girouard (1975) obtained similar results with Picea abies.

According to Alvim et al. (1976) and Nanda and Jain (1972) seasonal changes in rootability of cuttings appear to be related to the level of endogenous hormones. A variation in auxinic content of Populus x robusta (Schneid) cuttings during different seasons was associated with a decline or an increase in rootability (Smith and

Wareing 1972). Roberts (1969) associated certain yearly events, such as flower induction and dormancy, with seasonal changes in rootability. He suggested that cold treatment resulted in a decline in endogenous growth inhibitors and a release of rooting promoters. Nanda and Anand (1970) observed a relationship between profuse rooting of cuttings and active growth period of trees. However, they concluded that, in addition to growth inhibitors and histological features, root formation was governed by a complex of other factors.

Lanphear (1963) emphasized that one environmental factor that was particularly important in conjunction with seasonal rooting response was photoperiod. There have been numerous reports on the effect of photoperiod on rooting as previously mentioned, although its influence is not always clear. In 1966, Lanphear and Meahl observed variations in rooting of Andora juniper cuttings during different times of the year but the same variations in rooting took place under LD, SD or under natural daylength. In this particular case, daylength was not a factor accounting for seasonal variability in rooting.

4. Hormonal and other rooting substances

There are several reasons to assume the existence of allied substances causing the formation of roots in cuttings. The complexity and variability in rooting response indicate that the interaction of many substances must be considered. Bouillenne and Bouillenne-Walrand (1955) suggested a basic pattern explaining the interaction between

several compounds in the formation of roots. This pattern was adapted by Hartmann and Kester (1975) as described in Figure 1.

According to Tukey, Jr. (1979), three elements seemed to be necessary for root initiation: auxins, cofactors and complexing enzymes. Without any of these, rooting will not occur. Cofactors could differ from plant to plant and hormonal content could be specific for each species. The complexing enzymes in cuttings have been recently isolated in one species, namely the apple (Bassuk 1980).

4.1 Plant hormones

Plant hormones are closely involved in several aspects of plant propagation and their role is still incompletely understood.

4.1.1 Auxins

The most important substances regulating root regeneration are auxins (Lee et al. 1969). Auxins are synthetized in the apical meristems of flowering plants, in swelling buds, in expanding leaves during the growth season (Meyer et al. 1973; Wareing et al. 1964), and in root tips, pollen and fruits (Tukey, Jr. 1979). Auxins are transmitted through the plant by diffusion from cell to cell (Edmonds 1973). The basipetal translocation of auxin in cuttings results in its accumulation at the base of the cuttings, thus aiding root initiation (Tukey, Jr. 1979; Wareing 1973). Auxins serve to mobilize reserved food material, acting as a root-sink at the zone of its application (Nanda

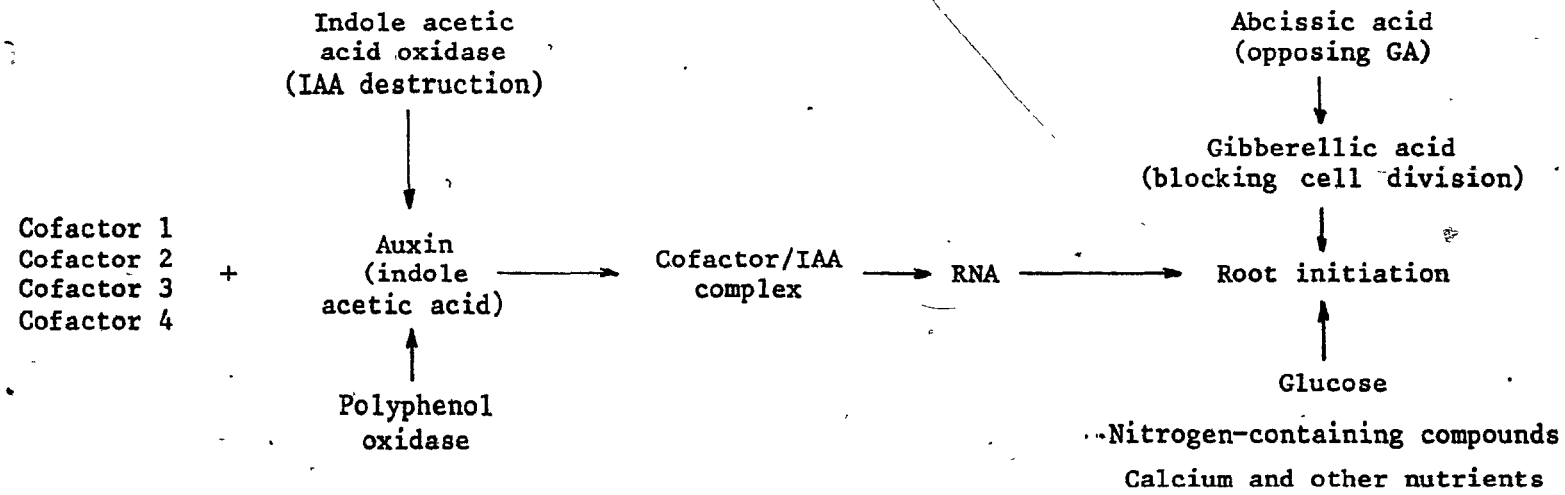

Figure 1. Hypothetical relationships of various components leading to adventitious root initiation. (Hartmann and Kester 1975)

and Anand 1970). They also affect the transport of assimilates indirectly by increasing sugar availability and initiate the breakdown of reserves (Atman and Wareing 1975). Furthermore, auxins enhance cell division, elongation and differentiation (Haissing 1970).

Indole acetic acid (IAA) was the first natural auxinic compound discovered and identified in the 1930's (Thimann and Went 1934; Went 1934). Within months of IAA identification, synthetic auxins were tested for their activity in promoting roots on stem segments. In 1935, several investigators demonstrated the practical use of these materials in stimulating rooting in cuttings (Cooper 1935; Thimann 1977). Researchers showed that the two related compounds, indole butyric acid (IBA) and naphthalene acetic acid (NAA), were even more effective than the naturally occurring IAA for rooting (Hess 1962; Hartmann and Kester 1975; Mes 1951; Wareing 1973; Zimmerman and Wilcoxon 1935), due to their higher stability and mobility (Tukey, Jr. 1979).

Although auxins are generally beneficial in promoting rooting of cuttings, for some species, auxins have been shown to have little or no effect, or may even be detrimental (Girouard 1971; Libby 1974; Whitcomb 1978).

4.1.2 Rhizocaline

In 1938, the work of Went was based on the certitude that auxin was not the only hormonal group involved in the rooting process and that the presence of an unknown substance referred to as «rhizocaline» was

involved (Bouillenne and Bouillenne-Walrand 1955; Cooper 1938; Galston 1948; Hess 1962; Kawase 1964; Thimann and Delisle 1939; Van Overbeek and Gregory 1945). Cooper (1938) found that lemon cuttings responded to auxin treatments by forming lateral roots. However, if the portion of the stem containing the roots was removed and auxins were applied a second time, there was no rooting response, even though leaves continued to provide nutrients. Cooper suggested that there was a limited supply of «rhizocaline» and that it was depleted with the first application of auxin. In subsequent years, several studies supported the «rhizocaline theory» but its isolation has not yet been accomplished (Hartmann and Kester 1975; Kawase 1964).

4.1.3 Other growth hormones

As reported by Hartmann (1977), roots (Wess and Waadia 1965) and especially root exudates (Kender 1965) are fairly rich in cytokinins. Their influence on root initiation may depend upon the particular stage of initiation and their concentrations (Hartmann and Kester 1975). Cytokinins are able to stimulate and to inhibit initiation and development of roots (Devlin 1966).

Kinetin, in the presence of casein hydrolysate, and IAA stimulated roots in tobacco stem cultures (Skoog and Miller 1957) and in lettuce leaf fragments (Meyer et al. 1973). Heide (1965) found that at a very low concentration, cytokinin stimulated the effect of auxin on rooting of Begonia leaf cuttings. This demonstrated synergism and antagonism with auxins.

The root system is the major source of gibberellins (Carr et al. 1964). This class of hormone seems to play a limited role in the rooting of cuttings. According to Brian et al. (1960) and Key (1969), gibberellins inhibited adventitious root formation in several pea varieties. They suggested that gibberellins interfered with the regulation of nucleic acid and protein synthesis. However, Tukey, Jr. (1979) showed that gibberellins limited root development because of growth competition and apparently had no effect upon initiation. In suitable concentrations, some gibberellins favored root elongation in certain species (Meyer et al. 1973). Ericksen (1974) observed root promotion with gibberellins at low concentrations (10^{-11} to 10^{-7} M) and an inhibiting effect between 10^{-6} and 10^{-3} M.

Abcissic acid is a hormone which influences rooting response of some cuttings by interfering and inhibiting the effect of gibberellins (Chin et al. 1969; Hartmann and Kester 1975; Tukey, Jr. 1979). Its main function is that of an inhibiting hormone (Torrey 1976).

4.2 Rooting cofactors and other substances

As shown in Figure 1 (p.12), rooting of cuttings is influenced by several hormones, working sometimes together at different concentrations and at different periods of the complex root formation process. The rooting phenomenon is the result of interaction and balance between several hormones and cofactors rather than apparently a direct response due to a single substance (Nitsch 1957; Thimann 1977; Waxman 1957).

Besides the hormones described above, there are many endogenous substances that seem to act as promoting and/or inhibiting factors in rooting (Basu et al. 1969; Girouard 1964, 1967; Hess 1962; Lee Choong and Tukey, Jr. 1971; Went 1938).

4.2.1 Phenolic compounds

Phenolic compounds were studied in relation to their root-promoting effect (Hess 1962). Basu et al. (1969) showed that none of the phenolic compounds promoted root initiation when used singly, but only when used in combination with auxins. The effect of phenolics on mung bean cuttings needs a particular structural qualification for root initiation, that is, the «presence of two hydroxyl groups in an ortho relationship and that the para position must be free» (Basu et al. 1969). Thimann (1977) has reported that monophenols inhibited both stem and root growth while o-diphenols promoted both. They concluded that the effect of phenols was not mediated primarily through those of IAA oxidation because addition of IAA did not restore normal root growth. Kefeli and Kadyrov (1971) suggested that phenolics acted primarily on metabolic systems rather than on hormonal systems. Gorter (1969) summarized the influence of phenolics as follows:

a) its inhibition of IAA oxidase system

b) a cofactor of tryptophane to IAA conversion

In other words, phenolic compounds interact with auxins (Thimann 1977); their synergistic action with auxins protected endogenous auxin from

oxidation. The concentration of phenols is almost 100 times that of hormonal levels in plants.

4.2.2 Carbohydrate and mineral nutrients

The presence of carbohydrates, translocated from leaves to stem, contributes in large part to root formation (Hartmann and Kester 1975; Hess 1962; Snyder 1974) and is a most important factor in root initiation (Thimann 1937). Researchers have demonstrated that exogenous nutrients supplied to cuttings, such as sucrose, increased the carbohydrate reserves in cuttings and improved rooting (Evans 1971; Howard and Sykes 1966; Loach and Whalley 1978; Nanda and Jain 1972; Went and Thimann 1937). According to Atman and Wareing (1975), IAA applied to the base of cuttings increased sugar accumulation at the site of root initiation.

Wott and Tukey, Jr. (1965) showed a positive influence of mineral nutrients on herbaceous and softwood cuttings upon root growth, but little effect upon root initiation. Swanson and Davies (1977) reported a significant effect of mineral nutrient on both root initiation and root development. Fluctuations in nitrogen nutrition were greatly reflected in the roots (Bosemark 1954), and calcium deficiencies characteristically suppressed root growth (Dvorak and Cernohorska 1972). Phosphorus is often involved in root growth (Singh and Singh 1971), but according to Black (1964) its response depends on whether or not the root is a storage-type tissue or an absorbing-type root tissue. Boron has been shown to stimulate root production in cuttings of some plant species (Hemberg 1951).

4.2.3 Other compounds

Other substances such as flavanoids, flavans, catechols, pyrogallol, chlorogenic acid, queratin, anthocyanins and leucocyanins, have been demonstrated to be useful in the rooting process (Bojarczuck 1978; Hackett 1969; Hartmann and Kester 1975; Lee Choong and Tukey, Jr. 1971). For instance, catechol seems to protect IAA from destruction, thus improving root initiation (Hackett 1969). Anthocyanins were reported to enhance root formation in cuttings. Bachelard and Stowe (1962) observed a high correlation between the total amount of anthocyanin present in the leaves of Acer rubrum cuttings and the number of roots formed in cuttings treated with IBA. Anthocyanins and flavanoids, which have a common structural ring similar to phenols, were considered as auxin-like substances (Lee Choong and Tukey, Jr. 1971). However, Thimann (1935) suggested a minimum interaction in in vivo culture between flavanoids and auxins because flavanoids were located in the vacuole and auxins in the cytoplasm; therefore a limited contact between them did not favor a strong interaction.

The B vitamins were found to stimulate root initiation. Bonner (1937) found that thiamin (B_1) was an important growth factor of isolated roots in synthetic sterile media. Bachelard and Stowe (1962) tried a basal pretreatment on Acer rubrum cuttings with riboflavin (B_2) and sucrose and these treatments did not increase rooting of these cuttings, but with Eucalyptus camadulensis tip cuttings some root promoting response was obtained. This interaction of riboflavin and sucrose was significant. Torrey (1976) found mixtures of thiamin and

pyridoxin (B_6) to be active in root formation of peas. In the study of Basu et al. (1970), using the vitamins B_1, B_2, B_3, nicotinic acid, and ascorbic acid, either alone or in conjunction with various auxins applied to several species, no rooting occurred except for treatments of nicotinic acid with NAA at 1 or 2 ppm in Justicia cuttings.

5. Plant extracts

That endogenous substances in plants act on physiological processes was recognized many years before the identification of auxins. Workers have tried to find hormonal activity in extracts from plants or plant parts. As reported by Hartmann (1977), Filting (1909) found that water extract of pollen inhibited floral abcission and stimulated ovary wall swelling in orchids. Went (1929) observed that leaf extracts from Acalypha plants induced root formation in Carica. Bouillenne and Went (1933) found substances in cotyledons, leaves, and buds which stimulated rooting of cuttings; they presumed these substances to be «rhizocaline.» Nelson (1959) tried a liquid extract of alfalfa containing an unknown active ingredient, called chloromone, on juniper cuttings. The chloromone benefitted rooting of junipers, both in speed and percentage.

According to Thimann (1937) crude extracts were frequently toxic. Hess (1961) suggested that the presence of four root-promoting substances named «rooting cofactors 1, 2, 3 and 4» (cf figure 1) in the extracts obtained from stem tissues of juvenile form of Hedera helix L. cuttings (Girouard and Hess 1964). Rooting cofactors also found in

chrysanthemum, hibiscus and camellia have been related to rooting ability of mung bean (Hess 1961, 1963; Richards 1964). Ouellet (1962) prepared extracts of boiled barley seeds plus water, boiled oat seeds and ground pieces of Ulmus twigs. These extracts promoted the rooting of Ulmus americana cuttings but none was equal to the effect of IBA treatments.

Coyama (1962), who applied water extracts of difficult-to-root species, Castanea crenata, Pinus densiflora, Myrica cubra and Cryptomeria japonica, on easy-to-root species of Salix babylonica, Amorpha fructicosa and Robinia pseudoacacia, obtained negative results in rooting of these easy-to-root species. The aqueous extracts contained some growth inhibitors able to inhibit the rooting of the easy-to-root species. These growth inhibitors were thermostable, alkaline for Cryptomeria and acidic for the three other species (Vieitez et al. 1980). Tognoni et al. (1977) found an acidic fraction of methanolic extract from Picea glauca which had root promoting activity. Modifications on the bioassay of Luckwill (1956) were done with lilac extracts to test the occurrence of promoters and inhibitors of rooting. These extracts were useful to detect the phenolic compounds and rootability of various cultivars (Bojarczuck 1978).

Kawase (1964) obtained a strong root promoting activity on many mung bean cuttings by applying diffusate of Salix alba cuttings steeped in water. The diffusate showed a strong synergistic effect with IAA on root formation of mung bean cuttings. The diffusate contained at least

four active rooting fractions and the most active fraction was extremely soluble in water but insoluble in chloroform or ethyl ether (Kawase 1964). Water soluble substances obtained from such woody plants as Cotoneaster racemiflora soongorica, Euonymus fortunei carrieri, Symplocos paniculata, Lonicera maacki, Ilex opaca, Physocarpus amurensis, Taxus cuspidata, and Viburnum bukwoodi were found to promote rooting when tested in mung bean bioassays (Kawase 1970, 1971). Kawase (1981) reported that the crude extract from only one-third of an ounce of willow twig stimulated production of 12 times as many roots per cutting of mung bean. The plant extract alone seemed to have the ability to stimulate rooting unmatched by any previously known rooting substance and did not cause injury to treated plants (Kawase 1981). The willow rooting substance is widely distributed in the plant kingdom, because a substance similar to willow rooting substance was found in all woody species tested at different concentrations and with different concentrations and with different efficacy (Kawase 1971).

6. Carbohydrate physiology

Sugars, the primary products of photosynthesis, are converted into starch and cellulose, stored in reserve, and transformed into utilizable energy as required by the plant (Devlin 1966). Loach and Whalley (1978) summarized the carbohydrate relationship in cuttings (Figure 2).

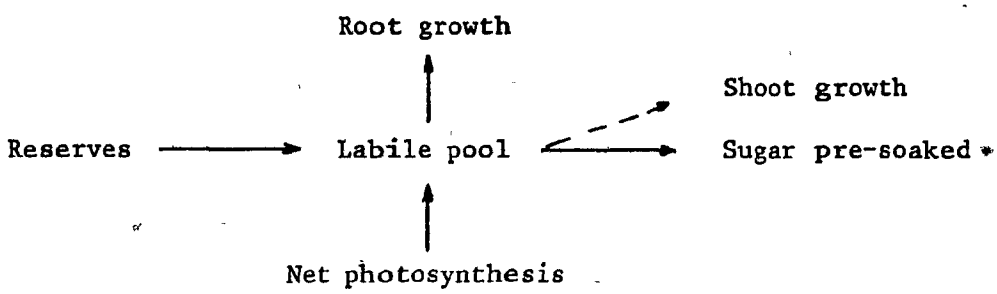

Figure 2. Carbohydrate organization in cuttings. (Loach and Whalley 1978)

A close relationship between carbohydrate content of cuttings and light exposure has been reported (Eliasson 1978; Howard and Sykes 1966; Loach and Whalley 1978). Light intensity must be high enough for carbohydrates to accumulate in excess over those used in respiration (Ellyard 1976; Hartmann and Kester 1975). Loach and Whalley (1978), Okoro and Grace (1976) and Vieiteiz et al. (1980) demonstrated that carbohydrate content of cuttings was an important determining factor in rooting success. Roots must be formed by cuttings before the existing food reserves are depleted; thus cuttings, very low in carbohydrate content, root poorly or with difficulty (Starring 1923).

6.1 Light and carbohydrate relationships

As previously discussed, LD treatments increase the ability of cuttings of many species to root. Apparently this increased rooting ability following extension of the light period is partially due to

greater accumulation of photosynthetates (Haugh 1978), at least up to a certain level of irradiance (Eliasson 1978; Howard and Sykes 1966).

Waxman (1965) reported that long photoperiods allowed cuttings a longer period of time to build up a supply of sugars and increased the size of the root system. However, Okoro and Grace (1976) observed in leafy cuttings that the total carbohydrate content increased under continued photoperiod but most of the increase was in the starch fraction. Haugh (1978) obtained significantly higher levels of starch in cuttings under LD treatment while content of total sugars was similar to that of cuttings under SD treatment. According to Basu and Ghosh (1974) the level of available sugars was more important than the concentration of starch in favoring rooting of cuttings; there was no correlation between starch content and rooting. The results seemed to support observations of Brandon (1939) and Cailloux (1943) showing that species having a high starch content did not root satisfactorily.

Lovell et al. (1971) observed that root formation was inhibited in detached cotyledons of Sinapsis alba L. under very high light conditions (10 k lux). It was also reported that under certain conditions a high carbohydrate level in cuttings can depress rooting (Hansen and Ericksen 1974). Okoro and Grace (1976) also obtained inhibited rooting associated with high levels of carbohydrate and high rate of photosynthesis. Thus, supraoptimal carbohydrate content can inhibit the rooting response.

Researchers reported that rooting was improved by exogenous application of sugar to cuttings at low light intensity levels (50 ± 10 foot candles) (Howard and Sykes 1966) but not at higher light intensity (1.5 MJ m^{-2}) (Loach and Whalley 1978). Contrastingly, Okoro and Grace (1976) reported that exogenous application of sugar on leafy cuttings stimulated root formation under light; cuttings under light contained more carbohydrate than those without light.

6.2 Auxin and carbohydrate relationships

Pearse (1963) and Nanda and Jain (1972) reported that both carbohydrate and auxin together influenced the ability of stem cuttings to root. One of the principal actions of auxins is stimulation of the hydrolyzing starch. Auxin exogenously applied or endogenously accumulated at the base of cuttings, can hydrolize accumulated starch, thereby improving root formation in cuttings. Atman and Wareing (1975) suggested that a threshold sugar content was required for the development of a given number of root primordia, and that this threshold was improved by IAA.

6.3 Seasonal response and carbohydrate relationships

Domanskaya and Kulivov (1976) reported that a seasonal accumulation of starch in broad-leaved evergreens was lowest in early autumn and highest in the spring. Nanda and Jain (1972) investigated the effect of auxins on the seasonal rooting response of cuttings of Populus nigra.

They found that seasonal response of cuttings was correlated with mobilization of reserve food material in cuttings. According to Nanda and Anand (1970), weak rooting response in winter between November and February was caused by low activity of the hydrolizing enzymes which can mobilize starch even when auxins were exogenously applied. On the other hand, vigorous rooting during the active growth period between February and October was caused by enzyme activity.

Okoro and Grace (1976) observed a decrease in carbohydrate reserve of hardwood cuttings when leaves and buds began to grow. This decrease continued even after the expansion of leaves in spring when net photosynthesis became positive. This continuous loss of carbohydrates from the hardwood stem segment was attributable to translocation to callus and developing roots.

MATERIALS AND METHODS

1. General details

1.1 Propagating environment

Three propagation experiments were conducted at Macdonald College under intermittent mist controlled by electronic leaf (Mac Penny, Plastic, Engineers, Ltd., Worthing, W. Sussex), either outdoors in shaded frames during the growing season or under greenhouse conditions during winter. Benches (each 2.5 m x 1.1 m) were provided with bottom heat thermostatistically set at 21°C in the region of the basal ends of cuttings (Hartmann and Kester 1975).

The rooting medium used was Canadian sphagnum peat moss and horticultural grade perlite mixed in equal volume. After sticking cuttings into the rooting medium, Benlate 50% WP (methyl-1 (butyl-carbamoyl)-2-benzimidazole carbamate) was applied at a rate of 1.5 liters/m^2 (2 g per liter) of bench space to prevent against rotting of cuttings. Thereafter, Captan 50% WP (cis-N-((trichloromethyl)thio)-4-cyclohexene-1, 2-dicarboximide) or Benlate, mixed and applied as described above, was applied alternatively every week.

1.2 Cutting preparation

The basal ends (lower 3 cm) of the cuttings were stripped of foliage, treated with extracts and/or IBA, as described below in each experiment, immediately stuck (2.5 cm deep x 2 cm apart) in rooting medium, and placed under intermittent mist. In these experiments, the rooting medium was contained in fiber flats (18 cm long x 13 cm wide x 7 cm deep).

1.3 Rooting evaluation

In each experiment, cuttings were evaluated according to the following parameters: (a) rooting index, a visual grade of the root system of each cutting within treatment, as exemplified in Figure 3 for Ribes alpinum; (b) rooting percentage; (c) total root length (cm) of all cuttings within treatments; (d) mean root length (cm) of each cutting within treatments; (e) mean root number of each cutting within treatment.

1.4 Willow and poplar extracts

1.4.1 Collection and preparation of twigs

The preparation and extraction of willow and poplar extracts, except when noted, were similar to procedures described by Kawase (1970).

On the dates November 10, 1980, January 12, 1981, March 30, 1981, May 1, 1981, June 13, 1981, and August 7, 1981, terminal twigs, 20 to 45 cm in length, were harvested from a 21-year-old weeping willow

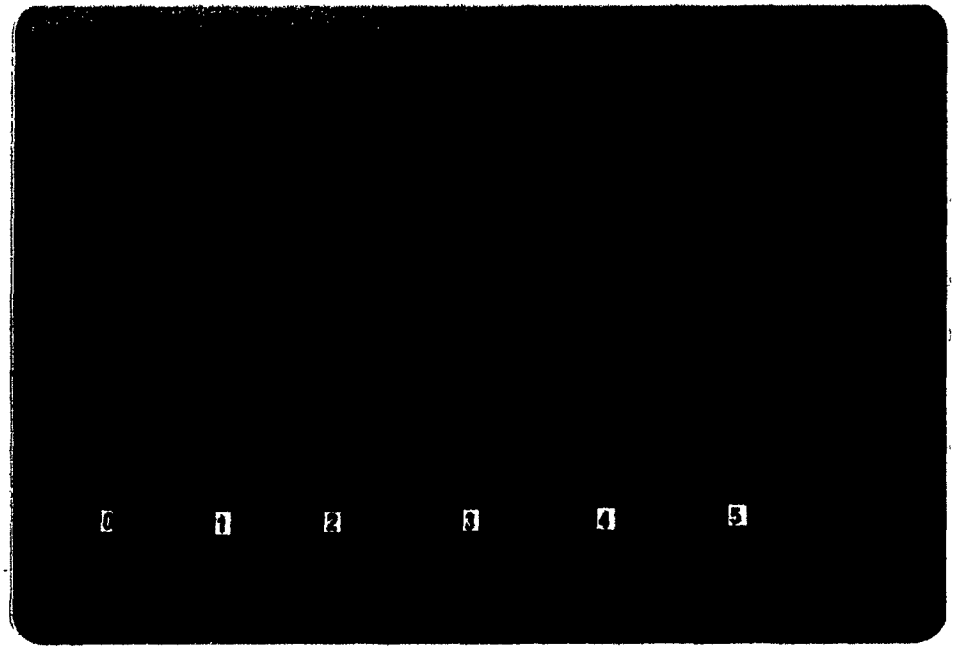

Figure 3. Rooting index of Ribes alpinum.
0 = dead or alive but no sign of activity; 1 = callusing only, but no roots; 2 = poor root system; 3 = few and short roots; 4 = good and uniform root system; and 5 = very extensive and well developed root system.

(Salix alba tristis Gaud) tree and a 45-year-old lombardy poplar (Populus nigra L. 'Italica') tree, both growing on the Macdonald Campus. Cuttings were taken from a single tree of each species to prevent inter-tree variation.

Cuttings were stripped of foliage, cut into pieces (5 to 7 mm), and frozen for 15 hours at -5°C (instead of being frozen for 48 hours at -20°C as reported by Kawase (1970)). The frozen tissues were freeze-dried for 42 hours (125 μ vacuum at -35°C, Labconco freeze-dryer Model 5). The lyophilized (freeze-dried) tissues were ground in a Wiley mill (Arthur H. Thomas Co., Philadelphia, Pa.) to pass through a 40-mesh wire screen (instead of being ground twice through a 20-mesh as reported by Kawase (1970)), and kept frozen at -5°C until used for extraction.

1.4.2 Preparation of extracts

Immediately before each experiment, extracts of the lyophilized powder were prepared by adding varying amounts of distilled water to the powder, depending upon the treatments in each experiment. The mixture was shaken for one hour on a reciprocating shaker (Eberbach Co., Ann Arbor, Michigan) at 270 to 280 strokes per minute in a cold room at 4°C (instead of at 0°C as reported by Kawase (1970)) to reduce possible enzymatic reactions.

1.4.3 Analysis of extracts

1.4.3.1 Total phenols

Extraction and determination of total phenols were by a modified method of Swain and Hillis (1959).

(a) Reagents

Folin-Denis.--The reagents were prepared according to the procedure of the A.O.A.C. (1975).

100% methanol.

50% methanol.--25 ml of 100% methanol with 25 ml of distilled water.

17% sodium carbonate.--To .18 g of Na_2CO_3, 10 ml of distilled water was added.

Phenols standard solution.--To 5 mg of tyrosine in a 50 ml volumetric flask, 50 ml of methanol 50% was added; aliquots with concentrations of 0, 1, 2, 3, 4, and 5 mg/50 ml were used for the standard curve.

(b) Procedure

To 10 mg of ground freeze-dried plant extract in a 4 ml volumetric flask, 2 ml of methanol was added and made to volume with distilled water. A 0.5 ml aliquot was diluted with distilled water to 7 ml in a 10 ml graduated test-tube. The contents were mixed well, 0.5 ml of the Folin-Denis reagent added, and the tubes thoroughly shaken again. After 3 minutes, 1.5 ml of Na_2CO_3 was added and the tubes shaken again. The absorptivity was determined after 1 hour by a spectrophotometer (Bausch

and Lamb Spectrophotometer, Model Spectronic 20) at 725 nm to a calibration curve prepared with solutions containing from 0 to 5 mg tyrosine/g dry weight.

1.4.3.2 Sugar and starch

Methods of analysis for total soluble sugars and starch are described below in section 2.1.3.2.

1.4.3.3 N, P, Ca, Mg, Fe, Mn, Cu, Zn and K

(i) Nitrogen

Total N was determined by the micro-Kjeldahl method (A.O.A.C. 1975).

(a) Reagents

H_2SO_4. Concentrated reagent, specific gravity 1.84, N-free.

K_2SO_4. Reagent grade, N-free.

Mercuric oxyde. HgO reagent grade, N-free.

Boric acid. H_3BO_3 saturated solution (6.35 g in 100 ml of water at 30°C).

Sodium hydroxide-sodium thiosulfate solution. A mixture of 60 g of solid NaOH and 5 g of $Na_2S_2O_3.5\ H_2O$ was made up to 100 ml with distilled water.

Indicator solution. A mixture of 2:1 by volume of 0.2% alcoholic methyl-red solution and 0.2% alcoholic methylene blue solution, respectively.

0.02 N HCl. 1.78 ml of HCl reagent 37.2% was made up to 1000 ml with distilled water.

(b) Oxidation procedure

To 50 mg of ground freeze-dried plant extract in a 30 ml regular Kjeldahl flask, 2 g of K_2SO_4, 50 mg of HgO and 2.5 ml of H_2SO_4 were added. The mixture was digested in the presence of boiling chips for 1.5 hours. After cooling, solids were dissolved with 2 ml of distilled water and then quantitatively transferred to a distillation apparatus. The distillate was received in a 125-ml Erlenmeyer flask containing 5 ml of saturated H_3BO_3 solution and 3-4 drops of indicator solution. The distillate was then carefully mixed with 10 ml of $NaOH-Na_2S_2O_3$ solution. A 15-ml aliquot was diluted to 50 ml with distilled water and total N determined by titration with 0.02 N HCl to the first appearance of violet.

(ii) P, Ca, Mg, Fe, Mn, Cu and Zn

The extraction for determination of P, Ca, Mg, Fe, Mn, Cu and Zn was done using the wet oxidahan procedure (Jackson 1958).

(a) Reagents

HNO_3. Concentrated reagent, specific gravity 1.42.

Ternary mixture of acids. A mixture of concentrated HNO_3 reagent, concentrated H_2SO_4 (sulfuric acid) reagent, and 70-72% $HClO_4$ (perchloric acid), in a ratio of 10:1:4 by volume.

2 N HNO_3. 128 ml of concentrated HNO_3 reagent was diluted to 1000 ml with distilled water.

(b) Procedure

To 0.40 g of dried ground tissue in a 30-ml beaker was added 2 ml of concentrated HNO_3. The beaker was covered with a watch glass, heated at 100°C over a hot plate for 30 minutes, after which 3 ml of the ternary mixture of acids were added and the temperature increased to 180°C. The digestion was continued until the ash residue in the beaker was white and nearly dry. After cooling, 1 ml of 2 N HNO_3 was added to the residue and the extract quantitatively transferred to a 10-ml volumetric flask and made up to volume with distilled water. The extract was stored in clear glass bottles at room temperature until used for P, K, Ca, Mg, Fe, Mo, Cu, and Zn determinations as described below.

(iii) Phosphorus

P was determined using the molybdovanado phosphoric acid method described by Greweling (1966).

(a) Reagents

Mixed reagent. To 25 g of $(NH_4)_6Mo_7O_{24}.4\ H_2O$ (ammonium molybdate tetrahydrate) dissolved in 300 ml of distilled water in a 1000-ml

volumetric flask, a solution of 1.25 g of NH_4VO_3 (ammonium metavanadate) in 500 ml of 5N HNO_3 was added while stirring, then made to volume with distilled water.

P standard solution. To 0.4393 g of KH_2PO_4 (potassium dihydrogen) in a 1000-ml volumetric flask, 5 ml of concentrated HNO_3 reagent was added and then made to volume with distilled water.

(b) Procedure

Two milliliters of extract and 5 ml of the mixed reagent were added to a 25-ml volumetric flask and made to volume with distilled water. Samples were allowed to stand at room temperature overnight for color development and then P was determined by comparing the transmittance at 460 nm to a calibration curve prepared with solutions containing from 0 to 500 µg of P (0 to 5 ml of P standard solution). It should be noted that full color development occurred after 15 minutes and remained stable for several days (Greweling 1966).

(iv) Potassium

The extraction for determination of K from freeze-dried ground samples was performed using the ammonium EDTA procedure (Baker and Greweling 1967).

(a) Reagents

0.1 M ammonium EDTA. To 292 g of ethylenedinitrilotetraacetic acid 500 ml of distilled water was added. Concentrated NH_4OH was then added until the acid was dissolved and then to excess to obtain a pH slightly

above 9. The solution was allowed to cool, diluted to 1000 ml with distilled water, and stored in plastic containers at room temperature. This solution was diluted 1 to 10 with distilled water immediately before extraction.

K standards. Reference solution 1000 µg K/ml (SO-P-351, Fisher Scientific Co.) was diluted with 0.1 M ammonium EDTA to 0.4 and 4.0 µg K/ml for the standard curve.

(b) Procedure

To 25 ml of 0.1 M ammonium EDTA extractant 0.25 g of freeze-dried ground tissue was added. The mixture was shaken for 45 minutes on a reciprocating shaker at 270 to 280 strokes per minute, then filtered through No.41 fast filter paper. The filtrate was diluted with 0.1 M ammonium EDTA 1 to 100 before determination of K by atomic absorption spectrophotometry, using the parameters shown in Table 1.

(v) Calcium and magnesium

(a) Reagents

Lanthanum solution 12.5%. To 147 g of La_2O_3 (lanthanum oxide) in a 1000-ml volumetric flask, a minimum amount of reagent grade HCl to dissolve and 100 ml of concentrated HCl were added and made to volume with distilled water.

Lanthanum solution 0.5%. One volume of the above solution was diluted with 24 volumes of 1+9 HCl.

TABLE 1. Operating parameters for K, Ca, Mg, Mn, Cu, and Zn determination

	K	Ca	Mg	Fe	Mn	Cu	Zn
Lamp	hollow cathode	hollow cathode	hollow cathode	hollow cathode	hollow cathode	hollow cathode	hollow cathode
Current (MA)	5-7	5-7	3	8-12	8-10	3-4	7.5
Wavelength (nm)	766.5	427.7	285.2	248.3	279.5	324.8	213.9
Spectral bandpass (nm)	.4-.8	.4-.8	.4-.8	.2-.4	.2-.4	.2-.4	.2-.4
Flame type	Air/Ac	N_2O/Ac	Air/Ac	Air/Ac	Air/Ac	Air/Ac	Air/Ac
Fuel	acetylene	acetylene	acetylene	acetylene	acetylene	acetylene	acetylene

Ca standards. Reference solution 1000 µg Ca/ml (SO-C-191, Fisher Scientific Fo.) was diluted with 0.5% lanthanum solution to 0 to 4 ppm for the standard curve.

Mg standards. Reference solution 1000 µg Mg/ml (SO-P-190, Fisher Scientific Co.) was diluted with 0.5% lanthanum solution to 0 to 2 ppm for the standard curve.

(b) Procedure

To a 1 ml aliquot of wet-ashed extract in a 25-ml volumetric flask, 1 ml of 12.5% lanthanum solution was added and made to volume with distilled water. The determination of Ca and Mg by atomic absorption spectrophotometry (SP 191, Atomic absorption spectrophotometer PYE UNICAM) was done using the parameters shown in Table 1.

(vi) Iron

(a) Reagents

Standard iron solution. To 1.755 g of Fe $(NH_4)_2(SO_4)_2.6\ H_2O$ (ferrous ammonium sulfate) in a 500-ml volumetric flask distilled water to dissolve the salt and 10 ml of concentrated HCl were added and made to volume with distilled water.

(b) Procedure.

Determination of Fe by the atomic absorption spectrophotometer followed instructions of Table 1.

(vii) Manganese

(a) Reagents

Standard manganese solution. To 0.5 g of pure manganese metal in a 1000-ml volumetric flask, a minimum amount of dilute HNO_3 was added to dissolve Mn and made to volume with distilled water and solutions to 0 to 4 ppm were prepared for standard curve.

(b) Procedure

Similar to iron procedure (Table 1).

(viii) Copper

(a) Reagents

Standard copper solution. To 0.393 g of $CuSO_4.5\ H_2O$ (copper sulfate pentahydrate) in a 1000-ml volumetric flask, deionized distilled water was added to make to volume and solutions to 0 to 2 ppm were prepared for standard curve.

(b) Procedure

Similar to iron procedure (Table 1).

(ix) Zinc

(a) Reagents

Standard zinc solution. Reference solution 1000 µg Zn/ml (SO-Z-13, Fisher Scientific Co.) was diluted with nitric acid (HNO_3) to 0.4 to 4 µg/ml for the standard curve.

(b) Procedure

To a 5-ml aliquot of wet-ashed extract in a 10-ml volumetric flask, deionized distilled water was added and made to volume. Determination was done following Table 1.

The contents of N, P, K, Ca, Mg, expressed in percentage, and the contents of Fe, Mn, Cu and Zn, expressed in ppm, were determined in duplicate.

2. Experiments

2.1 Interaction of willow and poplar extracts with IBA during rooting of evergreen cuttings under different photoperiods

2.1.1 Experiment 1 - Thuja occidentalis and Juniperus sabina

On November 10, 1980, terminal cuttings (9 to 10 cm long) were taken from hedge plantings of 10-year-old Thuja occidentalis L. and 6-year-old Juniperus sabina L. growing at Macdonald Campus. All cuttings were taken from vigorous current season's growth and from one plant per replication to minimize variation between individual plants. The mist frames were located in a greenhouse thermostatically set at 21°C day temperature and 18°C night temperature.

2.1.1.1 Rooting and photoperiod treatments

There were 12 rooting treatments as shown in Table 2. In this experiment only the November extracts of Salix and Populus were tested.

TABLE 2. Rooting treatments and extract preparation for rooting of *Thuja occidentalis* and *Juniperus sabina*

Rooting treatments			Ratio		
No.	Abbreviation	Description	a Powder(w)	b H_2O(v)	c IBA(v) 10,000 ppm
1	C	Control (50% ethanol)	0	0	0
2	IBA_5	IBA (5000 ppm)	1	1	0
3	S_1	*Salix*	1	25	0
4	$S_{2.5}$	*Salix*	1	10	0
5	P_1	*Populus*	1	25	0
6	$P_{2.5}$	*Populus*	1	10	0
7	SP_1	*Salix* + *Populus*	1	25	0
8	$SP_{2.5}$	*Salix* + *Populus*	1	10	0
9	$S_1 + IBA_5$	*Salix* + IBA (5000 ppm)	2	25	25
10	$S_{2.5} + IBA_5$	*Salix* + IBA (5000 ppm)	2	10	10
11	$P_1 + IBA_5$	*Populus* + IBA (5000 ppm)	2	25	25
12	$P_{2.5} + IBA_5$	*Populus* + IBA (5000 ppm)	2	10	10

a – w = weight in grams

b – v = volume in ml

c – IBA was prepared by dissolving crystals in 95% ethanol

All treatments were subjected to two photoperiods: natural photoperiod of approximately 9.5 hours between 0700 and 1630 hours, and extended photoperiod of 16 hours between 0500 and 2100 hours, using high pressure sodium lamps (Phillips HDK 602 Lu 400) suspended one meter above the rooting medium. Illumination at cutting level was 7000 lux (Weston Instrument Division, Model 756).

The experiment was a split split plot arranged in randomized complete block design, with one replication of light (main plot), 12 rooting treatments (sub-plot) and two extracts (sub-sub-plot). There were eight replications per experimental unit and nine cuttings in each unit.

Cuttings of Thuja occidentalis and Juniperus sabina were evaluated after eight and 10 weeks, respectively. Rooting parameters, as described previously under Material and Methods 1.3, were subjected to transformation to obtain normality and homogeneity of variances as described below:

(i) rooting index (RI): $\log (RI + 0.1)/(5.1 - RI)$

(ii) rooting percentage (RP): $\log (RP + 0.5)/(100.5 - RP)$

(iii) other parameters: powered to 1/3

Transformed data were subjected to analysis of variance. Differences among means were compared by the least significant test (LSD) method (Steel and Torrie 1980). Also conducted were correlation analyses of rooting parameters with contents of total sugars, starch, and sugar/starch ratio analyzed in cuttings, as described below in Materials and Methods section 2.1.3.2.

2.1.2 Experiment 2 - Juniperus sabina

On February 18, 1981, terminal cuttings of Juniperus sabina were taken and prepared as described under Materials and Methods section 2.1.1 for the previous experiment.

2.1.2.1 Rooting and photoperiod treatments

There were five rooting treatments as shown in Table 3. Similar to the previous experiment, only the November extract of each species was tested, and all treatments were subjected to the same two photoperiods. In that experiment, treatments 9, 10 and 11 in Table 1 (same as treatments 3, 4 and 5 in Table 3) appeared to yield the best rooting results.

This experiment was a split split plot in time arranged in a randomized complete block design with four replications and 10 cuttings per treatment. Main plots were harvest dates, sub-plots were photoperiods and sub-sub-plots were rooting treatments as described above.

Cuttings were harvested and evaluated on three different dates: April 16, April 30 and June 4, i.e., 8, 10, and 15 weeks after sticking, respectively. Data for rooting parameters were subjected to transformations as previously described. They were evaluated and analyzed following the procedures indicated under Materials and Methods section 2.1.1.1.

TABLE 3. Rooting treatments and extract preparation for rooting of *Juniperus sabina*

Rooting treatments			Ratio		
No.	Abbreviation	Description	a Powder(w)	b H_2O(v)	c IBA(v) 10,000 ppm
1	C	Control (50% ethanol)	0	0	0
2	IBA_5	IBA (5000 ppm)	0	1	1
3	$S_1 + IBA_5$	*Salix* + IBA (5000 ppm)	2	25	25
4	$S_{2.5} + IBA_5$	*Salix* + IBA (5000 ppm)	2	10	10
5	$P_{2.5} + IBA_5$	*Populus* + IBA (5000 ppm)	2	10	10

a - w = weight in grams

b - v = volume in ml

c - IBA was prepared by dissolving crystals in 95% ethanol

2.1.3 Chemical analysis

2.1.3.1 Tissue samples

Above-ground portions of cuttings of each sample within a replicate were dried for 36 to 48 hours at 60°C in a vacuum oven (20 cm of mercury; Precision Thelco, Model 29, Chicago).

Dried tissue samples were ground in a Wiley mill (Arthur H. Thomas, Co., Philadelphia, Pa.) to pass through a 40-mesh wire screen. Ground samples were kept in glass containers and stored at -5°C until analyzed. Before weighing portions for chemical analysis, ground samples were re-dried for 24 hours in the vacuum oven.

2.1.3.2 Sugar and starch determination

As described by Chong et al. (1979), extraction of total sugars was determined by a modified method of Dubois et al. (1956) and starch by a modified method of McCready et al. (1950).

(a) Reagents

80% ethanol (v:v). 1680 ml of 95% ethanol diluted to two liters with distilled water.

5% phenol (w:v): 150 g of phenol (C_6H_5OH) in 3000 ml of distilled water.

52% perchloric acid ($HClO_4$)(v:v). 270 ml of 72% $HClO_4$ with 100 ml of distilled water.

Concentrated sulfuric acid (H_2SO_4).

Glucose for standard curve. Stock solution (20 µg/ml) was prepared by adding 2 mg of glucose ($C_6H_{12}O_6.H_2O$) to 100 ml of distilled water; aliquots with concentrations of 0, 10, 20, 30, 40, 50, 60, 80, and 100 µg/5 ml were used for the standard curve.

(b) Procedure

Total sugars. Two hundred milligrams of the ground tissue was placed in a 15-ml centrifuge tube and soluble sugars were extracted during three successive 10-minute periods with 10 ml of 80% ethanol (v/v) over a hot water bath (80° to 90°C), with occasional stirring during extraction. After each period, the mixture was centrifuged and the extract decanted into a 100-ml volumetric flask. The ethanolic extracts were made up to volume with distilled water. Duplicate aliquots of 0.2 ml of the ethanolic extract were placed in cuvettes, and each diluted to 1.0 ml with distilled water. One milliliter of 0.5% phenol reagent was added, followed by 3 ml of concentrated H_2SO_4 added vigorously to ensure fast, rapid mixing.

Absorption values were read at 490 nm in a spectrophotometer (Coleman Junior, Model 6A). The content of soluble sugars, expressed in glucose units, was determined in duplicate by the phenolsulphuric acid method of Dubois et al. (1956).

A glucose standard curve was prepared in the range of 0 to 500 µg/5 ml of glucose as described above.

Starch. Starch was extracted from the ethanol-insoluble residue. In the centrifuge tube, 2 ml of distilled water was added and stirred. Starch was extracted by adding 6.5 ml of 52% perchloric acid ($HClO_4$) during two 10-minute periods at room temperature with occasional stirring. After each period, distilled water was added until the tube was nearly full to stop the digestion reaction. Following centrifugation after each 10-minute period, the starch extract was decanted into a 100-ml volumetric flask and the extracts made up to volume with distilled water.

The content of starch, expressed in glucose units x 0.9, was determined as above in duplicate by the phenolsulfuric acid method of Dubois et al. (1956).

2.2 Interaction of seasonal willow and poplar extracts with IBA on rooting of Cotoneaster acutifolius

On June 29, 1981, leafy semi-hardwood cuttings (9 to 11 cm long) of 11-year-old Cotoneaster acutifolius Turcz. growing at Macdonald Campus were taken from current season terminal growth of a series of shrubs, one shrub per replication. During cutting preparation, it was sunny with average day temperature of 16°C.

The mist frames were located outdoors.

2.2.1 Rooting treatments

This experiment consisted of 33 rooting treatments as shown in Table 4.

TABLE 4. Treatments with IBA and/or plant extracts for rooting of Cotoneaster acutifolius

Extracts	Rooting treatments		
	IBA (ppm)		
	0	5000	20,000
None	IBA_0 (0:0:0)[a]	IBA_5 (0:7:1)	IBA_{20} (0:1:1)
Salix			
Nov	$S_1 + IBA_0$	$S_1 + IBA_5$	$S_1 + IBA_{20}$
Jan	$S_1 + IBA_0$	$S_1 + IBA_5$	$S_1 + IBA_{20}$
Mar	$S_1 + IBA_0$	$S_1 + IBA_5$	$S_1 + IBA_{20}$
May	$S_1 + IBA_0$	$S_1 + IBA_5$	$S_1 + IBA_{20}$
Jun	$S_1 + IBA_0$ (1:25:0)[a]	$S_1 + IBA_5$ (8:175:25)	$S_1 + IBA_{20}$ (2:25:25)
Populus			
Nov	$P_1 + IBA_0$	$P_1 + IBA_5$	$P_1 + IBA_{20}$
Jan	$P_1 + IBA_0$	$P_1 + IBA_5$	$P_1 + IBA_{20}$
Mar	$P_1 + IBA_0$	$P_1 + IBA_5$	$P_1 + IBA_{20}$
May	$P_1 + IBA_0$	$P_1 + IBA_5$	$P_1 + IBA_{20}$
Jun	$P_1 + IBA_0$ (1:25:0)	$P_1 + IBA_5$ (8:175:25)	$P_1 + IBA_{20}$ (2:25:25)

[a]Ratio of powder (w) : H_2O (v) : IBA 40,000 ppm (v) where w = weight in grams and v = volume in ml.

Except for the first three treatments, which served as controls, Salix and Populus treatments were repeated for extracts collected from the following five dates:

Nov (November 10, 1980)

Jan (January 12, 1981)

Mar (March 30, 1981)

May (May 1, 1981)

Jun (Jun 13, 1981)

This was a 3 x 11 factorial experiment, arranged in a complete randomized design with seven replications and 10 cuttings per experimental factor combination. Factor A was concentrations of the powder : H_2O : IBA ratios (Table 3), and factor B, treatments at different months with Salix and Populus plant extracts.

Cuttings were evaluated August 7, 1981. Rooting parameters described previously were subjected to transformations as described under Materials and Methods section 2.1.1.1 before analysis of variance. Differences among means were compared by the LSD method.

2.3 Influence of seasonal willow extracts on rooting of three shrubs

On August 20, 1981, softwood cuttings (9 to 11 cm long) were taken from the following species: Philadelphus coronarius L. 'Aureus'; Cornus alba 'Elegantissima' Hort.; and Ribes alpinum L. All cuttings were taken from current season's terminal growth of a series of plants of each species growing on the Macdonald Campus. The approximate age

of these species was 10 years. During cutting preparation, it was cloudy with an average temperature of 16°C.

The mist frames were located outdoors.

2.3.2 Rooting treatments

There were seven rooting treatments as shown in Table 5.

The experimental design was a randomized complete block with 6 replications and 15 cuttings per experimental treatment.

Cuttings of Ribes and Philadelphus were evaluated October 1 and 2, respectively, and Cornus on October 8. Rooting parameters as previously described were subjected to analysis of variance. Data in this experiment did not require transformations. Differences among means were compared by LSD.

Initially, IBA alone was included as a second control. However, this treatment did not give the expected results. By trying the effectiveness of the hormonal solution with other species, it was concluded that the IBA solution had lost its effectiveness by degradation after storing.

TABLE 5. Rooting treatments and extract preparation for rooting of Philadelphus, Ribes, and Cornus

Rooting treatments			Ratio		
No.	Abbreviation	Description	a Powder (w)	:	b H_2O (v)
1	C	Control (50% ethanol)	0	:	0
2	S_1 Nov	Salix November	1	:	25
3	S_1 Jan	Salix January	1	:	25
4	S_1 Mar	Salix March	1	:	25
5	S_1 May	Salix May	1	:	25
6	S_1 Jun	Salix June	1	:	25
7	S_1 Aug	Salix August	1	:	25

a - w = weight in grams

b - v = volume in ml

RESULTS AND DISCUSSION

1. Interaction of willow and poplar extracts with IBA during rooting of evergreen cuttings under different photoperiods

1.1 Experiment 1 - Thuja occidentalis and and Juniperus sabina - Results

1.1.1 Influence of photoperiod

In this experiment conducted between November 10, 1980, and January 5, 1981, Table 6 summarizes results for rooting response and contents of total soluble sugars and starch in cuttings of Thuja occidentalis and Juniperus sabina rooted under extended and normal photoperiods. For both species, analysis of variance indicated significantly higher ($P = 0.05$) response under extended photoperiod than under normal photoperiod for all rooting parameters studied. Detailed data for rooting percentage, root length and root number are presented in Figure 4 for Thuja and in Figure 5 for Juniperus. Corresponding data not shown for the other rooting parameters (rooting index, root length per rooted cutting, and root number per cutting) showed similar trends.

In contrast to the rooting parameters, contents of total soluble sugars and starch were significantly lower ($P = 0.05$) in cuttings rooted under extended photoperiod, except for starch content of Thuja (Table 6). Detailed data for total soluble sugars and starch contents are presented in Figure 6 for Thuja and in Figure 7 for Juniperus.

TABLE 6. Rooting response and carbohydrate composition of cuttings rooted under extended and normal photoperiods

Parameters	Thuja occidentalis		Juniperus sabina	
	Extended photoperiod	Normal photoperiod	Extended photoperiod	Normal photoperiod
Rooting index †	2.0*	0.8	1.9*	0.7
Rooting percentage	79.0*	24.0	64.0*	22.0
Root length (cm)	1.0*	0.5	2.0*	0.8
Root length (cm) per rooted cutting	1.5*	1.1	3.6*	2.0
Root number per treatment unit	9.7*	2.4	3.5*	0.9
Root number per rooted cutting	14.8*	5.5	6.0*	2.4
Total soluble sugars (mg/g dry wt)	81.0*	106.0	65.0*	77.0
Starch (mg/g dry wt)	121.0 NS	127.0	84.0*	136.0

† Scale of 0 to 5, where 0 = dead or no sign of rooting activity; 1 = callusing; 2 = poor root system; 3 = few and short roots; 4 = good and uniform root system; 5 = very extensive and well-developed root system.

* Significantly different from normal photoperiod in analysis of variance on transformed values.

NS Not significantly different from normal photoperiod.

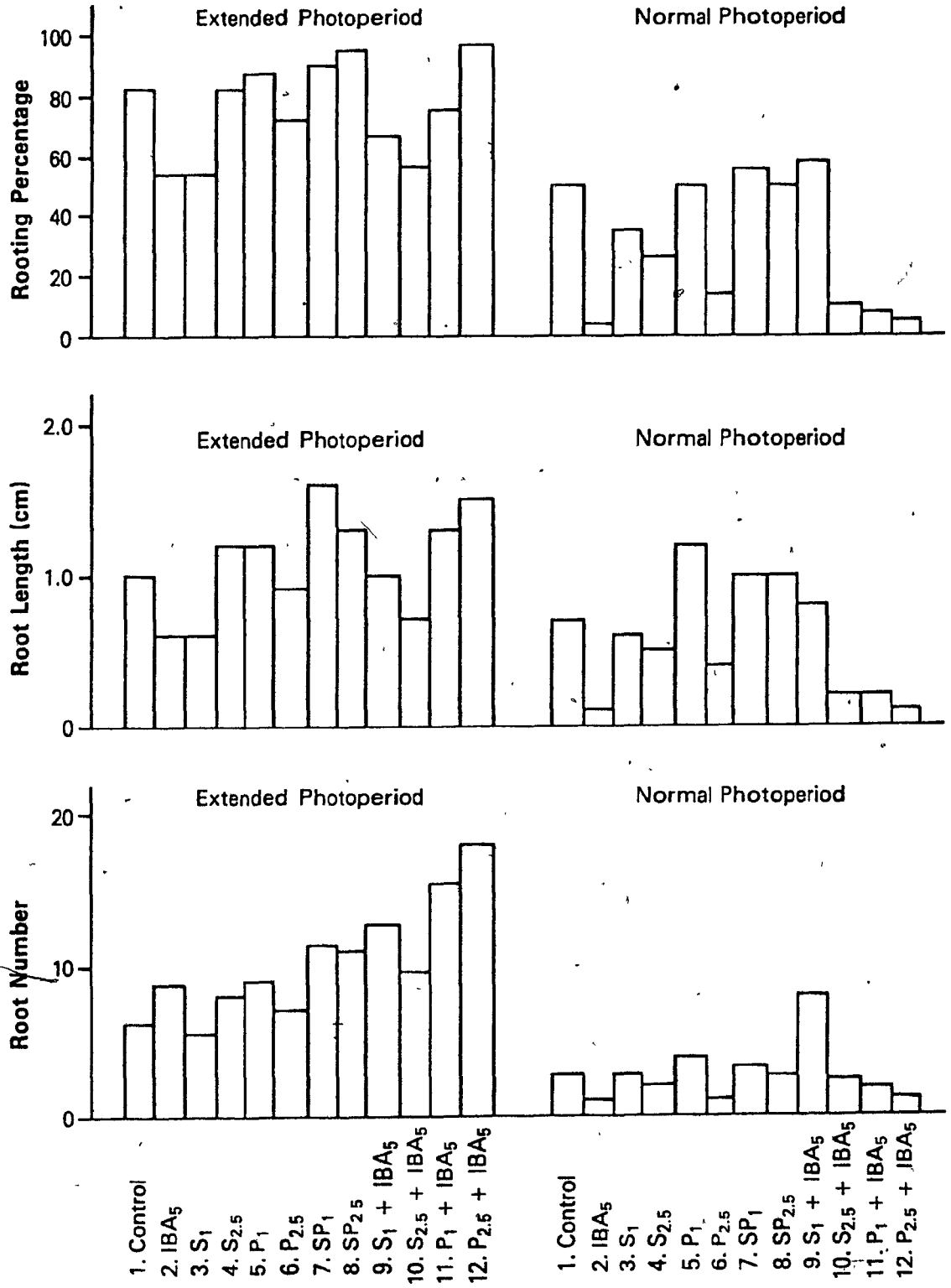

Figure 4. Rooting percentage, root length and root number of Thuja occidentalis rooted under extended and normal photoperiods (main effects). The numbers 1 to 12 correspond to rooting treatments (subplot effects) shown in Table 2.

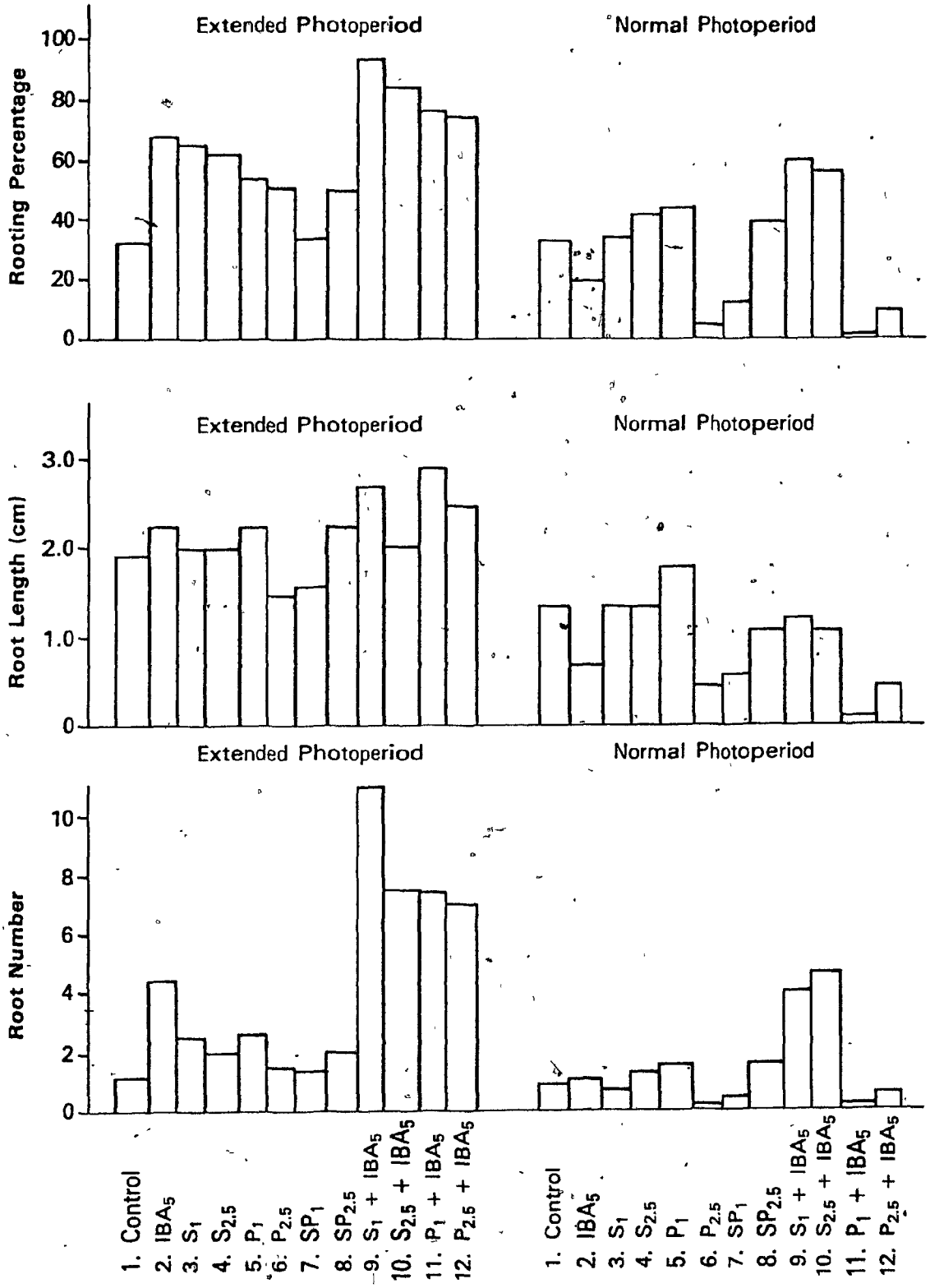

Figure 5. Rooting percentage, root length and root number of Juniperus sabina rooted under extended and normal photoperiods (main effects). The numbers 1 to 12 correspond to rooting treatments (subplot effects) shown in Table 2.

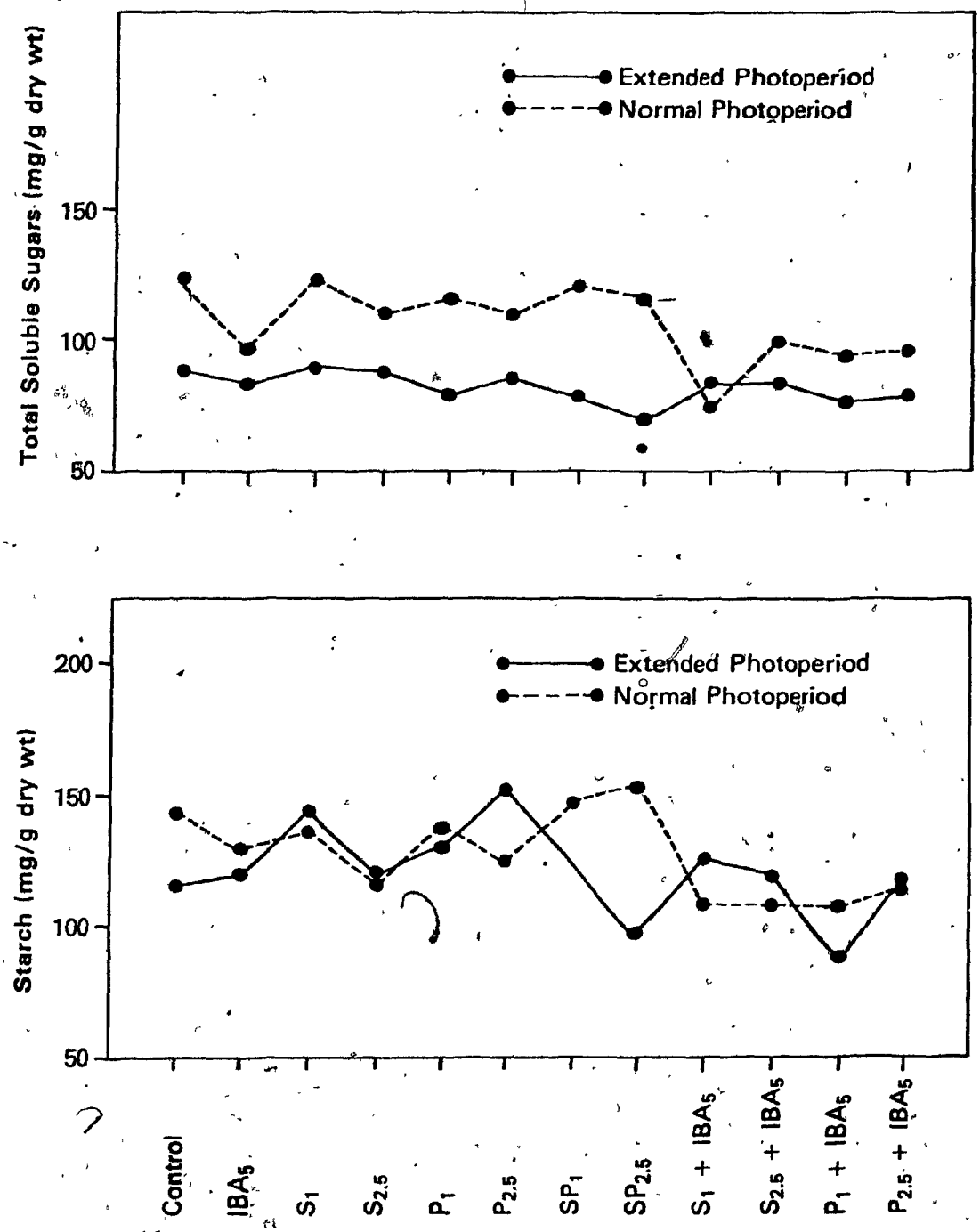

Figure 6. Contents of total soluble sugars and starch in Thuja occidentalis cuttings rooted under extended and normal photoperiods.

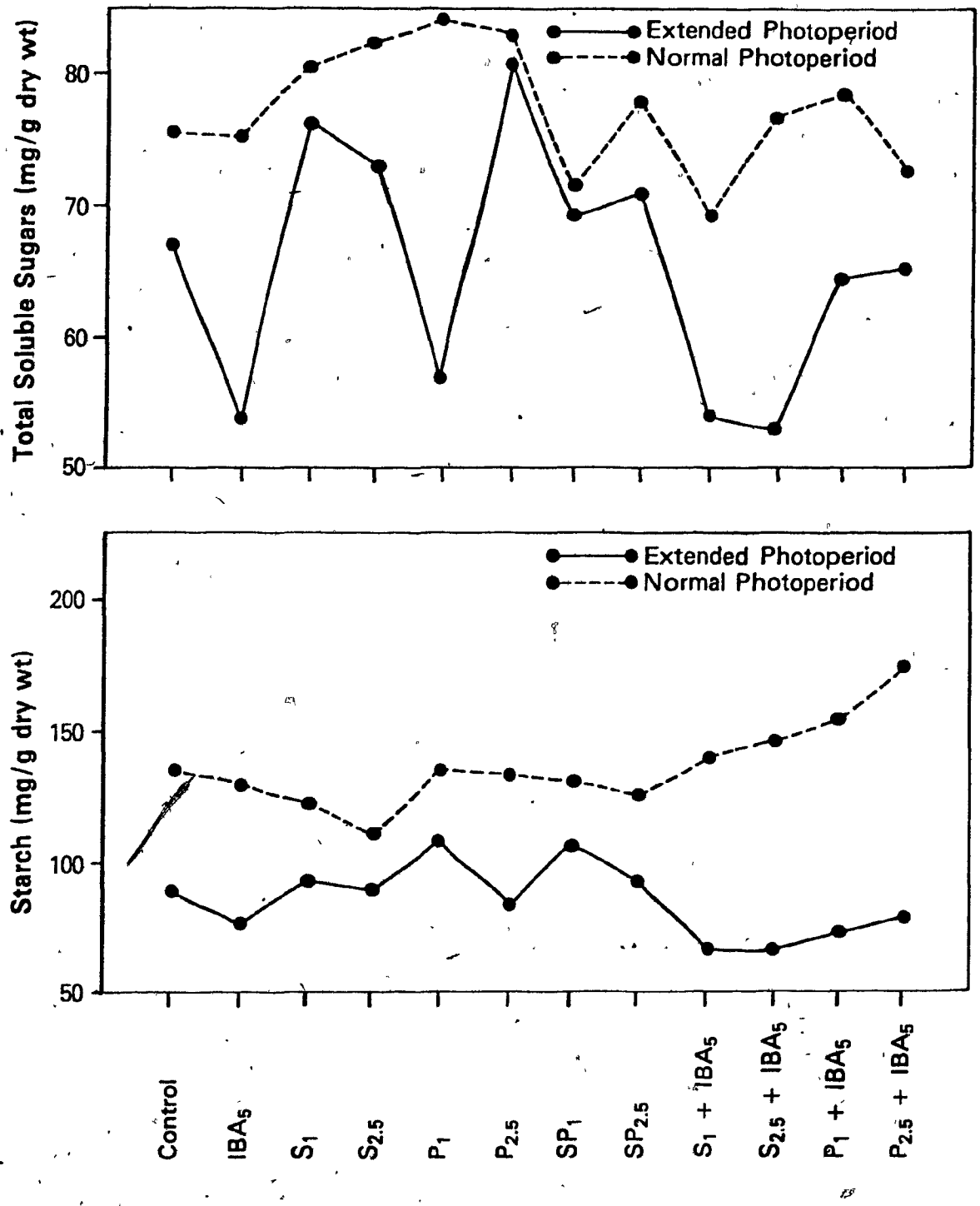

Figure 7. Contents of total soluble sugars and starch in *Juniperus sabina* cuttings rooted under extended and normal photoperiods.

Table 7 shows results for correlation coefficients between each of the three rooting parameters, rooting percentage, root length and root number, and amounts of total soluble sugars in cuttings of Thuja and Juniperus under each photoperiod. For Thuja there were significant correlations ($P = 0.05$) between all three rooting parameters and amounts of sugars in cuttings rooted under extended photoperiod (Table 7), although this relationship was not observed with cuttings rooted under normal photoperiod. In the case of Juniperus, a significant correlation ($P = 0.05$) was found only for root number under extended photoperiod (Table 7).

Table 8 shows results for correlation coefficients between each of the three rooting parameters, rooting percentage, root length and root number, and amounts of starch in cuttings of Thuja and Juniperus under both photoperiods. For Thuja there was no significant correlation with cuttings under extended photoperiod, although under normal photoperiod, significant correlations were observed ($P = 0.05$) for rooting percentage and root length. In the case of Juniperus, significant negative correlations ($P = 0.01$) were found for rooting percentage and root number under extended photoperiod but no relationship was observed under normal photoperiod.

1.1.2. Interaction of IBA and plant extracts

1.1.2.1 Rooting parameters

Table 9 summarizes results for rooting percentage, root length and root number of Thuja and Juniperus cuttings in response to 12 rooting

TABLE 7. Correlation coefficients between sugar content and rooting parameters in cuttings of Thuja occidentalis and Juniperus sabina rooted under extended and normal photoperiods

Rooting parameters	Correlation coefficients[a]			
	Thuja occidentalis		Juniperus sabina	
	Extended photoperiod	Normal photoperiod	Extended photoperiod	Normal photoperiod
Rooting percentage	.578*	.311	.518	.047
Root length	.650*	.493	.530	.314
Root number	.661*	.367	.657*	.320

[a] 10 df

* Significant ($P = 0.05$)

TABLE 8. Correlation coefficients between starch content and rooting parameters in cuttings of Thuja occidentalis and Juniperus sabina rooted under extended and normal photoperiods

Rooting parameters	Correlation coefficients[a]			
	Thuja occidentalis		Juniperus sabina	
	Extended photoperiod	Normal photoperiod	Extended photoperiod	Normal photoperiod
Rooting percentage	-.328	.577*	-.791**	-.369
Root length	-.426	.660*	-.445	-.516
Root number	-.543	-.076	-.737**	-.011

[a] 10 df

* Significant ($P = 0.05$)

** Significant ($P = 0.01$)

TABLE 9. Rooting percentage, root length and root number of Thuja occidentalis and Juniperus sabina in response to 12 rooting treatments, including IBA and extracts of Salix and Populus

Treatments		Thuja occidentalis						Juniperus sabina					
		Rooting percentage		Root length (cm)		Root number		Rooting percentage		Root length (cm)		Root number	
No.	Abb.	Extended photo.	Normal photo.	Extended photo.	Normal photo.	Extended photo.	Normal photo.	Extended photo.	Normal photo.	Extended photo.	Normal photo.	Extended photo.	Normal photo.
1	Control	82	49	1.0	0.7	6.1	2.7	32	32	1.8	1.3	1.2	0.9
2	IBA_5	52	3	0.6	0.1	8.6	0.8	68	19	2.2	0.6	4.4	1.0
3	S_1	53	35	0.6	0.6	5.5	2.7	65	33	2.0	1.3	2.5	0.7
4	$S_{2.5}$	82	26	1.2	0.5	7.9	1.9	62	42	2.0	1.3	2.0	1.2
5	P_1	87	50	1.2	1.2	8.8	3.8	53	44	2.2	1.7	2.6	1.5
6	$P_{2.5}$	72	13	0.9	0.4	6.9	1.2	51	4	1.4	0.4	1.5	0.1
7	SP_1	90	55	1.6	1.0	11.4	3.2	33	12	1.5	0.5	1.4	0.4
8	$SP_{2.5}$	93	50	1.3	1.0	10.7	2.6	50	39	2.2	1.1	2.0	1.4
9	S_1IBA_5	67	58	1.0	0.8	12.6	7.9	93	60	2.6	1.2	10.9	3.9
10	$S_{2.5}IBA_5$	56	10	0.7	0.2	9.5	2.3	84	56	2.0	1.1	7.4	4.7
11	P_1IBA_5	74	7	1.3	0.2	15.5	1.8	76	1	2.8	0.1	7.3	0.1
12	$P_{2.5}IBA_5$	97	4	1.5	0.1	18.0	1.2	74	9	2.4	0.4	6.9	0.5
LSD 0.05		*	*	NS	NS	*	*	*	*	NS	NS	*	*

photo. = photoperiod

* Treatments within columns significantly different at $P = 0.05$ in analysis of variance on transformed values.

NS Treatments within columns not significantly different.

treatments (sub-plot effects), including IBA and extracts of Salix and Populus.

Analysis of variance for Thuja cuttings indicated significant differences ($P = 0.05$) between the 12 rooting treatments with regards to rooting percentage and root length, but not for root number.

There was no significant main plot x sub-plot interaction for any of these three rooting parameters. For Juniperus, however, rooting percentage and root number, but not root length, were significantly different between the 12 rooting treatments. Interactions between sub-plot effects for rooting percentage and root length were significant ($P = 0.05$).

Transformed data on which analysis of variance was conducted for these two species are shown in Appendix Table 1.

Of the 12 rooting treatments, the two combinations of Salix extract + IBA (S_1 + IBA_5 and $S_{2.5}$ + IBA_5) (Table 9) had the most consistent effect on rooting response of both species with regards to rooting percentage and root number. This result was also similar for rooting index (data not shown).

Interestingly, a detailed examination showed that rooting percentage of cuttings of both species under extended photoperiod, treated with Populus extract + IBA, was dramatically high in comparison with corresponding treatments under normal photoperiod (Figure 8). A similar response was observed for root length in Thuja and root number in Juniperus (Table 9). This observation was not observed for corresponding Salix treatments (Figure 8).

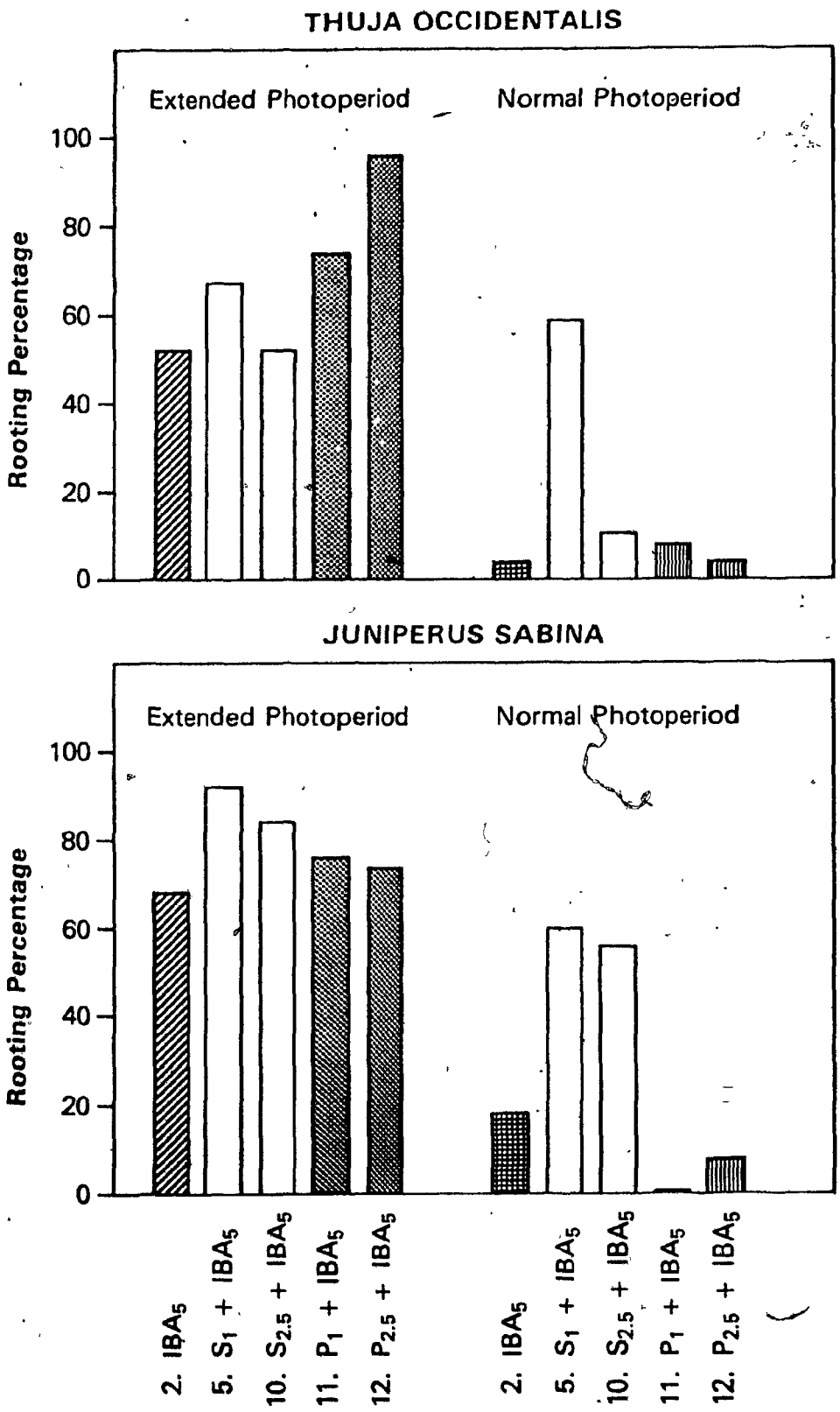

Figure 8. Comparative influence of treatments with IBA alone and IBA plus plant extracts on rooting percentage of Thuja occidentalis and Juniperus sabina under extended and normal photoperiods.

1.1.2.2 - Carbohydrate composition

Table 10 summarizes results for contents of total soluble sugars and starch analyzed in Thuja and Juniperus cuttings in response to the 12 rooting treatments. There was no significant difference in contents of total soluble sugars or of starch between treatments of both species rooted under both photoperiods, except for sugar in Thuja, which was significantly higher ($P = 0.05$) under normal photoperiod. In this instance, it is interesting to note that all treatments which contained IBA (IBA_5, $S_1 + IBA_5$, $S_{2.5} + IBA_5$, $P_1 + IBA_5$, and $P_{2.5} + IBA_5$) were lower in sugars than all other treatments solely with plant extracts (S_1, $S_{2.5}$, P_1, $P_{2.5}$, SP_1 and $SP_{2.5}$) (Table 10).

1.2 Experiment 1 - Thuja occidentalis and Juniperus sabina - Discussion

Extended daylength with supplementary light had beneficial effect on rooting of Thuja occidentalis and Juniperus sabina cuttings. Similar results have been obtained by other researchers. Nitsch (1957) reported that LD treatment increased rooting of several species. Lanphear and Meahl (1961) obtained increased rooting percentage and rooting quality with increasing length of photoperiod for Juniperus horizontalis 'Plumosa' cuttings rooted in the fall. Mcdonald (1969) and Canham (1972) reported that extension of daylength with high pressure sodium lamps stimulated rooting of Ilex aquifolium and Juniperus sabina. However, these positive effects of extended photoperiod on rooting of cuttings were not found by some researchers. Kamp and Van Dunen (1958)

TABLE 10. Contents of total soluble sugars and starch in cuttings of Thuja occidentalis and Juniperus sabina in response to 12 rooting treatments, including IBA and extracts of Salix and Populus

Treatments		Thuja occidentalis				Juniperus sabina			
		Sugar (mg/g dry wt)		Starch (mg/g dry wt)		Sugar (mg/g dry wt)		Starch (mg/g dry wt)	
No.	Abb.	Extended photo.	Normal photo.	Extended photo.	Normal photo.	Extended photo.	Normal photo.	Extended photo.	Normal photo.
1	Control	87	120	116	144	67	76	89	135
2	IBA_5	83	97	119	129	54	76	77	130
3	S_1	89	123	144	136	76	80	93	122
4	$S_{2.5}$	87	110	119	116	74	83	76	111
5	P_1	79	117	131	138	57	84	109	136
6	$P_{2.5}$	86	110	153	123	81	83	83	133
7	SP_1	77	120	122	147	69	71	107	131
8	$SP_{2.5}$	69	117	97	154	71	78	93	124
9	S_1IBA_5	84	74	126	108	54	69	64	138
10	$S_{2.5}IBA_5$	84	98	119	108	53	76	66	144
11	P_1IBA_5	75	93	88	108	64	78	74	156
12	$P_{2.5}IBA_5$	77	96	118	115	65	73	78	175
	LSD 0.05	NS	22*	NS	NS	NS	NS	NS	NS

photo = photoperiod

* Treatments within columns significantly different at $P = 0.05$ in analysis of variance.

NS Treatments within columns not significantly different.

reported that cuttings of some conifers rooted best under short days and Lanphear and Meahl (1961) reported that photoperiod had little effect on the rooting of Juniperus horizontalis 'Plumosa' cuttings. It appears that factors such as long photoperiod, which stimulated top growth of evergreens, inhibited rooting (Kamp and Van Dunen 1958; Lanphear and Meahl 1961). The above evidence suggests that inconsistency of rooting response with regards to photoperiod may in part be attributable to species difference.

Root promotion obtained under extended photoperiod (Table 5) could be due to an increase in auxins in stem tissues (Nitsch and Nitsch 1959) and in leaf tissues (Digby and Wareing 1966). This mechanism maintained cuttings in active growth and prevented dormancy, which in turn triggered a more rapid initiation of roots due to a greater supply of auxins coming from the growing shoots (Waxman 1955).

Ellyard (1976) reported that the light intensity and duration must be sufficient to ensure that carbohydrate production is in excess of that required for respiration. Thus this is another important reason why the photoperiod under which cuttings are rooted may affect root initiation. It was observed (Table 6) that the amounts of total soluble sugars were significantly lower under extended photoperiod and were negatively correlated with the three rooting parameters for Thuja and for root number only for Juniperus. This indicated that extended photoperiod favored rooting response.

The amounts of total soluble sugars decreased in the cuttings. Tukey et al. (1957) showed direct relationships between light intensity

and the leaching of carbohydrates. However, Loach and Gay (1979) and Hansen and Ericksen (1974) obtained similar negative correlations as those observed in Table 7. The decrease in reducing sugars of rooted cuttings could have been the result of their utilization for the regeneration of roots (Steponkus and Hogan 1967). Stromquist and Eliasson (1979) reported that the higher amount of total soluble sugars under normal photoperiod may be due to the absence of a sink, i.e., roots.

Significant correlations obtained in Table 7 for root number of both species (Thuja and Juniperus) under extended photoperiod were in agreement with results reported by Smith and Wareing (1972) who showed that favorable photoperiod affected mainly root initiation, i.e., formation of roots in cuttings.

The two other rooting parameters, root length and rooting percentage, were significant only for Thuja cuttings. According to Nelson (1959), Thuja cuttings rooted faster than Juniperus cuttings. Since rooting evaluation of Thuja and Juniperus was done about the same time, it is possible that similar relationships of Juniperus cuttings with regards to rooting percentage and root length with extended photoperiod, would have been discernable if cuttings of this species were to have been evaluated a little later in time. The relatively high negative non-significant correlation coefficients for rooting percentage and root length in this species seemed to support this view.

The correlation of starch content with photoperiod was more inconsistent. Whereas Molnar and LaCroix (1972) found a positive relationship between rooting capacity and content in starch, Brandon (1939) and Cailloux (1943) did not. Thus, there is an apparent controversy with respect to the relationship between starch content and rooting ability in woody species. As a storage compound, starch constitutes the major source of carbohydrates for providing the energy required for the initiation and development of root primordia. This process is regulated by hydrolytic enzymes (Gibbs 1940). According to Nanda and Anand (1970) these enzymes may be more important than the starch content in rooting ability of cuttings. However, departures from this theory as obtained in Juniperus and Thuja (Table 8) may be due to a different enzymatic activity associated with different species and with different environmental conditions during rooting.

Table 9 shows results with IBA treatments alone and with plant extracts + IBA combinations on Thuja and Juniperus cuttings. The evidence from this table suggests a synergistic response between Salix and Populus extract with IBA under extended photoperiod. Similarly, it was demonstrated by Hess (1962) that substances in extracts of difficult-to-root grape cuttings after leaching water-soluble inhibitors exhibited a greater amount of activity when supplied to the cuttings in combination with IAA. Kawase (1964) showed that Salix diffusate had a strong synergistic effect with IAA on the root formation of mung bean cuttings. Several reasons could be hypothesized to explain the synergism with auxins with plant extracts. For instance, Atman and Wareing (1975)

reported that accumulation of endogenous IAA at the base of the cutting, or an exogenous auxin treatment, might immediately affect the accumulation of other factors that are needed for root formation. The plant extracts may contain one or more of these factors, which when applied with IBA or other auxin, stimulates root formation of cuttings.

Since 5000 ppm IBA was the best concentration for rooting of these two species (Thuja and Juniperus), the synergistic effect of plant extract + IBA (Table 9, Figure 8) was probably due to the presence of complexing enzymes or of cofactors (Hess 1962). Possibly the plant extracts contain one or more enzymes, which in the presence of auxin, favored biochemical processes with more efficiency towards rooting. It should be noted that enzymes are generally thermolabile and plant extracts are heat-resisting (Van der Lek 1925), i.e., effective even after boiling. Although in this study precaution was taken to preserve the integrity of enzymes and thermolabile factors, i.e., freeze-drying, mixing with water and shaking at 4°C, it is possible that some destruction of these thermolabile compounds could have occurred during preparation and use, i.e., during grinding of the freeze-dried extract at room temperature, or during use of the extract just before sticking of cuttings.

A more feasible possibility is that the synergism was due to cofactors. According to its definition, a cofactor promotes rooting of activity of IAA (Hess 1962). Cofactors are divided into four groups, with the most active ones being terpènes and phenolics (Bouillenne and Bouillenne-Walrand 1955). The data of Table 9 and Figure 8 seem to

support this synergistic hypothesis (Thimann 1977). Similar results were obtained by Kawase (1970) who investigated the composition of plant extracts to explain synergism. The four subfractions of Salix diffusate corresponded to root promoting activity of cofactor 2 and to cofactor 4. Cofactor 4 was identified to be a phenolic compound (Hess 1961). Phenolic compounds protect the breakdown of auxins (Hess 1965). Tognoni et al. (1977) reported that the water extract should contain substances in the form in which they exist in the cuttings.

Figure 8 shows the responses of cuttings treated with Populus extract in combination with IBA. While these cuttings rooted well only under extended photoperiod, and cuttings treated with Salix extracts in combination with IBA rooted well under both photoperiods, the differences between willow and poplar extract combinations and under extended and normal photoperiods were not due to the composition of the extracts. This is because in Figures 4 and 5, treatments with extracts alone (treatments 3, 4, 5, 6) under both photoperiods responded similarly for willow and poplar extracts and both extracts increased rooting relative to the control. The difference occurred when extracts were used in combination with IBA. A possible explanation could be the presence of cofactors specific for each plant extract. In this sense, willow cofactor(s) activity appears insensitive to light, whereas poplar cofactor(s) activity is influenced by light.

Perhaps there was a change in the balance of promoters and inhibitors in the cutting tissue, where the ratio of promoters to inhibitors decreases under normal photoperiod compared with extended

photoperiods (Table 6). The inhibitors produced could be more specific to counteract the effect of promoters present in poplar extract compared with promoters present in willow extract. Or alternatively, these inhibitors acted synergistically with the inhibitors present in poplar extracts.

Table 9 shows significant differences between treatments in terms of rooting parameters. However, Table 10 does not show difference between treatments in terms of amount of total soluble sugars and starch. This evidence indicates that the carbohydrates were not responsible for the differences between treatments and that the amounts of these carbohydrates analyzed in cuttings were due to the effect of treatments.

A detailed examination of Table 10 shows that all treatments which contained IBA (IBA_5, S_1 + IBA_5, $S_{2.5}$ + IBA_5, P_1 + IBA_5, and $P_{2.5}$ + IBA_5) were lower in total soluble sugars than all the other treatments with plant extracts (S_1, $S_{2.5}$, SP_1, $SP_{2.5}$, P_1, and $P_{2.5}$). These results corroborated the previous data in Figure 7 and also indirectly, that of the synergistic response of plant extract plus IBA combinations, which could be responsible for a decrease in sugar content in cuttings. In plant extract plus IBA combinations similar correlations were obtained, i.e., increase in rooting response was associated with lower amounts of total sugars in cuttings. Atman and Wareing (1975) suggested that one of the roles of IAA in promoting rooting of cuttings, is to increase sugar availability at the site of root formation.

1.3 Experiment 2 - Juniperus sabina - Results

In this experiment conducted between February 18 and June 4, 1981, photoperiods (main plot effects) showed no influence on rooting response or in content of total soluble sugars and starch in Juniperus sabina cuttings. However, as shown in Table 11, summarizing results for rooting response and contents of total soluble sugars and starch in Juniperus cuttings evaluated on harvest dates 1, 2 and 3, i.e., 8, 10 and 15 weeks, respectively, after sticking (sub-plot effects), analysis of variance indicated significant difference ($P = 0.05$) for harvest dates with regards to rooting percentage, root length and root number. There was no significant difference in rooting response between harvest dates 1 and 2, but between harvest date 3 and the other two dates ($P = 0.05$). Results were also similar for rooting index, root length of rooted cuttings and root number of rooted cuttings (data not shown).

Amounts of total soluble sugars in Juniperus cuttings increased significantly ($P = 0.05$) between harvest dates 1 and 2, but were not significantly different between harvest dates 2 and 3 (Table 11). There was no significant difference in the amounts of starch analyzed in cuttings between the three harvest dates (Table 11).

1.3.1 Interaction of IBA and plant extracts

1.3.1.1 Rooting parameters

Table 12 summarizes results for rooting percentage, root length and root number at each of the three harvest dates for Juniperus cuttings in response to five rooting treatments, including the control

TABLE 11. Rooting responses and contents of total soluble sugars and starch in *Juniperus sabina* cuttings evaluated on harvest dates 1, 2, and 3, i.e., 8, 10 and 15 weeks

Parameters	Harvest date 1 (8 weeks)	Harvest date 2 (10 weeks)	Harvest date 3 (15 weeks)	LSD = 0.05
Rooting percentage	14.3	21.75	56.7	
	(-3.123)†	(-2.386)	(0.285)	(1.225)
Root length (cm)	0.27	0.54	2.74	
	(0.383)‡	(0.551)	(1.248)	(0.219)
Root number	1.11	1.19	3.04	
	(0.594)†	(0.703)	(1.247)	(0.320)
Total soluble sugar (mg/g dry wt)	59.56	77.87	80.45	5.70
Starch (mg/g dry wt)	102.35	110.72	105.50	NS

† Data transformed to log (RP + 0.5)/(100.5 - RP) for analysis of variance.

‡ Data transformed to powered to 1/3 for analysis of variance.

NS Not significantly different between harvestings.

TABLE 12. Rooting responses for rooting percentage, root length and root number at harvest dates 1, 2, and 3, for Juniperus sabina cuttings

Treatments	Rooting percentage			Root length (cm)			Root number		
	Harvest date 1	Harvest date 2	Harvest date 3	Harvest date 1	Harvest date 2	Harvest date 3	Harvest date 1	Harvest date 2	Harvest date 3
Control	8.8 (-4.197)†	7.5 (-4.187)	27.5 (-1.924)	0.27 (0.236)‡	0.20 (0.228)	1.36 (0.806)	0.37 (0.261)‡	0.29 (0.261)	1.08 (0.746)
IBA_5	12.5 (-2.876)	26.3 (-1.635)	66.3 (1.497)	0.22 (0.409)	0.91 (0.750)	4.03 (1.467)	0.63 (0.590)	1.33 (0.807)	3.46 (1.341)
$S_1 + IBA_5$	17.5 (-2.698)	21.3 (-2.866)	59.8 (0.392)	0.46 (0.487)	0.37 (0.427	2.57 (1.227)	1.58 (0.692)	1.32 (0.633)	3.83 (1.338)
$S_{2.5} + IBA_5$	23.8 (-2.379)	33.8 (-1.142)	61.3 (0.433)	0.33 (0.490)	0.81 (0.770)	2.90 (1.387)	2.36 (0.932)	2.25 (1.086)	4.15 (1.507)
$P_{2.5} + IBA_5$	90.0 (-3.466)	20.0 (-2.098)	68.8 (1.027)	0.11 (0.292)	0.39 (0.578)	2.83 (1.351)	0.62 (0.494)	0.74 (0.728)	2.68 (1.301)
LSD 0.05	*	*	*	*	*	*	*	*	*

* Treatments within columns significantly different at $P = 0.05$ in analysis of variance on transformed values.

† Data of rooting percentage transformed to log (RP+0.5)/(100.5-RP) for analysis of variance.

‡ Data of root length and root number transformed to powered to 1/3 for analysis of variance.

(sub-sub-plot effects). Analysis of variance indicated significant differences ($P = 0.05$) between the five rooting treatments with regards to rooting percentage, root length and root number for each of the three harvest dates. There was no significant sub-plot x sub-sub-plot interaction.

In comparison with the control, all of the four rooting treatments (IBA and combinations of Salix or Populus extract plus IBA) at each of the three harvest dates showed significant differences ($P = 0.05$) with regards to rooting percentage, root length and root number, except for treatment $P_{2.5} + IBA_5$ on harvest date 1 (Table 12).

1.3.1.2 Carbohydrate composition

Table 13 summarizes results for total soluble sugars and starch analyzed in Juniperus cuttings rooted under five rooting treatments, at three harvest dates. There was no significant difference between treatments in the amounts of total soluble sugars or of starch analyzed in these cuttings (Table 13).

As shown in Figure 9, each rooting parameter increased progressively with each consecutive harvest date. With time, the influence of rooting treatments with extracts of Salix and Populus seemed to show some interesting differences in their stimulatory effect on rooting (Figure 9; Table 13). For rooting percentage and root length, treatments IBA_5, $S_1 + IBA_5$ and $S_{2.5} + IBA_5$ were equally effective and tended to be superior to treatment $P_{2.5} + IBA_5$ after 8 weeks of rooting. In contrast,

TABLE 13. Content of total soluble sugars and starch in cuttings of Juniperus sabina in response to five rooting treatments at three harvest dates, 1, 2, and 3

Treatments	Total soluble sugars (mg/g dry weight)			Starch (mg/g dry weight)		
	Harv. 1	Harv. 2	Harv. 3	Harv. 1	Harv. 2	Harv. 3
Control	61.34	78.50	83.59	106.86	114.53	110.30
IBA_5	60.39	77.95	77.35	103.24	105.50	100.09
$S_1 + IBA_5$	54.65	73.04	77.68	99.64	106.21	103.01
$S_{2.5} + IBA_5$	63.64	76.21	74.05	99.41	112.59	102.69
$P_{2.5} + IBA_5$	57.80	83.64	80.45	102.60	114.78	111.68
LSD 0.05	NS	NS	NS	NS	NS	NS

*Treatments within columns significantly different at $P = 0.05$ in analysis of variance.

NS Treatments within columns not significantly different.

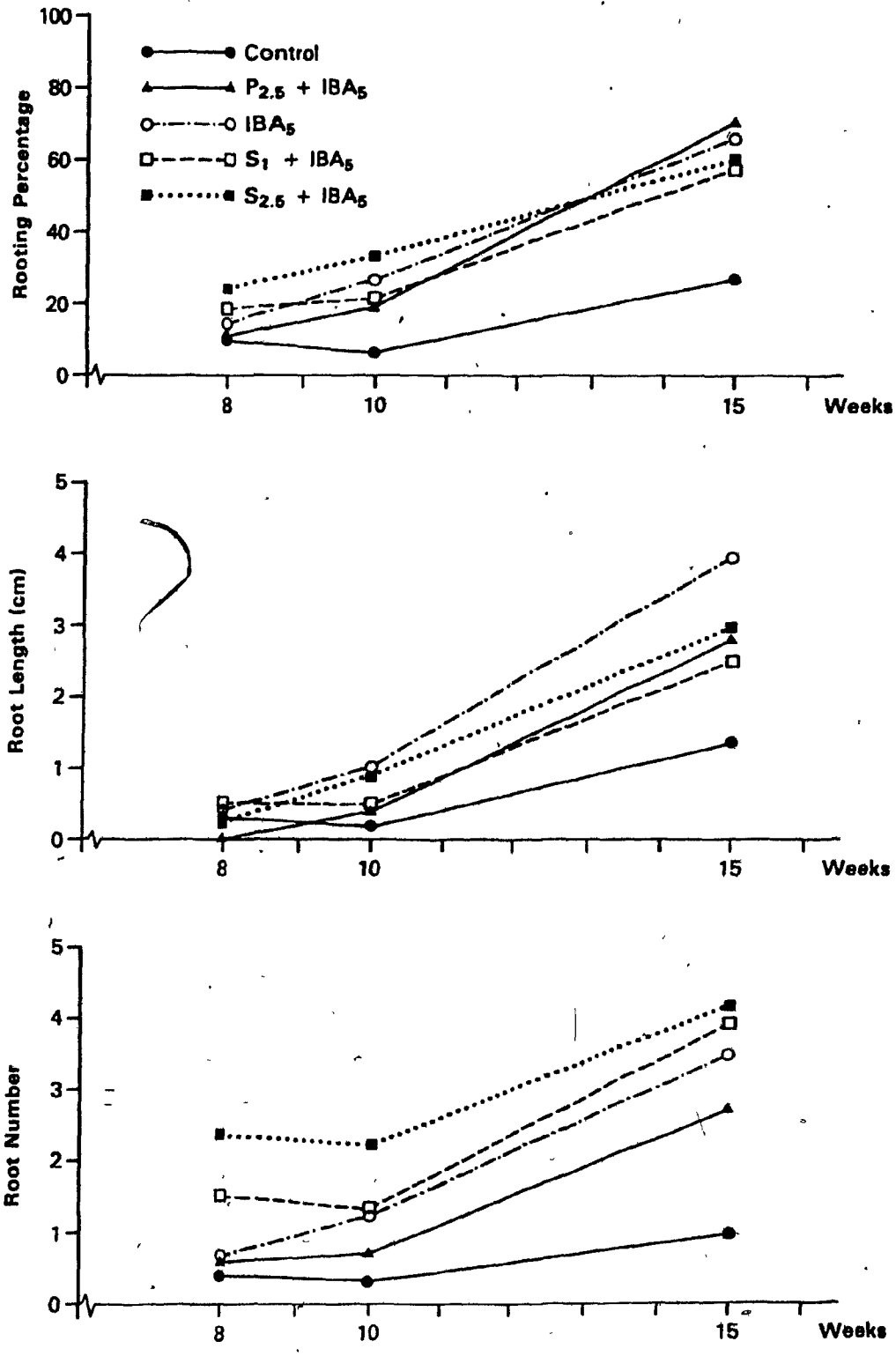

Figure 9. Rooting percentage, root length and root number of Juniperus sabina cuttings at three harvest dates, 1, 2 and 3 (8, 10 and 15 weeks).

the reverse situation was observed after 15 weeks of rooting. In terms of root number, however, treatments IBA_5, S_1 + IBA_5 and $S_{2.5}$ + IBA_5 were superior to $P_{2.5}$ + IBA after 15 weeks of rooting.

In terms of rooting percentage, Salix + IBA treatments were equally as effective as IBA after 8 weeks, but this stimulatory rooting effect of the Salix + IBA treatments diminished after 15 weeks, while that for IBA remained stable. Salix + IBA treatments responded faster in root number than those treated with IBA_5 alone. After 15 weeks, no difference between Salix + IBA_5 treatments and IBA alone was observed. The treatment combination $S_{2.5}$ + IBA_5 was the most consistent and effective treatment for early and later rooting.

Notwithstanding lack of statistical difference in rooting response due to photoperiods, previously described, closer examination (Figure 10) of data for rooting percentage of Juniperus cuttings, and amounts of total sugars, starch, and sugar/starch ratio, analyzed in the cuttings at harvest date 3 (15 weeks) under extended photoperiod and normal photoperiod, showed some interesting relationships of rooting treatments and photoperiods. Under normal photoperiod a rooting percentage of 22% was accompanied by starch content of 85 mg/g dry weight, while under extended photoperiod, a 33% rooting was accompanied by 135 mg/g dry weight of starch. Under normal photoperiod, content of total sugars and sugar/starch ratio showed an inverse relationship with rooting percentage resulting from the five rooting treatments; however, a similar relationship was not observed under extended photoperiod.

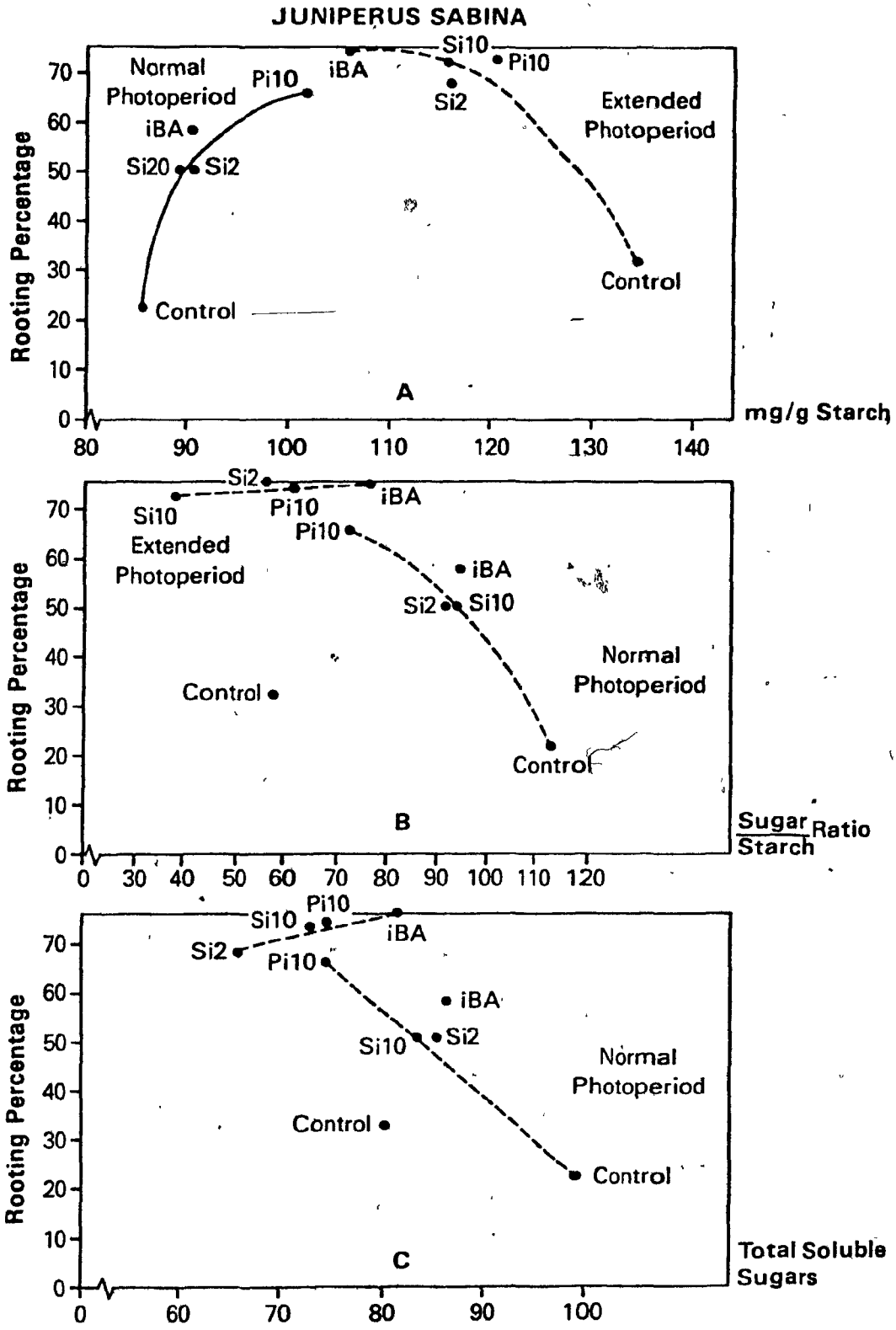

Figure 10. Relations between rooting percentage response of Juniperus sabina cuttings with (A) the amount of starch, (B) sugar/starch ratio, (C) the amount of total sugars analyzed in cuttings, for harvest date 3 under extended and normal photoperiods.

1.4 Experiment 2 - *Juniperus sabina* - Discussion

In the Results and Discussion section 1.1 with *Thuja occidentalis* and *Juniperus sabina*, photoperiod (main effect) showed an influence on rooting response and on carbohydrate content (both total sugars and starch) in the cuttings. However, under the conditions of this second experiment with *Juniperus sabina* conducted three months later, the above parameters were not found to be statistically significant. Lanphear and Meahl (1961) reported similar results with *Juniperus horizontalis* 'Plumosa' cuttings when the influence of photoperiod on the rooting of various woody ornamental species rooted during the fall and winter period. In this study, the difference in response of *Juniperus* cuttings, when rooted in November and in February under natural and extended (16-hour) photoperiods, indicates the importance of time of year on rooting response (Girouard 1975; Hartman and Kester 1975; Hartman and Loreti 1965; Still 1981; Wyman 1930). The change of natural daylength in these two experiments seems to explain the reason why extended photoperiod increased rooting responses of *Thuja* and *Juniperus* cuttings rooted between November and January and not *Juniperus* rooted in February to June. In the first experiment with *Thuja* and *Juniperus*, the normal photoperiod varied between 9 and 9.5 hours of daylight, in the second experiment with *Juniperus* the normal photoperiod varied between 10.5 and 15.5 hours.

As previously discussed, difference in the carbohydrate content in cuttings was a response to root initiation in the cuttings. The significant increase in total soluble sugars in *Juniperus* cuttings

between harvest dates 1 and 2 (8 to 10 weeks, Table 11), was probably due to the absence of a sink, i.e., roots (Hansen et al. 1978). Okoro and Grace (1976) reported a substantial accumulation of carbohydrates until roots formed. The close relationship between the rooting of cuttings and the mobilization of reserve food material is clear. When roots began to develop, between 10 and 15 weeks, the decreasing augmentation of total soluble sugars suggests a very active utilization of sugars and increased metabolic activity in cuttings (Chong et al. 1979). Carbohydrates were utilized primarily in developing roots and callus (Hartmann and Kester 1975). The constant content of starch in cuttings was similar to those reported in the Results and Discussion section 1.1.

The rooting response of cuttings was higher for those treated with IBA and with plant extracts plus IBA, than for those of the control (Table 12, Figure 9). According to Smith and Wareing (1972) the degree of rooting was correlated with the level of IAA at the cutting base. In the present study, all treatments contained auxin except the control. As previously discussed, auxin is one of the three factors needed for root initiation (Bouillenne and Bouillenne-Walrand 1965). Thus, results obtained in Table 12 were in agreement with previous workers (Basu et al. 1969, 1970; Bojarczuck 1978; Cooper 1935; Gil-Albert and Boix 1978; Gorter 1969; Hartman and Kester 1975; Hess 1963).

Figure 9 shows an increasing difference between treatments with IBA or with plant extracts plus IBA in comparison with the control during consecutive harvest dates. According to Thimann and Koepfli (1935),

Thimann and Went (1934), and Went (1929), auxins stimulated rooting of cuttings and required a certain time to become active for root initiation.

The endogenous promoting factors, added when cuttings were treated with combinations of willow extracts plus IBA (Figure 9) resulted in more rapid initiation of roots than cuttings treated with IBA alone (Table 12). Exogenous auxins affect the accumulation of other substances needed for root initiation (Atman and Wareing 1975) and rooting substances may take more time to initiate roots in cuttings. Thus, after 10 weeks no difference between plant extract plus IBA and IBA alone was observed in root length and root number responses.

Furthermore, as seen previously, poplar extracts interact differently than willow extracts with IBA under different photoperiodic conditions (Figure 9). A possible difference in endogenous promoting factors in plant extracts between willow and poplar species, may explain why poplar extract plus IBA takes a longer time to be more effective than IBA alone. Another possible explanation is, as discussed in Results and Discussion section 1.1, the ratio of promoters to inhibitors acted synergistically under extended photoperiod but counteracted the effect of poplar extract plus IBA combinations under normal photoperiod and the synergism of poplar plus IBA combinations was not very strong; but at harvest date 3 the difference between extended and normal photoperiods was less, and poplar extract plus IBA reacted as under extended photoperiod and the synergistic rooting response occurred (Table 12).

Under both photoperiods, cuttings from the control treatments contained less total soluble sugars and starch than cuttings treated with IBA alone or with plant extract plus IBA combinations (Figure 10). Root-promoting treatments applied on cuttings favored an active metabolism which increased the formation and utilization of carbohydrates. Similar results were shown by Hansen et al. (1978), who reported very low amounts of carbohydrates associated with a poor rooting development of cuttings.

According to the review of literature, there seems to be no satisfactory explanation to account for the metabolic phenomena shown in Table 11.

2. Interaction of seasonal willow and poplar extracts with IBA on rooting of Cotoneaster acutifolia

2.1 Results

Figure 11 shows results for rooting response of Cotoneaster acutifolia cuttings rooted in response to 33 treatments consisting of IBA treatments alone (IBA_0, IBA_5, and IBA_{20}), extracts of Salix and Populus alone, and extracts of both species in combination with IBA treatments.

Analysis of variance indicated significantly higher rooting response in terms of rooting percentage, root length and root number for the IBA treatments in comparison with plant extract treatments and treatments with plant extracts in combination with IBA. Actual data and

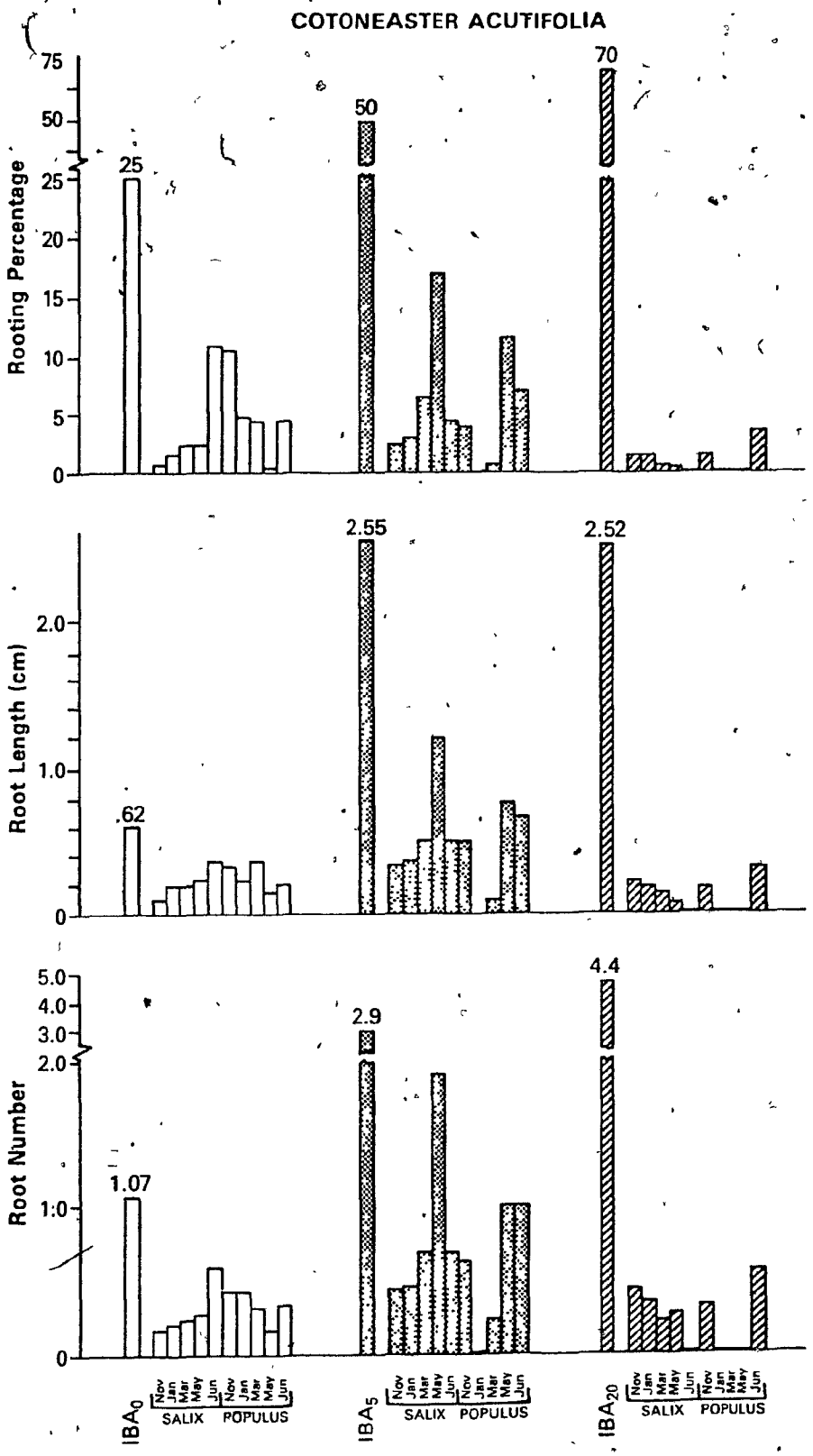

Figure 11. Rooting percentage, root length and root number of Cotoneaster acutifolia cuttings in response to 33 treatments consisting of IBA alone, extracts of Salix and Populus alone, and extracts of both species in combinations with IBA (5000 and 20000 ppm).

transformed data on which analysis of variance was conducted are shown in Appendix Table 2.

For the IBA treatments, rooting percentage and root number increased with increasing IBA concentrations to maximum values of 70 and 4.4, respectively, with the 20,000 ppm IBA (IBA_{20}) treatments. In comparison with cuttings treated without IBA (IBA_0), root length of those treated with IBA at 5000 ppm (IBA_5) and 20,000 ppm (IBA_{20}) was equally and significantly greater ($P = 0.05$).

For both root length and root number (Figure 11), treatment combinations of plant extracts of both species plus 5000 ppm IBA, were significantly higher ($P = 0.05$) than corresponding treatments with 20,000 ppm IBA or with plant extracts alone. In contrast, combinations of plant extracts with 20,000 ppm IBA acted antagonistically on rooting response.

In terms of their effect on rooting response, there was no significant difference between Salix and Populus extract.

Table 14 shows results for plant extract injury (death) of cuttings observed at the end of the experiment. While plant extracts of Salix and Populus alone caused little or no injury to cuttings, treatments with combinations of plant extracts plus 5000 ppm or 20,000 ppm IBA resulted in significant numbers ($P = 0.05$) of dead cuttings. A mean of 95% of dead cuttings was obtained with plant extracts plus 20,000 ppm IBA and a mean of 83% with plant extracts plus 5000 ppm IBA compared with 0.25% with plant extracts alone.

TABLE 14. Percentage of dead cuttings of Cotoneaster acutifolia in response to 33 treatments consisting of IBA alone (no extracts), extract of Salix and Populus alone, and extracts of both species in combinations with IBA (5000 ppm and 20000 ppm)

Treatments	IBA		
	0 ppm	5000 ppm	20000 ppm
No extracts	0.3	17	4
S_1 Nov	1.7	91	23
S_1 Jan	0.0	96	98
S_1 Mar	0.0	90	99
S_1 May	0.0	40	96
S_1 Jun	0.0	75	100
P_1 Nov	0.5	85	98
P_1 Jan	0.3	94	100
P_1 Mar	0.9	99	100
P_1 May	0.3	54	100
P_1 Jun	0.0	81	95
Means	0.25	83	96
LSD 0.05	NS	*	*

* Significantly different in analysis of variance on transformed values.

NS Not significantly different.

Differences between treatments due to extracts in different months were inconsistent and not interesting.

2.2 Discussion

An increase in the rooting response of Cotoneaster acutifolia cuttings was associated with an increase in the concentrations of IBA treatments applied to cuttings. High hormonal concentrations have proven to be a significant factor in the successful rooting of certain difficult-to-root species. Quercus robur 'Fastiagata' cuttings rooted with 20,000 ppm IBA (Flemer 1962), Malus floribunda with 10,000-30,000 ppm IBA, Malus 'Hopa' (Brown and Dirr 1976; Chong 1982), Malus 'Selkirk' and Malus sieboldii zumi var. calocarpa (Brown and Dirr 1976), Taxus spp, and Cotoneaster acutifolia (Chong 1982) with IBA treatments between 10,000 ppm and 40,000 ppm.

Unlike easily rooted species, such as the willows, which possess preformed root primordia in their stems (Bullock 1973; Densmore and Zasada 1978) root primordia must be biochemically induced in many species (Libby 1974). Difficult-to-root species may lack the necessary active enzymes or substrates to induce a meristematic state and thus the initiation of root primordia (Libby 1974). Continuous sclerenchyma rings between the phloem and cortex exterior to the point of origin of adventitious roots may constitute an anatomical barrier to rooting of difficult-to-root species (Edwards and Thomas 1980). Sachs et al. (1964) showed that with mist and auxin treatments, all expansion and proliferation in the cortex, phloem and cambium resulted in breaks in

continuous sclerenchyma rings. Evidence further suggests that, like other growth processes, each step of the rooting process is controlled by a delicate balance of growth hormones (Tognoni and Lorenzi 1972; Tukey, Jr. 1979) and that the optimum hormonal levels must be determined empirically for each species or cultivar (Burd and Dirr 1977; Hartmann and Kester 1975).

IBA treatments increased significantly the rooting response of Cotoneaster cuttings compared with plant extracts alone or with plant extracts in combination with 5000 or 20,000 ppm IBA. For Cotoneaster cuttings, the plant extracts probably interfered with the balance of promoters and inhibitors required to induce root formation, and decreased the rooting ability of cuttings. Alternatively, the plant extracts may contain compounds that block adventitious root formation (Hartmann and Kester 1975). Cuttings without any treatments (IBA_0), rooted better than those treated with plant extracts (Figure 11). Rooting response of cuttings treated with combinations of plant extracts plus 5000 ppm IBA were significantly higher than those treated with plant extracts alone or with 20,000 ppm IBA. This increasing rooting response was due to the positive influence of 5000 ppm IBA in the combination. Interestingly, 20,000 ppm IBA in combination with plant extracts, responded differently than with 5000 ppm IBA, and further reduced the rooting ability. An excessive concentration of auxins reduced the rooting of cuttings (Cooper 1935) or an antagonistic response with the highest concentration of IBA and plant extracts. This seems to emphasize our present uncertainty in the use of growth

regulators and also our lack of understanding of the sequence of rooting events which allows growth regulators to be used effectively (Cameron and Rook 1974; Libby 1974).

Brown and Dirr (1976) and Burd and Dirr (1977) indicated that high concentrations of IBA between 20,000 and 30,000 ppm often resulted in defoliation, significant injury or death of crabapples taxa. Chong (1981) reported similar results with woody ornamental species, i.e., basal portions of cuttings tended to be injured with 20,000 and 40,000 ppm IBA. In the present study, it was noted that the death percentage of cuttings was extremely high with cuttings treated with plant extracts and IBA, compared with plant extracts alone or with IBA alone (Table 14). This evidence indicates that the endogenous substances in plant extracts are toxic when mixed with IBA and applied to Cotoneaster cuttings.

3. Influence of seasonal willow extracts on rooting of three shrubs

3.1 Results

Figure 12 summarizes results for rooting percentage, root length and root number of Philadelphus coronarius, Ribes alpinum and Cornus elegantissima cuttings treated with extracts of Salix harvested at different times of the year (Nov 10, 1980, and Jan 12, Mar 30, May 1, Jun 13, and Aug 7, 1981).

Detailed data on which analysis of variance was conducted for these three species are shown in Appendix Table 3.

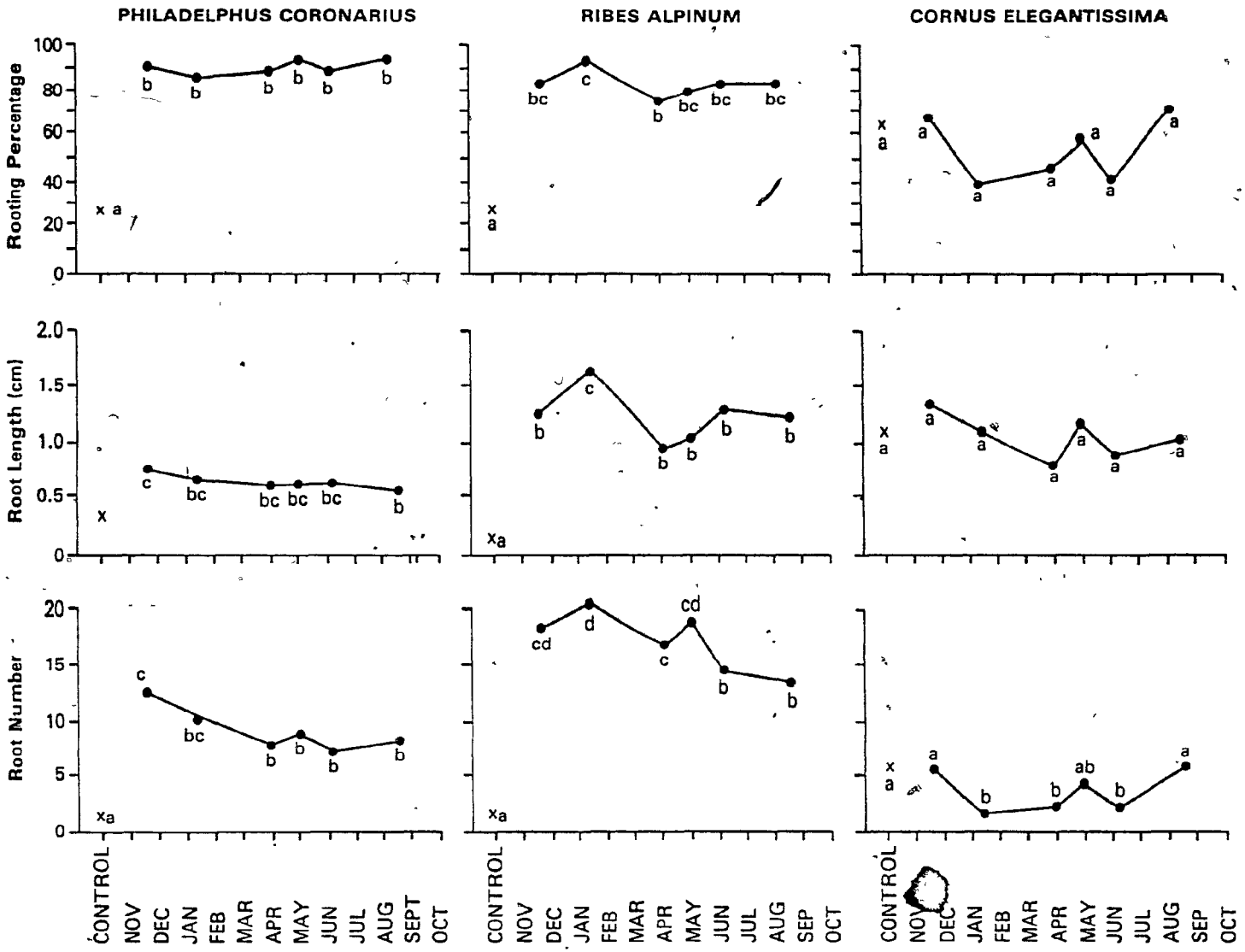

Figure 12. Rooting percentage, root length and root number of Philadelphus coronarius, Ribes alpinum and Cornus elegantissima in response to seasonal Salix extract treatments.

While cuttings of Philadelphus and Ribes showed similar rooting response in terms of rooting percentage, root length and root number, Cornus responded differently (Figure 12). Analysis of variance for Philadelphus and Ribes indicated a significantly higher ($P = 0.05$) rooting percentage, root length and root number of all extract-treated cuttings in comparison with the control. In contrast, the rooting response of Cornus was variable, and analysis of variance indicated no significant difference between treatments for each of the rooting parameters except for root number.

While there was no significant difference in rooting percentage of Philadelphus coronarius due to seasonal extracts treatments between November 1980 and August 1981, root number showed a general decreasing trend in cuttings treated with extracts collected during this period. For Ribes alpinum, there was a tendency for increased rooting percentage, root length and root number of cuttings treated with the January extract in comparison with those treated with extracts from other months of the year. Similar to Philadelphus, root number showed a decreasing trend for cuttings treated with extracts between January and August. Although rooting percentage and root length of Cornus elegantissima cuttings showed no significant difference due to seasonal extract treatments, root number was significantly higher ($P = 0.05$) in cuttings treated with the November and August extracts.

3.1.1 Nutritional and biochemical status of seasonal extracts

Figure 13 shows results of three compounds (phenols, total sugars and starch) and for nine elements (N, P, K, Ca, Mg, Fe, Mn, Cu and Zn) analyzed in Salix extracts harvested at the different times of the year (Nov 10, 1980, and Jan 13, Feb 17, Mar 30, May 1, Jun 7, Aug 7, Sep 10, 1981). Table 15 shows correlation coefficients between the three rooting parameters of Philadelphus coronarius, Ribes alpinum and Cornus elegantissima and total phenols, total soluble sugars, starch and sugar/starch ratio, and of the nine mineral elements analyzed in Salix extracts.

The amounts of phenols varied considerably during the year but a general decreasing trend occurred between November and May, with an increase in June and a decrease until August. Variations in total sugars generally followed a similar trend as the phenols but variations were smaller. Starch content in Salix extract showed light variation during the year.

The contents of Ca, Mg, Fe, Mn, Cu, and Zn showed somewhat similar trends, a net decrease between November and January, followed by an increase in February, and then a decreasing trend to June until August. The content of N was higher during the winter months than between May and September. The content of phosphorus was stable between November and February, followed by wide variations until August. Potassium was quite stable, except there was a sharp increase in July.

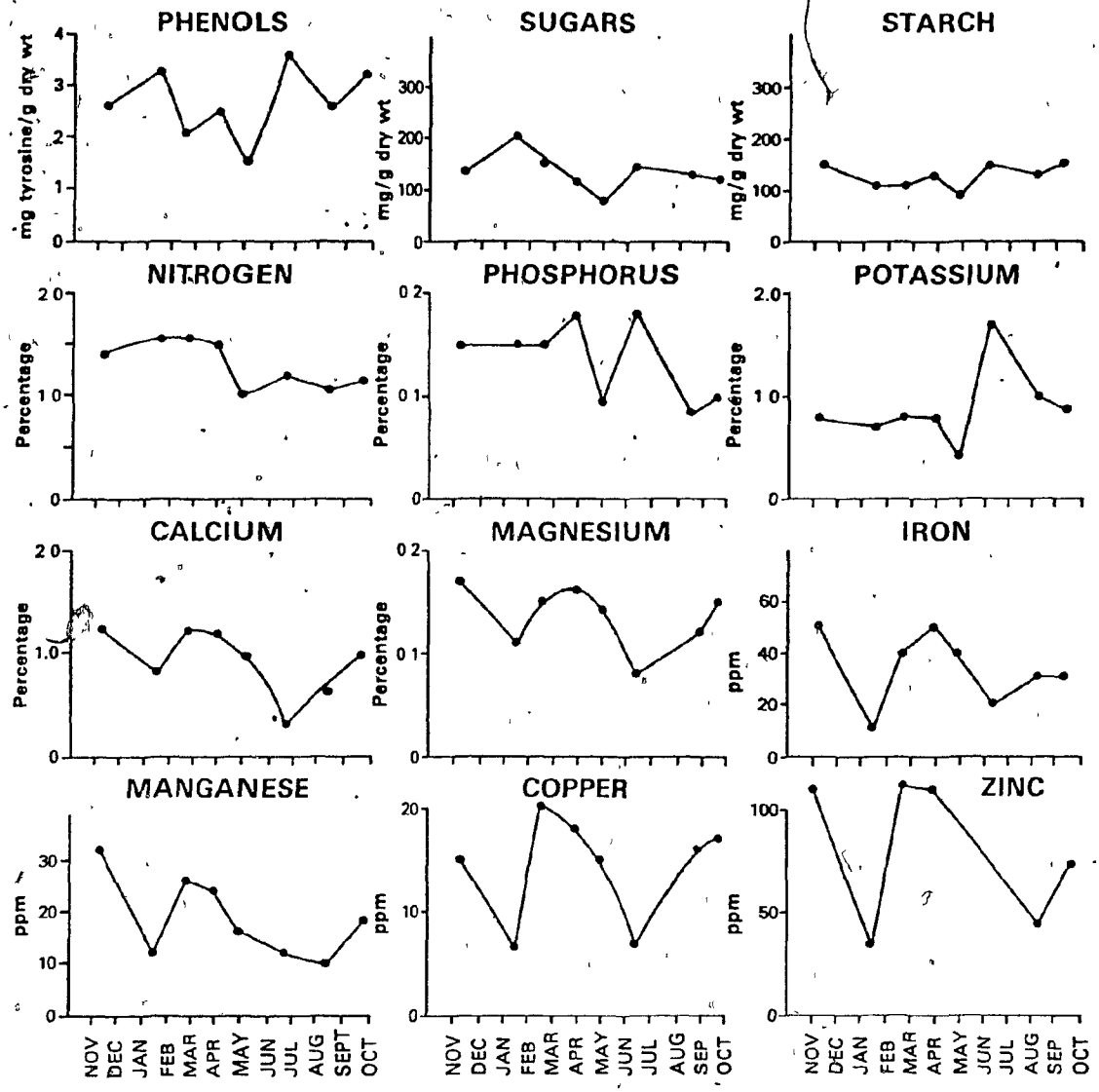

Figure 13. Amounts of three compounds (phenols, total sugars and starch) and of nine mineral elements (N, P, K, Ca, Mg, Fe, Mn, Cu, and Zn) in Salix extracts harvested at different times of the year. N, P, K, Ca, and Mg are expressed in percentages and Fe, Mn, Cu and Zn are expressed in ppm.

While there was a significant negative correlation ($P = 0.05$) between root percentage of Philadelphus coronarius and amounts of total phenols analyzed in Salix extracts, interestingly, a positive correlation was found for Ribes alpinum (Table 15). Furthermore, significant correlations were found between rooting percentage and total soluble sugars for Philadelphus and between rooting percentage and sugar/starch ratio for Ribes. There were no significant correlations between root length and root number of the three species and total phenols, total soluble sugars, starch and ratio sugar/starch.

There was no significant correlation between rooting parameters with each of the nine mineral elements analyzed.

3.2 Discussion

It is well established that time of the year in which cuttings are taken influences rooting ability (Alvim et al. 1976; Girouard 1975; Hartmann and Kester 1975; Nanda and Jain 1972). In willow and poplar cuttings, Alvim et al. (1976) reported variations in hormone content and Nanda and Jain (1972) reported considerable variations in their ability to root with season. Smith (1964) reported that seasonal variations in the rooting propensity of Populus cuttings were related to endogenous hormones in the cuttings.

In certain woody species (Thimann 1977), growth inhibitory substances were higher during dormant periods (Alvim et al. 1976). ABA was shown to be present in the xylem sap of willow (Davison 1965) and of several other woody species (Alvim et al. 1976). Levels of ABA started

to decrease at the end of December in Salix viminalis L. (Alvim et al. 1978). It has been found that ABA acted synergistically with auxin to induce root formation (Basu et al. 1970; Chin et al. 1969; Tognoni et al. 1977). Thus, higher rooting potential of the November and January extracts could have been due to an accumulation of growth inhibitory substances, which, together with endogenous auxins and/or other rooting cofactors, favored root formation in Philadelphus and Ribes, but not in Cornus.

Another possible explanation may be related with the synthesis and rooting cofactors or related substances. According to Smith and Chiu (1980), root promoting substances are synthesized in the leaves. In autumn, root-promoting substances produced by the leaves could possibly accumulate in stem tissues. Thus, plant extracts from November and January stem tissues probably contained rooting substances in larger concentrations which favored rooting. On the contrary, increasing metabolic activity in the spring favored transport of these root-promoting substances to the roots to initiate new root formation of the tree. Thus, plant extract from March and April stem tissue was not as effective in promoting rooting as November and January extracts.

As previously discussed, large differences exist among species in the rooting ability of cuttings (Hartmann and Kester 1975), and endogenous rooting factor(s) in plant extract treatments reacted differently with each species (Results and Discussion sections 1.1 and 1.3). In this study, seasonal willow extracts significantly increased the rooting response of Philadelphus and Ribes cuttings but did not

affect significantly the rooting response of Cornus cuttings (Figure 12). Generally, softwood cuttings of deciduous species taken during spring or summer usually tend to root more readily than hardwood cuttings (Hartmann and Kester 1975), because the level of endogenous auxin is higher in the spring (Nanda and Anand 1970). Furthermore, unlike the other two species, no significant correlation was observed with regard the three rooting parameters and the amounts of phenols, total soluble sugars, starch and sugar/starch ratio contained in the extracts (Table 15). Similar results were obtained by Lipecki and Dennis (1972) who reported lack of a correlation between phenols and rooting response of apple cultivars. It is also possible that the extracts probably did not contain the specific cofactors to promote rooting, or possibly the required balance between promoters and inhibitors in this species. However, the two other species, Philadelphus and Ribes, showed significant correlations between rooting percentage with phenols and total soluble sugars for Philadelphus and correlation between rooting percentage with phenols and sugar/starch ratio for Ribes.

It would be easier to explain these correlations with phenols if the relationships were similar for both species, i.e., correlation negative with Philadelphus and positive with Ribes. It is possible that phenols were not the only factors in the plant extracts which promoted rooting activity. It is also possible that in Philadelphus stem tissues, a substance which interacted with phenol activity was lacking, with the reverse situation for Ribes. As previously described, several factors like polyphenol oxydase and abcissic acid (Chin et al. 1969) could play

TABLE 15. Correlation coefficients between three rooting parameters of Philadelphus coronarius, Ribes alpinum, and Cornus elegantissima and total phenols, total soluble sugars, starch, sugar/starch ratio and nine mineral elements analyzed in Salix extracts

Analysis	Philadelphus coronarius			Ribes alpinum			Cornus elegantissima		
	Rooting percentage	Root length	Root number	Rooting percentage	Root length	Root number	Rooting percentage	Root length	Root number
Total phenols[a]	-.828*	.161	.206	.818*	.153	.110	.632	.492	.455
Total sugars[b]	-.892*	.076	.537	.654	.522	.280	.543	.478	.429
Starch[b]	.267	.576	.049	.680	.486	.658	.008	.150	.163
Sugar/starch ratio[b]	.738	.005	.573	.884*	.796	.580	.542	.552	.516
Nitrogen (N)	.757	.148	.391	.418	.762	.451	-.551	.569	.585
Phosphorus (P)	.677	.020	.136	.369	.182	.129	.726	.532	.685
Potassium (K)	.155	.430	.429	.317	.573	.736	.194	.057	.006
Calcium (Ca)	.271	.665	.404	.204	.226	.664	.382	.335	.226
Magnesium (Mg)	.239	.081	.240	.540	.404	.262	.045	.114	.003
Iron (Fe)	.583	-.641	-.153	-.689	-.400	-.008	.558	.512	.441
Manganese (Mn)	.191	.667	.336	-.267	.003	.291	.261	.324	.174
Copper (Cu)	.541	.005	-.380	-.443	-.518	-.273	.610	.446	.502
Zinc (Zn)	.038	.195	-.216	-.643	-.373	-.122	.144	.217	.007

[a] mg tyrosin/g dry weight

[b] mg/g dry weight

df = 4

* Significant ($P = 0.05$)

NS Not significant

the role of cofactors, and were not analyzed in this experiment. The role played by the phenolic rooting cofactors in adventitious root initiation is controversial (Basu et al. 1969; Thimann 1977).

As previously discussed (Results and Discussion section 1.2), the amount of carbohydrates in cuttings had an effect on rooting response. In willow extracts, high concentrations of carbohydrates were correlated with low rooting percentage. Jones and Bradlee (1933) reported in a monthly carbohydrate analysis of maple tree, that results were divided into two groups: November to April, and May to October, the former representing the dormant and the latter the growing and storage periods. Results showed maximum soluble sugars occurring in January and a minimum in June. Gibbs (1940) reported a minimum value in September for birch twigs. Data in this experiment showed maximum amounts of total sugars in willow extracts occurring in January but lower amounts in May.

According to Winkler and Williams (1945) starch is transformed to sugars during the winter, and the increase in total soluble sugars accounts for essentially all of the decrease in starch. Generally, starch and other types of storage compounds accumulate during the period of photosynthetic activity and are used for rejuvenation of growth in the spring (Siminovitch et al. 1953).

Seasonal variations in nutritional analysis of tissues were reported by several researchers. Cannon et al. (1960) reported that levels of the nutrient elements (N, P, K, Ca, Mg, Fe, and Mn) changed considerably in ornamental trees during the summer. Kelly and Schier

(1965) reported variations in stem tissues of Taxus media. Their data were similar to those reported in the literature (Cannon et al. 1960; Davidson 1960) but they found that stems used as a plant part for sampling over longer periods had a higher coefficient of variation than for leaves.

In this study, seasonal variations in Salix extract seem to agree with the above reports. However, no correlation was found with the rooting parameters for the three species (Philadelphus, Ribes and Cornus). Swanson and Davies (1977) reported that although K is considered as an important factor in the activity of enzymes, its deficiency did not affect root initiation and development. Similar results were obtained with Mg and S deficiencies. Graca and Hamilton (1981) observed that root growth of Cotoneaster divaricata was not improved with phosphorus applications.

The beneficial effect of Salix plant extracts on rooting was not mediated through the mineral contents of their extracts.

SUMMARY

Willows (Salix spp) and poplars (Populus spp) are two related species which root easily. Exogenously-applied plant extracts of willows and many other species have been known to induce rooting of cuttings. This study investigated the influence of Salix and Populus extracts as rooting promoters, the interaction of these plant extracts with IBA on rooting of cuttings, and also the influence of photoperiod on rooting and carbohydrate physiology of cuttings during propagation.

In the first study, there were 12 rooting treatments with Salix and Populus extracts used alone or in combination with IBA. Extracts were derived from freeze-dried twig samples collected in November. Treatments were applied to Thuja occidentalis and Juniperus sabina cuttings, which were rooted between November and January under natural and extended (16-hour) photoperiods. Higher rooting responses in terms of rooting percentage, root length and root number, and generally lower contents of total soluble sugars and starch were observed under extended photoperiod. This root promotion effect was probably due to an increase in auxins in stem and leaf tissues, and the accompanying lower amounts of carbohydrates due to their utilization for root regeneration. The treatment combinations of Salix extracts plus IBA had the most consistent effect on rooting, suggesting a synergistic response possibly associated with the right balance of auxins or the presence of cofactors and/or enzymatic reactions.

In a related study, five rooting treatments were applied only to Juniperus sabina cuttings rooted between February and June under the same two photoperiods and with cuttings harvested 8, 10, and 15 weeks (harvest dates 1, 2, and 3, respectively) after sticking. In this experiment, photoperiod showed no influence on rooting response or in contents of total soluble sugars and starch in Juniperus cuttings. This was probably associated with the change of natural daylength in the experiment, which increased progressively between February and June. Differences with regards to rooting parameters were observed between harvest dates 1, 2, and 3. Amounts of total soluble sugars in Juniperus cuttings increased between harvest dates 1 and 2 but not significantly between harvest dates 2 and 3. Starch content remained nearly constant between the three harvest dates. In comparison with the control treatment, all of the other four rooting treatments plus IBA, at each of the three harvest dates, showed significant differences with regards to the rooting parameters. Generally, treatments with IBA or Salix extracts plus IBA were equally effective and tended to be superior to treatments with Populus extract plus IBA after 8 weeks, with the reverse situation occurring after 15 weeks. As previously observed, the treatments with Salix extract plus IBA were the most consistent. Under normal photoperiod content of total soluble sugars and sugar/starch ratio showed an inverse relationship with rooting percentage, resulting from the five rooting treatments, although a similar relationship was not observed under extended photoperiod.

The interactions of seasonal willow and poplar extracts with different concentrations of IBA on rooting of Cotoneaster acutifolia were studied. Higher rooting responses were observed for the IBA treatments in comparison with plant extract treatments alone or in combination with IBA. In this case, the plant extracts probably interfered in the balance promoters and inhibitors required to induce root formation or they may have contained compounds that block adventitious root formation. For the IBA treatments, rooting increased with increasing (0, 5000, and 20,000 ppm) IBA concentrations. While plant extracts of Salix and Populus alone or IBA alone caused little or no injury to cuttings, all treatments with combinations of plant extracts plus IBA resulted in significant numbers of dead cuttings. This indicated that the endogenous substances in plant extracts were toxic when mixed with IBA and applied to Cotoneaster cuttings. Differences between treatments due to the extracts in different months were inconsistent and not interesting.

The influence of seasonal willow extracts (collected at intervals over a one-year period) on rooting of Philadelphus coronarius, Ribes alpinum, and Cornus elegantissima, was studied. For Philadelphus and Ribes cuttings, higher rooting responses were observed with plant extracts in comparison with the control. In contrast, the rooting response of Cornus was variable and plant extract treatment showed less influence on rooting. A negative correlation was obtained between rooting percentage of Philadelphus and total phenols of Salix extract and a corresponding positive correlation for Ribes. Furthermore,

correlations also were found between rooting percentage of Philadelphus and soluble sugar content of Salix extracts and between rooting percentage of Ribes and sugar/starch ratio of the extracts. No significant correlation was found between the nine elements (N, P, K, Ca, Mg, Fe, Mn, Cu, and Zn) in seasonal Salix extracts and rooting responses of the three species. It is possible that phenols were not the only factors in the plant extracts which promoted rooting activity.

SUGGESTIONS FOR FUTURE RESEARCH

Further studies need to be conducted with cuttings of other species taken at other periods of the year to increase our knowledge of the influence of willow and poplar extracts on rooting of trees and shrubs. It would be interesting, also, to identify with more precision the plant extract components which favor or inhibit the rooting response of cuttings. A precise hormonal study of the plant extracts for auxins, cytokinins, gibberellins, abcissic acid, specific phenolic compounds, and specific carbohydrate constituents (i.e., sugars) would elucidate the identity of the rooting factors of these plant extracts. Furthermore, the association of plant extracts with other root promoting substances, such as vitamins and other hormones, would yield interesting and useful information.

APPENDIX TABLES

APPENDIX TABLE 1. Transformed data for rooting percentage, root length and root number of Thuja occidentalis and Juniperus sabina in response to 12 rooting treatments, including IBA and extracts of Salix and Populus

Treatments		Thuja occidentalis						Juniperus sabina					
		Rooting percentage		Root length		Root number		Rooting percentage		Root length		Root number	
No.	Abbr.	Ext photo	Normal photo	Ext photo	Normal photo	Ext photo	Normal photo	Ext photo	Normal photo	Ext photo	Normal photo	Ext photo	Normal photo
1	Control	1.498	-0.026	0.930	0.824	1.690	1.221	-0.735	-0.730	0.941	0.957	0.993	0.878
2	IBA	0.111	-3.218	0.779	0.286	1.928	0.668	0.730	-1.439	1.240	-0.720	1.597	0.903
3	S_1	0.138	-0.619	0.789	0.783	1.623	1.220	0.593	-0.682	1.912	-0.997	1.296	0.797
4	$S_{2.5}$	1.498	-1.048	1.006	0.701	1.864	1.041	0.481	-0.339	1.186	-1.019	1.205	1.033
5	P_1	1.863	-0.005	1.006	1.014	1.944	1.407	0.116	-0.232	1.241	-1.109	1.310	1.065
6	$P_{2.5}$	0.936	-1.886	0.917	0.629	1.772	0.846	0.026	-3.017	1.042	0.639	1.085	0.370
7	SP_1	2.202	-0.201	1.118	0.919	2.136	1.297	-0.709	-2.001	1.073	-0.667	1.045	0.616
8	$SP_{2.5}$	2.519	0.000	1.039	0.930	2.094	1.198	0.000	-0.455	1.230	-0.943	1.242	1.055
9	S_1 + IBA_5	0.682	0.317	0.931	0.835	2.218	1.867	2.455	0.391	1.301	0.978	2.186	1.528
10	$S_{2.5}$ + IBA_5	0.227	-2.117	0.802	0.500	1.999	1.134	1.605	0.249	1.180	0.936	1.908	1.629
11	P_1 + IBA_5	1.021	-2.455	1.020	0.414	2.393	1.022	1.164	-4.287	1.345	0.189	1.901	0.241
12	$P_{2.5}$ + IBA_5	3.218	-3.017	1.089	0.360	2.526	0.833	1.048	-2.318	1.270	-0.590	1.868	0.706
LSD 0.05		1.781*	1.781*	0.250*	0.250*	NS	NS	1.899*	1.899*	NS	NS	0.392*	0.392*

Ext = extended; photo = photoperiod

* Treatments within columns significantly different at $P = 0.05$.

NS Treatments within columns not significantly different.

APPENDIX TABLE 2A. Transformed data for rooting percentage, root length and root number of Cotoneaster acutifolia in response to 33 rooting treatments

Treatments	IBA concentrations								
	IBA_0			IBA_5			IBA_{20}		
	Rooting percentage	Root length	Root number	Rooting percentage	Root length	Root number	Rooting percentage	Root length	Root number
Control	-1.06	0.63	0.79	-0.01	1.22	1.25	0.82	1.22	1.50
S_1 Nov	-4.09	0.19	0.20	-3.53	0.41	0.46	-3.84	0.31	0.46
S_1 Jan	-3.84	0.28	0.25	-3.42	0.44	0.46	-3.85	0.28	0.37
S_1 Mar	-3.59	0.29	0.27	-2.52	0.53	0.61	-4.29	0.21	0.25
S_1 May	-3.59	0.36	0.32	-1.45	0.87	1.04	-4.40	0.17	0.27
S_1 Jun	-2.08	0.45	0.56	-3.01	0.54	0.60	-5.30	0.00	0.00
P_1 Nov	-2.10	0.41	0.45	-3.05	0.54	0.58	-3.85	0.26	0.37
P_1 Jan	-2.89	0.33	0.45	-5.30	0.00	0.00	-5.30	0.00	0.00
P_1 Mar	-2.97	0.45	0.37	-3.95	0.18	0.28	-5.30	0.00	0.00
P_1 May	-4.29	0.22	0.19	-2.00	0.70	0.77	-5.30	0.00	0.00
P_1 Jun	-3.01	0.31	0.38	-2.49	0.65	0.79	-3.34	0.43	0.53
LSD 0.05	1.33	0.27	0.30	1.33	0.27	0.30	1.33	0.27	0.30

APPENDIX TABLE 2B. Actual data for rooting percentage, root length and root number of Cotoneaster acutifolia in response to 33 rooting treatments

Treatments	IBA concentrations								
	IBA_0			IBA_5			IBA_{20}		
	Rooting percentage	Root length	Root number	Rooting percentage	Root length	Root number	Rooting percentage	Root length	Root number
Control	25.6	1.07	0.63	49.8	2.90	2.56	69.7	4.44	2.53
S_1 Nov	1.2	0.15	0.12	2.4	0.43	0.32	1.6	0.44	0.21
S_1 Jan	1.6	0.20	0.19	2.7	0.44	0.34	1.6	0.33	0.19
S_1 Mar	2.2	0.22	0.20	7.0	0.67	0.47	0.9	0.20	0.13
S_1 May	2.2	0.27	0.26	18.7	1.89	1.22	0.7	0.22	0.10
S_1 Jun	10.7	0.58	0.36	4.2	0.65	0.48	0.0	0.00	0.00
P_1 Nov	10.5	0.43	0.31	4.1	0.62	0.47	1.6	0.32	0.17
P_1 Jan	4.8	0.43	0.23	0.0	0.00	0.00	0.0	0.00	0.00
P_1 Mar	4.4	0.33	0.36	0.4	0.23	0.11	0.0	0.00	0.00
P_1 May	0.9	0.15	0.14	11.5	1.02	0.76	0.0	0.00	0.00
P_1 Jun	4.2	0.33	0.22	7.2	1.07	0.65	3.0	0.54	0.33
LSD 0.05	*	*	*	*	*	*	*	*	*

APPENDIX TABLE 3. Data for rooting percentage, root length and root number of Philadelphus coronarius (A), Ribes alpinum (B), and Cornus elegantissima (C), in response to seasonal Salix plant extract treatments

Treatments	Rooting percentage			Root length			Root number		
	(A)	(B)	(C)	(A)	(B)	(C)	(A)	(B)	(C)
Control	34 a*	28 a	66 a	0.30 a	0.22 a	1.10 a	2.72 a	1.84 a	5.5 b
S_1 Nov	94 b	85 bc	68 a	0.76 c	1.27 b	1.36 a	12.56 cd	19.57 c	5.6 b
S_1 Jan	90 b	95 c	40 a	0.67 bc	1.68 c	1.10 a	10.30 d	22.20 bc	1.7 a
S_1 Mar	92 b	78 b	48 a	0.60 bc	1.07 b	0.74 a	8.26 c	17.42 b	2.5 ab
S_1 May	96 b	82 bc	62 a	0.60 bc	1.13 b	1.22 a	9.24 bc	19.57 b	4.3 a
S_1 Jun	92 b	85 bc	42 a	0.60 bc	1.25 b	0.90 a	7.36 b	15.17 b	2.5 a
S_1 Aug	94 b	85 bc	71 a	0.54 b	1.17 b	1.02 a	8.50 b	14.12 b	6.7 b
LSD 0.05	15.5	15.0	NS	0.19	0.26	NS	3.25	4.39	2.9

* Treatments with same letters are not significantly different.

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