PROPAGATION OF ACTINIDIA CHINENSIS (PLANCH.) BY STEM AND ROOT CUTTING

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ABSTRACT

Basal wounding, bottom heat, light with IBA treatments were found to be beneficial for rooting of Actinidia chinensis (Planch.). IBA treatment was effective only when there was a high natural ability to initiate root in Summer and Spring.

Seasonal fluctuations in rooting ability was pronounced. This seasonal variation seems to be related to the levels of endogenous IAA, ABA and cofactor 2. No correlation between root initiation and bud activity or IAN level was established.

IAA seems to be the fundamental physiological promoter of adventitous root formation. IBA plays only a supporting role in promoting root formation, by protecting the endogenous IAA level in the cutting base.

Leaf tissue is an important factor for rooting to be successful. The role of leaf tissue is not just to produce auxin or synthesize nutrients but rather some unknown factor in the leaf can produce a synergistic interaction with auxin in root formation processes.

Root cuttings of Abbott variety were sequentially harvested and planted over a period from late Autumn (1.4.77) until mid Summer (8.1.78). Root cuttings of different thickness and length were compared to evaluate their effect on regeneration. The effect of various growth regulators was investigated too.

Root diameters of 0.5 - 1.5 cm. out performed that of the thinner or thicker ones. Shorter cuttings (5 cm) of equivalent total length were found to be more productive than a single long cutting (15cm). Strong polarity was observed with shoots only arising from the proximal end of the cutting.

Regenerative capacity was highest in late Autumn and Winter and lowest in Summer. This seasonal fluctuation

can be altered by exogenous application of growth regulators. IBA suppressed shoot regeneration, whereas cytokinin and sucrose promoted it, while GA3 did not have any significant effect.

For commercial use, the practical and economic aspects of this techique require further investigation.

Glossary of Abbreviations

IAA = indoleacetic acid

IAN = indoleacetic nitrile

IBA = indolebutyric acid

ABA = abscisic acid

GA_3 = gibberellic acid

GA = gibberellin

BAP = benzylaminopurine

DNA = deoxyribonucleic acid

RNA = ribonucleic acid

PPM = parts per million

P = probability

X = 0.1%

XX = 0.05%

XXX = 0.001%

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CHAPTER I

REVIEW OF LITERATURE

1.1 INTRODUCTION

The Propagation of Actinidia chinensis (Planch.) is by budding or grafting of clonal wood on to seedling stocks (Bailey 1961, Fletcher, 1976). However the production of seedling in United States and some European countries has been difficult because of poor and erratic germination of seed (Opitz and Beutel, 1975).

An alternative method of propagation is by stem cuttings, which at present is still at its infant stage of development as far as Actinidia chinensis is concerned.

Recent developments in tissue culture techniques open up new options for vegetative propagation. The use of tissue culture for the propagation of Actinidia chinensis has still a long way to go (Harada, 1975).

1.2 PROPAGATION BY STEM CUTTINGS

Propagation of plants by stem cuttings is one of the oldest and perhaps the most employed means of vegetative propagation. It is of immense value in horticulture and forestry being able to mass produce in a relatively short period, genetically uniform plants from a specially selected parent.

The rootability of stem cuttings varies from species to species and from time to time. This involves a complex physiological process under the control of a complex of natural plant growth regulators, the role of which is not well understood yet.

The ability to propagate from cuttings is clearly significant for the nursery industries. For

centuries plant propagators had been refining the methods used. Rooting substances were postulated as early as 1880 by sachs. Since then tremendous technological advances, in particular the discovery of the role of IAA by Went (1934) had revolutionised the science of plant propagation. Critical reviews of adventitious root formation are now abundant, Selim (1956), Fernqvist (1966), Hyun (1967), Hess (1968), Sung (1969), Westwood (1973), Hartmann (1974), Haissig (1974b & c), Hartmann and Kester (1975), Heuser (1976) and many others.

In the following sections, an attempt is made to discuss various aspects of propagation by cuttings in order to focus the various hypotheses on present objectives. The topic will be discussed under several subtitles :

1. The anatomical basis of rhizogenesis.
2. Environmental factors.
3. Physiological factors.
4. Hormonal factors.
5. Rooting cofactors.
6. Nutritional factors.

These subdivisions are purely for convenience of discussion as all the factors listed in the final analysis may interact in the events leading to rhizogenesis.

1.2.1 ANATOMICAL DEVELOPMENT OF ROOTS IN STEM CUTTINGS

Adventitious root formation is the result of 3 processes - dedifferentiation, differentiation of the root initial, and emergence of the root meristem.

In most plants, the formation of adventitious roots takes place after the cutting is made (Hartmann and Kester, 1975, Girouard, 1967b). The origin of the adventitious roots is in the pericycle i.e. from cells immediately adjacent to immature xylem. They can easily

acquire auxin as a local product of the xylem, or auxin can be transported with other nutrients from the stem apex. In woody plants, parenchyma xylem is the preferred origin (Haissig, 1974a). This group of normal thin walled parenchyma cells must regain their capacity to divide : this is termed dedifferentiation. In these groups of cells, the root initials divide irregularly at first followed by regular divisions, forming groups of small cells which divide into root primordia. Cell division and differentiation continues, and soon each group of cells takes on the appearance of a root tip. A vascular system develops in the new root primordium and becomes connected to the adjacent vascular bundle (Esau, 1965). The root tip continues to grow outward, through the cortex, emerging from the epidermis or cork of the stem.

In herbaceous cuttings the root initials are located just outside and between the protoxylem. In woody perennial plants, in which there are one or more layers of secondary xylem and phloem roots usually originate in the young secondary phloem tissue, although such roots also arise from other tissues such as cambium, vascular ray or pith (Corbett, 1897; Kraus, Brown, Hammer, 1936; Van Tieghem Dauliot, 1888).

Certain plants possess latent or preformed root primordia, whose location is generally the same as that of non-preformed root initials, (Carpenter, 1961; lek, Van der, 1925). The preformed root primordia form during stem development and usually remain dormant until cuttings are made and are places under favourable environmental conditions, after which they grow and develop as adventitious roots (Carlson, 1950; Van de Lek, 1925).

In conifers, 4 different ways in which adventitious roots arise are described by Satoo (1956). In most conifers difficult to propagate by stem cuttings, root primordia usually originate from within callus tissue

(Cameron & Thomson, 1969).

The anatomical processes of adventitious root formation in juvenile Hedera helix (Girouard, 1967a) Helianthus tuberosa tissue cultures (Gautheret, 1969), Hydrangea macrophylla (Molnar and La Croix, 1972), Pinus radiata (Smith and Thorpe, 1975), seedling hypocotyls of Lycopersicon esculentum (Byrne, Collins, Cashau, and Aung, 1975) and Pecan (Brutsch, Allan and Wolstenholme, 1977) are well documented. A substantial general review of the origins of adventitious root formation in twigs and branches of gymnosperms and dicotyledons is also reported (Haissig, 1974a).

1.2.1.1 Mechanical Hinderance

In stem cuttings of some species there is a sclerenchymatic ring of tough cells surrounding the active meristems of adventitious root origin. There is some evidence (Beakbane, 1969; Ciampi and Gellini, 1958; Ciampi, 1962), easily rooted types were characterized by discontinuity of sclerenchymatous ring. While leafy cuttings of difficult-to root types have a continuous ring of sclerenchyma. Instances of a heavily lignified continuous sclerenchymatous ring in cuttings of other species have also been reported (Nanda, Anand and Kumar 1970; Vieltez 1974; Vieltez and Vieltez, 1976). Hardwood cuttings of Vaccinium corymbosum L. are difficult to root because of hinderance by the lignified pericycle and the epidermis of closely packed cultinized cells (Mahlstede and Watson, 1952).

While a sheath of lignified tissue in stem may in some cases act as a mechanical barrier to root emergence, there are so many exceptions that this certainly cannot be a primary cause of rooting difficulty.

In difficult-to-root cuttings of mature Hedera

helix, Girouard (1969) observed intense groups of discontinuous sclerenchyma fibres in the cortex, but adventitious roots had no difficulty growing through them. Sachs, Loreti and De Bie (1964) also found no correlation between density and continuity of sclerenchyma and ease of rooting. Although a continuous sclerenchymatous ring is present in carnation, it is relatively easy to root. Probably because in this case root growth occurs through the base of the cuttings.

1.2.2 ENVIRONMENTAL FACTORS AFFECTING ROOTING ABILITY OF CUTTING

The rooting ability of cuttings is influenced by several environmental factors : season of the year, photoperiod, light intensity, temperature, aeration and humidity. These environmental factors will have influence eventually on some physiological parameters or mechanism which control root regeneration.

1.2.2.2 SEASONAL VARIATION

Seasonal fluctuation of rooting ability in cuttings is a common phenomenon and the optimum season for taking cuttings varies immensely between species and even varieties (Hitchock and Zimmerman 1930; Howard, 1968a; Tustin 1976; Porlingis and Therios, 1976; and other authors here-after cited.)

Cuttings of apple, peach, pear, cherry and apricot cultivars root better in Autumn than Winter (Howard, 1966; Doud and Carlson, 1872). A rapid rise in rooting ability occurs in Spring. In some cases the Autumn peak is not present, but the Spring flush is always recorded. Many woofy ornamental species also show seasonal variations in rooting ability (Baker and Link, 1963). Actively growing softwood cuttings frequently show optimal

rooting in mid-summer whereas cuttings from hardwoods which become dormant are best taken in Autumn (Smith and Wareing, 1972a).

Many workers found a high correlation between bud activity and seasonal rhythm of the rooting capacity of cuttings. The root initiation on ' Old Home ' hardwood cuttings was best in Autumn and was correlated with the ability of the cutting to root. (Fadl and Hartmann, 1967a). Van der Lek (1934) found that buds which stimulated rooting in Summer and Autumn inhibited rooting in Winter, with inhibition decreasing progressively during Winter. Similar trends have been observed in Willow cuttings by Kefeli and Turetskaya (1965). However, the promotive role of the active bud is not a general rule, some contrary evidence has been reported by, Snyder (1955), Biran and Halevy (1973). For further discussion in this area refer to bud activity in the Literature Review (1.2.3.3).

Seasonal variation in roots initiation whether caused by day length, dormancy or bud activity, have in many cases been associated with fluxes in endogenous auxin levels under these conditions, and correlations have been established between ease of rooting and high auxin status. Spiegel (1955) noted an increase in auxin level occured just prior to bud break in Spring while inhibitor levels were low in the case of grape cuttings. In Populus nigra rooting ability was low in Winter, with the onset of dormancy, but with the renewed growth in Spring a high level of rooting occured. Also the response to exogenous auxins was greatest in the Winter months. They proposed that auxin levels in the Winter were sub-optimal for root initiation, and added auxin thus facilitated root initiation. In Spring, added auxin inhibited rooting, presumably because of supraoptimal levels, since cuttings normally root readily at this time. They concluded that root initiation was determined by the

physiological condition of the cutting material, and that any response to exogenous auxin was governed by the endogenous auxin status of the cuttings, which fluctuated with season.

In the propagation of hardwood cuttings of apple varieties, Tustin (1976) concluded that root initiation potential appeared to be directly related to the level of endogenous IAA. No correlation exists between root initiation and bud dormancy, endogenous abscisic acid, cytokinin or rooting cofactors.

Seasonal variability in rootability of Populus nigra, Hibiscus rosasinensis and Fiscus infectoria has been related to bud acitivity, auxin content and the mobilization of starch (Nanda, Anand, and Kumar 1970). The seasonal fluctuation in rooting potential of Impatiens balsamina cuttings are related to change in endogenous auxin, cambial activity and ability to mobilize food reserves governed by the activity of hydrolytic enzymes (Gupta, Kochhar and Nanda, 1975).

For Hibiscus schizopetalus, the seasonal rhythm rootability is not limited by the seasonal fluctuations in carbohydrates or natural daylength but can be ascribed to changes in auxin content (Kachecheba, 1975, 1976).

However for Bryophyllum tubiflorum and Delbergia sissoo rooting potential was found to be positively correlated with hydrolytic enzyme activity and negatively correlated with the starch content (Bala, Anand, Nanda, 1969).

Rootability can also be related to long day, Wareing and Roberts (1956), Bhella and Roberts (1974), Smith & Wareing (1972b). Rootability of leaf cuttings of

Populus X robusta Schneid cultured under longday, declined over Summer period which was associated with a decrease in endogenous IAA (Smith and Wareing, 1972b).

Nesterov (1968) noted that the successful propagation of stem cuttings of quince depended on the amount of chilling the mother plant had undergone. Best rooting results had been obtained with Picea abies if cuttings were taken in Spring just before or during bud bursting or in mid-Autumn when plant had been exposed to cool temperature for several days (Girouard, 1975).

The difficulty in rooting Pinus radiata was found to be associated with a high concertration of one of the inhibitors as determind from bud extracts (Cameron and Rook 1974; Zabkiweicz and Steele, 1974). In Willow cuttings, the sprouting buds in Spring promoted rooting, and the removal of dorment buds in Autumn also promoted rooting.

To futher complicate the situation, Tizio, Moyano and Morales (1968) found that the amount and quality of rooting substances change during the vegetative cycle of the mother plants and vary from year to year. Hartmann (1946) reported that Olive cuttings form roots more readily in Winter than in late Spring. Contrary to this, Porlingis and Therios (1976) said that olive cuttings root best in Summer and worse in Autumn and Winter. The difference presuamably being due to a locality factor.

The rooting potential of cuttings of different species, varies considerably with reason and this is also evident in the easy rooting species. In many cases, the succulent new growth appearing during the active seasons is favourable material for rooting (Doud and Carlson, 1972; Howard, 1968a). Although some woody plants can be more easily propagated in the late Summer or Winter months, (Smith and Wareing, 1972a).

As a whole, the seasonal variations in rooting capacity and the effectiveness of auxins treatment can be attributed to one or more of the following factors either separately or in combination.

1) Changes in the bud activity, promoters and inhibitors contents. Roberts, Tomasovic and Fuchigami, 1974; Cameron and Rook, 1974; Zabkiewicz and Steele, 1974; Bhella and Roberts, 1974 and 1975; Girouard, 1975).

2) Endogenous levels of auxin (Spiegel, 1955; Tustin, 1976; Vieltez and Pena, 1968; Nanda, Anad and Kumar, 1970; Evans, 1971; Smith and Wareing, 1972a & b; Kachecheba, 1975, 1976).

3) Photoperiod (Kachecheba, 1975, 1976; Wareing and Robert, 1956; Bhella and Roberts, 1974; Smith and Wareing, 1972b).

4) Chilling (Nesterov, 1968 and Girouard, 1975).

5) The content of hydrolytic enzymes and starch. (Reines and Bamping, 1964; Bala, Anand and Nanda, 1969; Nanda and Anand, 1970; Nanda, Anand and Kumar, 1970; Evans, 1971; Gupta, Kochhar and Nanda, 1975).

6) The status of cambial activity in the cuttings (Nanda, Purohit and Bala, 1968; Anand and Herberlein, 1975; Gupta, Kochhar, Nanda, 1975)

1.2.2.2 PHOTOPERIODS

The photoperiods experienced by the stock plant or by the cutting after placement in the rooting bed, have a distinct effect on rooting. The photoperiods most favourable for rooting vary from plant to plant, with some species rooting best under long and some under short photo-

periods (Stoutemyer and Close, 1946; Maskow and Koschezhenko, 1939). Some evidence suggests that the photoperiods most favourable for rooting are those in which carbohydrate reserves are accummulated in the cuttings, But may be lower on interaction with N - that is, cuttings high in N will not show as good a response to carbohydrate accummlation in terms of rooting - still the photoperiod influence appears to be largely a consequence of its effect on the carbohydrate of the plant (Smith, 1926). The photoperiods experienced by the stock plant before the cuttings are made have a more profound effect on the ability of the cutting to root than the photoperiods experienced by the root initiation itself, Wareing and Roberts (1956) and Bhella and Roberts (1974) both related longday treatments with improved cambial activity and the possible link of endogeneous IAA production as the effector of this process.

Stoutemyer and Close (1946) reported that radiation in the orange-red end of the spectrum gave fewer rooted cuttings. Treatment of green liliac cuttings with ultra-high frequency and x-ray irradiation increases rooting (Syrovatko, 1972).

Plant species do not respond equally to increased light intensity or daylength because of differing carbohydrate reserves and photosynthetic efficiency. Light effects mediated through the leaves are usually beneficial i.e. through a facilitation of photosynthesis (Fernquist, 1966; Hansan and Eriksen, 1974). However, it has not been possible to establish a correlation between the amount of reducing sugars, total N or growth regulators of stock plant on cuttings of Abelia grandiflora Rehd 'Prostrata' and the rooting response with respect to photoperiod (Steponkus and Hogan, 1967).

Long photoperiod depresses the rooting of few cuttings which can be restored by applied auxin (Lanphear

and Meahl, 1963). Active and dormant leafy cuttings of Populus robusta Schneid root to the same extent under long days as both types of cuttings contain similar amounts of IAA (Smith and Wareing, 1972b).

Rooting trials with 26 woody ornamentals have demonstrated that a long photoperiod is advantageous only to dormant or hardwood cuttings (Baker and Link, 1963) and long day conditions enhance the rooting of Douglas-fir stem cuttings by hastening bud break (Bhella and Roberts, 1974).

Short days suppress root formation in Begonia leaves while long day promote it by increasing auxin; cytokinin ratio (Heide 1965, 1967)

Short day treatment of the parent plant or cuttings of Bryophyllum tubiflorum improves rooting (Nanda, Purohit and Bala 1967). In Anagallis arvensis clone, short days maintain juvenility, vegetative growth, high RNA content and rooting capacity, whereas long days elicit the opposing effects (Trippi and Brulfert, 1973).

On the other hand the rooting of stem cutting of Pinus radiata is not influenced by photoperiod (Cameron and Rook, 1974).

1.2.2.3 OTHER LIGHT EFFECT

Light itself has direct effect on root initiation. If the entire piece of plant material is exposed to light, root initiation commonly is inhibited and furthermore, root growth is inhibited once root initiation has taken place (Went, 1935; Fernqvist, 1966); Hess, 1968; Pierik, 1969; Olienan, Van der Meer, Pierik and Roest, 1971). On the other hand, if light is applied only to the above ground part of the plant, a stimulation

of rooting is sometimes produced, (Stoutemyer and Close, 1946). They also found that red light is more effective in promoting rooting than blue light.

Light exerts its effects upon the organisation of cambium in Helianthus tuberosa tissue culture and this effect is independent of photosynthesis; (Gautheret, 1969). The light inducible rooting factor was found to be transmittable from cell to cell. Light influence the production of inhibition which directly or indirectly affects root formation (Hansen and Eriksen, 1974). 2, 4 - dinitrophenol promotes the rooting of dark grown bean hypocotyls (Krul, 1969) DNP is photoinactivated.

Bastin (1966) found that differences in rooting ability of Abies balsamia cuttings were due to differences in extractable IAA. Light grown cuttings rooted better than dark grown and this was attributed to increased synthesis of diphenol compound which inhibit IAA oxidise activity. A linear increase of diphenol content and number of roots and cuttings was established. Bastin suggested that diphenols acted as competitive inhibitors of IAA oxidation rather than by complexing with IAA as suggested by the rhizocaline and cofactor theories of Went and Hess.

The rooting inhibitory effect of light may be due to inactivation of light-labile factors, (Hartmann and Kester, 1975).

Etiolation of stock plants or tissue prior to or during planting as a cutting is ofter beneficial to rooting (Kawase, 1965; Zimmerman and Hitchock, 1937; Kawase 1965; Herman and Hess, 1963, 1966; Pierik, 1969) and the emergence of preformed root primordia (Shapiro, 1958), presumably by the preservation of auxin at the site of etiolation, IAA was able to replace the etiolation effect in non-etiolated cuttings (Kawase 1965; Turetskaya and Kof,

1965; Nanda, Purohit and Mehrotra, 1968; Nanda and Kochhar, 1968). In etiolated cuttings it was found that much lower dosages of IAA inhibited root initiation suggesting a near optimum IAA level existed in these cuttings for root regeneration.Harman and Hess (1963) had noted a slightly higher lever of IAA in etiolated tissues compared to non-etiolated tissue of Phaseolus vulgaris and Hibiscus rosasinensis.

Etiolation of red kidney bean and Hibiscus greatly enhances the rooting of cuttings by a reduction in starch content, mechanical strengthening tissue and cell wall thickness and an increase in less differentiated tissues, parenchyma cells, endogenous auxin level, sucrose, glucose, fructose, protein and rooting cofactors. Etiolated bean cuttings contain greater amount of value threonine, aspartic acid and asparagine than green tissues and etiolated Hibiscus tissues contain greater quantities of glutamic acid than green tissues (Herman and Hess, 1963, 1966). Turetskaya and Kof (1965) have found that etiolation results in high auxin and low inhibitor contents in bean seedlings while illumination results in high inhibitor and low auxin contents. The low auxin content may be related to increased peroxidase activity in light.

1.2.2.4 PHYSICAL ENVIRONMENT FOR ROOTING

Basal temperature, humidity, oxygen and PH of the medium are the most important components of the rooting environment.

A temperature range of 21-27^{o}C is sufficient for the rooting of most cuttings (Esper and Roof, 1931; Hartmann 1946; Hatcher and Garner, 1956; Pierik, 1969; Bhella and Roberts, 1974; Hartmann and Kester, 1975). Many workers found high temperature can greatly enhance

the effect of growth substance application (Hartmann et al, 1965; Ashiru and Carlson, 1968; Doud and Carlson, 1972).

Auxin treatment and temperature treatment were found to promote rooting independently but it was necessary to combine both treatments to give the greatest benefit (Howard, 1968b).

Although basal heat improves the rooting of cutting through the enhanced utilization of carbohydrate, its extensive use may result in reduced establishment (Cheffins, 1975). A high temperature of 25°C has been found to increase the rooting of hardy hybrid Rhododendron cultivars but precautions must be taken to prevent rotting (Whalley and Loach 1977).

Heide (1967) reported that high temperature (24-27°C) increases root formation in Begonia leaves to an optinum by increasing the auxin Cytokinin ratio.

A low night temperature of 5-10°C is advantageous to the rooting of Pinus radiata stem cuttings (Cameron and Rock, 1974).

Excessively high air temperatures tend to promote bud development in advance of root development, and increase water loss from the leaves. It is important to have root development ahead of shoot development (Hartmann and Kester, 1975). Therefore basal heating to maintain a higher base temperature than that of the buds is beneficial.

High humidity is often required for root induction (Hartmann, 1946). For this purpose constant or intermittent mist is used. By the presence of mist, cuttings can be exposed to high light intensity without causing a concommittant rise in the ambient temperature (Hess and Snyder, 1955; Howard, 1965). The respiratory

rate is reduced while the photosynthetic rate is increased in a build up of rooting potential owing to the accummulation of carbohydrate. Intermittent mist is more effective than constant mist such that the temperature of the medium is not reduced excessively (Hess and Snyder, 1955).

Propagation of Euonymus alatus 'Compactus' cuttings during dormancy under intermittent mist results in increasing the effective levels of substances such as carbohydrate, phenolics, flavonoid compounds, auxin-like substance and rooting cofactors, presumably due to the leaching of certain inhibitory substance present (Lee, 1969).

In Balsam poplar pH7 promotes root formation while PH 11 is deleterious (Hartmann and Kester, 1975). pH 7 is best for Thuja occidentalis while lower pH's inhibit.

While oxygen is not essential for root primordium initiation, it is essential for root growth (Zimmerman, 1930; Kordan, 1976). Willow cuttings form roots readily in water with an oxygen content as low as 1ppm, but English ivy requires about 10ppm for adequate root growth (Zimmerman, 1930).

1.2.3 PHYSIOLOGICAL ASPECTS

1.2.3.1 JUVENILITY

Cuttings from young plants invariably strike root more easily and readily than those from mature plants. The juvenile form can often be distinguished from the mature form by certain distinct morphological features such as growth form, leaf shape and appearance of the bark and buds.

Vekhov and Iljin (1934) studied 600 varieties of trees and shrubs and concluded that shoots from young plants rooted better than those from older plants. The decrease in rooting capacity with increase age of the stock plants has been reported by many others (Gardner, 1929; Muzik and Gruzado, 1958; Hare, 1965; Quamme and Nelson, 1965; Nelson and Pepper, 1965; Libby and Conkie, 1966; Cameron, 1968; Pierik, 1969; Bilan, 1974; Girouard, 1974; Porlingis and Therios, 1976; Riding, 1976.)

Juvenile factors appear to be graft-transmitable. Grafting a juvenile stock with an adult scion can enhance rootability of cuttings secured from the latter (Muzik and Cruzado, 1958).

Juvenility in relation to rooting may possibly be explained by the increasing production of rooting inhibitors as the plant grows older (Paton and Pryor, 1971; Paton, Willing, Nichols and Pryor, 1970; Vieltez and Vieltez, 1976).

Also reduced rootability on ontogenetic aging of Hedera helix was reported to be associated with lower phenolic levels in the mature forms than in the juvenile forms (Girouard, 1969).

While Hess 1957, 1961, 1962(b), 1963, 1968; Stoutemyer and Britt, 1962 reported that decreasing rooting potential as plants age may possibly be lowering in rooting cofactors.

In Hedera helix, GA3 spray can induce rejuvenation of the mature phase (Frydman and Wareing, 1974). In fact juvenile shoot apices contain higher levels of extractable GA like substance than the adult (Frydman and Wareing, 1973).

Trippi and Brulfert (1973) concluded that

ontogenetic aging is associated with a decline in the ability to synthesize RNA.

In rooting cuttings of difficult-to-root species it would be useful to induce rejuvenation of the easily rooted juvenile stage from plants in the adult phase. This can be done by several methods:

1) hedging or shearing treatments (Libby, Brown and Fielding, 1972; Garner and Hatcher, 1962).

2) using root cuttings from aged plants (Stoutemyer, 1937a).

3) grafting adult forms on to juvenile forms; Stoutemyer, Britt and Goodwin, 1961; Muzik and Cruzado, 1958).

4) Gibberellin sprays (Muzik and Cruzado, 1958; Robbins, 1960).

As a plant matures, the seat of juvenility is not lost, but is retained at the basal part of the plant. Species of some trees, such as oak, spruce and beech, leaf retention late into the Autumn occurs at the basal parts of the tree and indicates this that part still has juvenile characteristics. Cuttings should be taken from these zones (Hackett, 1970; Grace, 1939; Hyun, 1967; O'Reuveni and Adato, 1974; Riding, 1976).

1.2.3.2 THE EFFECTS OF LEAVES

The role of leaves in rooting is primarily thought to be because they provided certain nutritive materials beneficial to root formation (Zimmerman and Hitchcock, 1933; Girouard and Hess, 1964; Altman, 1972; Ohta and Furukawa, 1975). Other materials such as vitamins and nitrogenous materials are supplied by the

leaves as well. However, the root promoting effects of leaves are not merely for nutritive reasons, but leaves are also known to be powerful hormone producers (Bouillenne and Went, 1933; Bouillenne and Bouillenne-Warland, 1955; Girouard, Hess 1964; Okoro and Grace, 1976).

It has long been known that the photosynthetic activity of the leaves contribute to rooting, and there has been considerable supporting experimental evidence. In Hibiscus rosa-sinensis, it has been shown that the chief effect of the leaves in root formation is through their supply of nutritional factors to the base of the cuttings (Zimmerman and Hitchcock, 1933; Girouard and Hess, 1964). The rooting of many plant species is proportional to the leaf surface exposed (Calma and Richey, 1930; Esper and Roof, 1931; Altman, 1972; Bilan, 1974). A combination of auxin, sucrose and a suitable source of nitrogen can replace the root forming effect of leaves suggesting the effect of leaves is primarily nutritive (Nanda, Jain and Malhotra, 1971; Ohta and Furukawa, 1975). The nutritive effect of leaves has also been reported in hop cuttings (Howard, 1965), Justicia gendarussa cuttings (Sen and Basu, 1960), olive cuttings (Porlingis and Therios, 1976) and in Pinus radiata (Cameron and Rook, 1974).

The labelling experiments of Altman and Wareing (1975) shown that the presence of leaves is absolutely essential for root formation in Phaseolus vulgaris cuttings. Carbohydrate transported from the leaves was found to be the main limiting factor rather than unknown cofactors of an hormonal nature, and that a minimum sugar content must be available for the formation of a given number of root primordia.

The carbohydrate resulting from photosynthetic activity of the leaves, have been shown to contribute to rooting. However the rooting promoting effect of leaves like buds is due chiefly to auxin production (Hartmann and

Kester, 1975). While Hibiscus rosa - sinensis is difficult to root due to lack of auxin and unknown factor from the leaves (Overbeek and Gordon and Gregory, 1946).

Bouillenne and Went (1933) and Went (1938) formulated the rhizocaline hypothesis and revised by Bouillenne and Bouillenne-Warland in 1955. They postulated 3 components :

1) A specific factor, characterized by orthodiphenolic groups, originating from the leaves.

2) A non specific factor, auxin translocated, at low physiological levels from the leaves.

3) A non-mobile enzyme factor of the polyphenol oxidase type, located at particular viable cells (pericycle, phloem, cambium) Which is probably of the polyphenol oxidase type. Interaction between auxin and orthodiphenols wherever the enzyme is present, giving rise to the complex "rhizocaline".

Portudal, a bicyclic diterpene with a per-hydroazulene nucleus, extracted from Portulaca leaves has been found to promote the rooting of Azukia argularis Vigna catjang Var.Sinersis, mung bean and Raphanus sativus var. acanthiformis 'Riso daikon' (Mitsuhashi, Shibaoka and Shimokoriyama, 1969).

In Hedera helix, the presence of leaves not only improves rooting by synthesizing photosynthates but also serving as a mean of retaining intrinsic rooting substances which have a tendency of diffusing out in their absence (Girouard, Hess, 1964).

It was also reported that leaves supply thiamine (Bonner, 1942), and pyridoxine (Bonner and Dorlard,

1943) necessary for root formation.

1.2.3.3 EFFECT OF BUDS

The idea that bud activity may influence root initiation has been evidenced by several workers (Van der lek, 1925; Went, 1929; Harada and Nakayama, 1957; Fadl and Hartmann, 1967a; Roberts et al, 1964). The presence of active buds on a cutting has a strong promotive effect on rooting. Removal of active buds from cuttings or if all the buds are dormant in certain plants will stop root regeneration almost completely, especially in species without root initials (Went, 1929, Ver der Lek, 1925, 1934).

Van der Lek (1934) found that buds which stimulated rooting in Summer and Autumn inhibited rooting in Winter, with inhibition, decreasing progressively by late Winter. The low rooting in Winter of cuttings with buds was postulated to be due to the accummulation of inhibitors, such compounds arrest bud development and also inhibit the formation of endogenous root promoting substances, such hypothesis is supported by Kefeli and Turetskaya (1965) in studying rooting of Willow cuttings, where they found the sprouting buds in Spring promote rooting and the removal of dormant buds in Autumn promote rooting.

Hemberg (1949) proposed that inhibitors may be involved in bud dormancy. They have been found in many plant parts other than buds (Kefford, 1955, Luckwill, 1952). A definite pattern of inhibitor levels in buds seems to exist before, during, and after the rest period, increasing in late Summer, reaching a maximum in late fall, and decreasing during the Winter. Minimum levels occur in Spring when buds are expanding (Hemberg, 1949, Philips and Wareing, 1958). Most of these inhibitors have been identified as part of the inhibitor-beta complex (Bennentt-

Clark and Kefford, 1953) and have been shown to be phenolic compounds (Hemberg, 1949; Hess, 1964).

Treatments which induce bud burst (eg. chilling and day length manipulations) have been known to enhance root initiation (Fadl and Hartmann, 1967; Smith and Wareing, 1972, a,b; Roberts et al; 1974; Bhella and Roberts, 1974; Whitehill and Schwabe, 1975). Chilling shortened bud dormancy and increased root initiation (Smith and Wareing, 1972a). Endogenous auxin levels are higher in chilled than unchilled buds, Smith and Wareing (1971) also correlated bud size with root initiation; bud size affects the quantitative output of IAA. The greater the number of buds present on the cuttings, the greater was the chilling effect on rooting. Changes correlated in terms of inhibitor and promoter levels assayed from treated cuttings.

It was postulated that hormone like substances formed in the developing buds and transported through the phloem to the base of the base of the cuttings where they stimulated root formation. In cuttings with only 1 bud, roots appear directly below that bud. In some plants, if a ring of bark is removed down to the wood just below a bud, root formation is reduced, indicating that some influence travels through phloem from the bud to the base of the cutting, where it is active in promoting root initiation (Haradan and Nakayama, 1957).

The application of auxin has not been found to replace entirely the presence of buds in causing root formation (Went and Thimann, 1937). This indicated that a factor other than auxin presumably produced by the bud, is needed for root formation.

In contrast Cahlalizah and Nekrasova (1962 cited by Biran and Halevy 1973) have shown that peach cuttings bearing sprouting buds do not root, while those

with dormant buds root satisfactorily, and removal of sprouting buds facilitated rooting.

While Snyder 1955 found no correlation between rooting response and the degree of lateral bud activity in Taxus cuspidata. Howard (1968a) demonstrated that both chilling and removing of potentially active buds of plum did not depress rooting. In fact disbudding was shown to stimulate rootings similar to that of wounding effect.

In cuttings made from shoots with preformed root initials, the further development of the roots seems to be little influenced by the presence or absence of buds (Hartmann and Kester, 1975).

Biran and Halevy (1973) proposed an explanation for this controversial phenomenon. They suggested that growing buds affect rooting of Dahlia cuttings in two opposed directions i.e. they inhibit rooting, on the one hand, by diverting certain metabolites away from the rooting zone, and on the other, they also promote rooting by enhancing cambial activity and creating a fine balance of phytochoromes favourable for rooting. Promotion or inhibition therefore depends on which influence is stronger.

To sum up, the primary role of buds in adventitious root formation is their ability to synthesize auxin, promotory and inhibitory substances, and act as metabolically competitive systems to adventitious root formation during their development.

1.2.3.4 EFFECT OF FLOWERING BUD

Initiation of flowers, or the presence of flower buds on a plant indicate the maturity of that plant.

Flower buds usually reduce root regeneration capacity (Biran and Halevy, 1973; Gorter, 1956).

In some cases, such as Chrysanthemum, Fuchsia and carnation, pinching off of flower buds at an early stage eliminates their inhibitory effects (Riehl, 1956; Kraus, 1953).

Flowering and root regeneration are antagonistic due to redistribution of auxin. High auxin level, promotes root formation and inhibits flowering (Selim 1956; Johnson, 1970). Data also suggest that competition exists between developing flowering buds and root initiation, especially in difficult-to-root cultivars (Adams and Roberts, 1967). However, in easy-to-root plants, the presence of flower buds or cuttings may have little effect (Singh, 1962).

Contrary to the above, lilac cuttings collected during the onset of flowering, root best (Bojarczuk, 1975) and Gibberellin treatment stimulates both flowering and rooting of Bryophyllum tubiflorum cuttings (Nanda, Purohit and Bala, 1967).

1.2.4 HORMONAL BASIS FOR ROOTING

Successful rooting is dependent upon the presence in the cuttings a number of natural plant growth substances. There are several groups of such substances, including auxins, cytokinins, and gibberellins of these, auxins are of the greatest interest in repect to the formation of roots on cuttings. It is evident that the auxins stimulate the formation of roots by an interaction involving organic materials in the plants, especially carbohydrates and nitrogenous compounds. In addition to these groups there probably are other naturally occuring

substances which have a part in promoting initiation of adventitious roots. The balance between auxin and these plant constitutents as the control of differentiation, is the basis for the rooting of cuttings as we understand it today.

HORMONAL ASPECTS OF ADVENTITIOUS ROOT FORMATION

1.2.4.1 ENDOGENOUS AUXINS

Since the original demonstration ny Went (1934) and Thimann and Went (1934), of the stimulation of adventitious root initiation on stem cuttings by plant extracts, and their correlation and identification of Hetero-auxin (IAA) (Kogl *et al*, 1934) as being the active component, much research had been carried out to determine the effect of both endogenous and exogenous auxin on plant propagation by cuttings.

The classical work of Warmke and Warmke (1950) firmly established the role of endogenous auxins in the differentiation of roots compared with shoots in plants. A higher distal auxin content in root cuttings of *Taraxacum* and *Cichorium* accounted for the regeneration of new roots. This process could be reverted to shoot formation to a limited extent, by exhaustive leaching of the cutting base in water, or by treatment with an auxin inhibitor. In a range of herbaceous species, Odom and Carpenter (1965) related rooting ability to the presence or accummulation of 'free auxin' in the cutting bases. Rapidity and vigour of rooting parallel the level of endogenous auxin. An inverse relationship has been shown between the endogenous acidic auxin level and the known beneficial response to added root promoting substances suggesting that endogenous auxin plays a primary role.

Root formation is coupled with a steady decline in endogenous auxin, this indicates that IAA was instrumental in the initiation of roots on cutting (Saito and Ogasawara, 1960; Feucht and Dausend, 1976; Smith and Wareing, 1972a).

Warmke and Warmke (1950) detected an increase in the 'neutral', 'free acidic' and 'bound' auxin during the rooting of chicory and dandelion root sections. Hemberg (1954) has found an increase in both the 'free' and 'bound' auxin during rooting of Phaseolus vugaris hypocotyls. In the rooting of Populus nigra, the amount of auxin increases at first, reaching a maximum after root emergence and then declines (Michniewicz and Kriesel, 1970).

Many difficult-to-root plants were found to be lack of endogenous auxin (Cooper, 1935; Vieltez, Seoane, Dolores, Gesto, Mendez, Mato and Vazquez, 1964). Thus suggested that endogenous auxin was a limiting factor in rooting.

Sin and Sung (1968) confirmed that, in a range of Pinus species and Populus alba, IAA was an important rooting promoter and that easily rooted brachyblast cuttings had a higher auxin status than normal cuttings. In some cases IAN was also considered to be an active root promoter.

In Brittle willow, root primodium initiation and early development of root primodia depend to a greater extent than subsequent growth on IAA (Haissig 1970). Removal of the stem apex, leaves and axillary buds (Sites of auxin production) adjacent to the site of root initiation inhibited early process in root formation which could be remedied by exogenous auxin application (Haissig, 1970).

The Process of root formation on cutting consisted of 2 phases - the initiation phase and root growth and elongation phase (Lovell et al, 1977; Eriksen, 1974). The initiation phase may have 2 stages, an auxin active and an auxin - inactive stage (Haissig 1970. 1972; Eriksen and Mohammed, 1974 and Mohammed and Eriksen, 1974). The initiation phase was dependent upon a continuous supply of IAA rather than its availablity at one time in high concentration (Haissig, 1970; Eriksen, 1974; Eriksen and Mohammed, 1974; Mohammed and Eriksen, 1974; Ryugo and Breen, 1974; Mohammed, 1975).

Root formation in Vigna cuttings constituted consecutive histologically different stages. The effects of inhibitory (400mg/1) and promotory (50mg/1) concentrations of IAA on these stages had been investigated (Sircar and Chatterjee 1973, 1976, 1977). An inhibitory IAA concentration lengthens the duration of the various stages but its effect declined towards the later stages, and decreased total sugar, nitrogen, promoter, RNA, DNA contents presumably by causing injury. A promotory IAA concentration was particularly effective at the later stages especially at stage III, the incipient disorganisation stage of parenchymatous tissues, and augments the accumulation of sugars, nitrogenous substances, promoters, RNA and DNA to a maximum (Sircar and Chatterjee 1973, 1977).

Considerable evidence existed indicating that IAA influenced growth processes by triggering the synthesis of specific new RNA's and emzyme proteins (Nanda, Jain and Malhotra 1971; Nanda, Kumar and Kochhar, 1974). Glucose and IAA were found to increase the protein and nucleic acid pool above those of the control and in the presence of protein and nucleic acid antimetabolites. In the presence of glucose, IAA induces the synthesis of two new low molecular weight RNA's of the messenger or transfer type which through some unknown mechamism lead to

rhigogenesis (Nanda, Kumar and Kochhar, 1974).

IAA enhances the activity of a protein complex of multiple enzymatic functions including polyphenoloxidase, IAA oxidase and peroxidase activities, prior to root initiation in mungbean hypocotyl tissues, indicating that some early metabolic events probably involving the turn over of growth substances may be required for root initiation (Frenkel and Hess, 1974).

By feeding redioactive precursor of amino acid mungbean hypocotyl tissues, Kuraishi (1973) concluded that IAA does not stimulate protein synthese. However, it protects the degradation of some protein fractions.

From current knowledge, it seems likely that auxin may influence root regeneration by regulating nucleic acid and protein metabolisms. The actual mechanism in this aspect requires further elucidation.

However, several lines of evidence suggest that the adventitious root regeneration from cuttings is more complex than a simple regulation by auxin. There are instances in which rooting ability fail to correlate with endogenous auxin level (Tyce, 1957). Biran and Halevy (1973) reported that endogenous auxin is not the determinant in the rooting of easy-and difficult-to-root Dahlia cuttings. The difference in root-ability of hard- and easy-to-root ortets of sugar is not due to endogenous auxin content (Greenwood, Atkinson and Yawney, 1976). Foong (1977) also concluded that process of R.ponticum and R.brittana.

With the improvements in plant hormone detecting techiques, it might be more relevant today to examine auxin : inhibitor ratios and ratios between auxins and other plant hormones which are known to antagonize root initiation. Spiegel (1955) found in Vitis cuttings

that easy rooting cuttings always correlate with high auxin : low inhibitor ratio and the converse was true for difficult-to-root cuttings. This relationship between rooting ability and auxin ; inhibitor ratio was also reported by Ogasawara (1960) in Pinus densiflora, Heide (1967) in Begonia, Sarkisova (1972) in apple, peach, pear and quince cuttings.

The auxin : cytokinin ratio had also been reported to show growing importance in rooting. Heide 1965, 1968; Heide and Skoog, 1967 noted that day length and temperature influenced the regeneration of Begonia leaves by altering endogenous auxin : cytokinin ratio.

1.2.4.2 CYTOKININ

The differentiation of a meristem into a root primordium is the first step in root formation. Skoog and his colleagues (1951) showed that the type of differentiation that occurs in a meristem is dependent on the proportion of auxin to cytokinin or to other substances, such as adenine, that stimulate cell division. Tobacco stem sections tend to form buds and leaf primordia when auxin : cytokinin and adenine ratio is low. However, when ration is high, root primordia form. When ratio is intermediate, a simple callus is formed. Heide (1965) reported similar trend in Begoina leaf cuttings.

Humphries (1960) reported the inhibition of root initiation on petioles and hypocotyls of dwarf bean by Kinetin and it counteracts the effect of NAA in root formation. Schraudof and Reinert (1959) noted that the addition of Kinetin would inhibit root development and counteract root promotion by the auxin, 2,4-D. Others who found cytokinin inhibitory are Kaminek, 1967; Fellenberg, 1969; Besemer, Harden and Reinert, 1969; Mullins, 1970; Smith and Thotpe, 1975).

Although cytokinins would not be expected to stimulate root development, since they usually stimulate shoot development and are antagonstic to rooting, there have been some reports that low concentrations of cytokinins do stimulate root initiation. Kinetin at a concentration of 0.1ppm more than doubles the rooting of terminal cuttings of one clone of the difficult-to-root Feijoa sellowiana (Meredith, Joiner, Biggs, 1970). Working on Acer rubrum and Eucalyptus camaldulensis (Bachelard and Stowe, 1963) found Kinetin inhibits rooting when applied to the base but it is promoting when applied to the leaves. These workers found no evidence that the stimulatory effect is due to a change in the balance between auxin and Kinetin.

Low BA concentration promotes the early stage of root initiation of decapitated pea cuttings, whereas high BA concentration inhibits early root initiation of both decapitated or decapitated and disbudded pea cuttings. Probably due to positive and negative interaction with auxin. The inhibitory effect is lost during later phase of root initiation. The developing root primordia are now capable of regulating the endogenous level of active cytokinin and therefore does not responde to exogenous cytokinin (Eriksen 1974). Kriesel (1976) suggested that newly formed roots of willow cuttings are capable of cytokinin synthesis.

Like other hormones, cytokinin effect is been postulated to be affecting the metabolism of nucleic acid and protein metabolism (Kaminek and Stemberova, 1969; Kaminek, 1967; Haissig, 1971b, 1974c).

1.2.4.3 GIBBERELLINS AND ROOTING

GAs are notable for their inhibition of root initiation in cuttings and tissue culture explants,

Salix purpurea cuttings -(Gundersen, 1959);
Acer rubrum cuttings, Eucalyptus camaldulensis Dehn. - (Bachelard and Stowe, 1963);
Cinchona ledgeriana Moens cuttings -(Chatterjee, 1966);
tomato cuttings -(Hansen, 1967);
stem segments of Populus nigra -(Nanda, Purohit, Mehrotra, 1968);
Azukia cuttings -(Mitsuhashi and Shibaoka and Shimokoriyama, 1969);
Mung bean hypocotyl cutting -(Anzai, Shibaoka and Shimokoriyama, 1971);
tobacco callus tissue -(Thorpe and Meier, 1973);
Vigna hypocotyl cuttings -(Sircar and Chatterjee, 1973, 1974);
Pinus radiata seedling cuttings -(Smith and Thorpe, 1975);
black currant and sour sherry cuttings -(Turetskaya, et al 1976).

The involvement of GA in rooting is substaintiated by the fact that many GA antagonists improve root production in the cuttings of many plant species by lowering their endogenous GA status by inhibiting synthesis and or promoting their breakdown (Jankiewicz, Bojarczuk and Piatkowski, 1973).

SEVERAL GA ANTAGONISTS FOUND TO ENHANCE ROOTING :

GA Antagonists	References
(2-chloroethyl) trimethyl-ammonium chloride	Doede, 1969; Read, Durham & Fieldhouse, 1972; Turetskaya, et al 1976.
ABA	Chin, Meyer & Beevers, 1969; Basu, Roy & Bose, 1970.
SADH	Read & Hoysler, 1969; Bojarezuk & Jankiewicz, 1975.
n-dimethylamino succinic acid (Alar)	Doede 1969.
α-cyclopropyl-α-(4-methoxyphenyl)-5-pyrimidine methanol (EL 531).	Kefford, 1973

The promotory effect of these GA antagonists can only be confined to certain plant species. As found by Jankiewicz, Bojarczuk and Piatkowski (1973) that SADH is not active in the rooting of Syringa meyeri Schnoid.

GA_3 depresses the rooting of tobacco callus tissues with the concomitant reduction of starch levels and inhibition of the rise in respiratory rate associated with increased metabolism (Thorpe and Meier, 1973). In the rooting of tomato leaf cuttings, localized starch accummulation in the petiole prior to or during rhizogenesis is a prerequisite. GA_3 causes inhibitor by interfering with such an accummulation (Coleman and Greyson, 1977a and 1977b).

In the rooting of Pinus radiata seedling cuttings, the effect of GA_3 depends on its time of application. Early application during the time of meristemoid formation promotes this process. GA_3 application post-meristmoid formation retards their development (Smith and Thorpe, 1975). In the regeneration of Brittle willow cuttings, initiating root primordia are least affected by GA_3 whereas the cell number per primordium is reduced through GA_3 inhibition of intraprimordium cell division which normally leads to the development of primordia. It is probable that GA_3 blocks the action or the early process of primordium development subsequent to the initiation phase (Haissig, 1973). These findings are contrary to that of Brian, Hemming and Lowe (1960) where they have proposed that GA_3 exerts its inhibitory effect only after root primordia foundation in pea and bean cuttings.

GA_3 exhibits both promotory (0.1 ppm) and inhibitory (50 ppm) influences in the rooting of Vigna hypocotyl cuttings which is characterized by 5 distinct histologically differentiated stages. Inhibitive GA_3 causes lengthening of the durations of stages of applied after the first stage but this effect declines progressively over the stages. Unlike the promotory effect of IAA which progressively become more marked, the promotory effect of GA_3 decreases sequentially. At stage 1, promotory GA_3 causes substantial accumulation of sugars,

nitrogenous substances and growth promoters but this effect is slowly lost at later stages. The absence of inhibition by GA_3 at later stages is associated concomitantly with lower levels of growth inhibitory substances at the hypocotyl base (Sircar and Chatterjee, 1973, 1974).

Hence experimental evidence suggests that the influence of GA either promotory or inhibitory is exerted at some early process of rhizo-genesis.

Two theories on the mode of action of gibberellin inhibition of root inhibition have been proposed. An early theory proposed by Brian (1957) was the nutrient diversion hypothesis. This proposed that GA, by inducing stem elongation, or bud burst and elongation, set up a new sink for nitrogenous compounds and carbohydrates, which compete more effectively for these food reserves than did meistematic sites of root regeneration. Several workers subscribed to this idea, Galston, 1948; Brian, 1957; Chatterjee 1966; Nanda, Purohit and Mehrotra, 1968; Sircar and Chatterjee, 1974, Turetskaya, Polikarpova, Kefeli, Koe and Kichina, 1976. Brian, Hemming and Lowe (1960) have disputed this theory from their observations that

a) small does of GA_3 applied basally inhibit rooting without affecting stem elongation.
b) disbudding cuttings prevents stem elongation but inhibition of root formation still persist and
c) apical applications of GA_3 causes stem elongation without affecting rooting, and concluded that GA inhibition is a direct localized effect, preventing dedifferentiation of adult stem tissues. Brian et al (1960) is supported by other workers Mitsuhashi et al (1969); Coleman and Greyson (19771, 1977b) also noted that basal sucrose application cannot overcome the GA_3 inhibitory effect.

There are some interesting reports on a

rooting promotion effect of GA. GA_3 stimulates the rootings of Bryopyllum tubifloum cuttings under short day but fails to do so under long day conditions (Nanda, Purohit and Bala 1967). The promotory effect of GA_3 in the concentration range of 10^{-11} - 10^{-7}m is probably due to increased auxin production and/or mobilization of a large food reserve sufficient for both root and shoot growth (Eriksen 1970, 1971) cited by Hartmann and Kester, 1975).

There are also reports of rooting promotion by high GA_3 concentration and root formation in petioles of Phaseolus vulgaris leaves is accentuated by GA_3 (5 and 10 ppm) and has been found to be an effect mediated by GA_3 - induced IAA synthesis from tryptophan in the lamina which is subsequently transported to the petiole (Varga and Humphries, 1974).

GA_3 at concentration 10^{-8} - 10^{-4}m increase the rooting of tomato leaf disc kept in the dark in the presence of tryptamine by effecting an increase in IAA synthesis through the pathway:-

tryptamine → Indole-3-acetadehyde → IAA.

This promotion is concentration dependent and several cofactors of the enzyme indolved in IAA bio-synthesis e.g. ∧-Ketoglutonic acid and pyriodoxal phosphate, have a beneficial effect (Coleman and Greyson 1977a). GA_3 induced IAA synthesis is manifested in bean hypocotyls in the presence of tryptophan (Varga, Koves, Sirokman and Bito, 1968).

In still other cases GA has no effect on rooting e.g. Sinapis alba seedling cutting (Gundersen, 1959) and Cichorium intybus leaf culture (Besemer, Harden and Reinert, 1969).

Some reports suggested that GA_3 action is light dependent. Hansen (1975) noted that low GA_3

(10^{-3} - 10^{-8}m) treatment promoting rooting of cuttings from stock pea plant grown under low irradiance only, whereas under high concentration inhibition occur in both high and low radiation. Coleman and Greyson (1977a and 1977b) also reported the light dependant nature of GA_3 effect.

GA was postulated to exert its effect through nucleic acid metabolism. At concentration 10^{-4} - 10^{-3}M GA_3 decreases the transfer messenger of purified DNA of pea seedlings, thereby, facilitate transcription and translation (Bamberger, 1971). Sircar and Chatterjee (1977) also reported that GA increase the nucleic acid fractions of Vigna hypocotyl, but the increase is not specific for the rooting process.

1.2.4.4 ABSCISCI ACID

Abscisic acid has been found to both promote root initiation (Chin and Beevers, 1969; Basu et al, 1970; Bojerczuk and Jankiewicz, 1975) or alternatively inhibit, root formation (Heide, 1968; Eliason, 1969; Pierek and Stegmans, 1975).

At lower concentration (10 and 20 mg/L) ABA has an additive effects with IAA (8.76 mg/ml) in promoting rooting of mung bean hypocotyl cuttings, (Basu, Roy and Bose, 1970). Bojarczuk and Jankiewicz, (1975) also found a promoting effect of ABA on lilac cuttings.

One of the functions of ABA may be to antagonize the inhibitory effect of high gibberellin level in adventitious root formation - Chin, Meyer and Beevers (1969) have reported that ABA at 1-50 Mg/ml promotes the rooting of mung bean hypocotyl cuttings but no synergism or additive effects of ABA at the above concentration and IAA (0.876 Mg/ml) have been observed. ABA application can

partially overcome the inhibitory effect of GA_3 but not of Kinetin. Thorpe and Meier (1973) also noticed the inhibition of root development in tobacco callus culture by GA_3 is partially overcome by ABA.

ABA which exibits promotory effects in root induction in Myrobolan B Plum has been shown to be an inhibitor in the coleoptile bioassay (Lipecki and Dennis, 1972).

In contrast to reports of ABA promoting of root initiation, several reports demonstrate root-inhibition activities of ABA. ABA has no effects with IAA or NAA in the rooting of Phaseolus vulgars L. and only negligible effect in combination with IBA. 50mg/l ABA in conjuction with IBA or NAA completely inhibits rooting (Basu, Roy and Bose 1970).

Heide (1968) noted that bud formation is promoted by ABA but reduces root formation in Begonia leaves.

Pierek and Steegmans (1975) found the addition of ABA always has a negative effect or no effect at all on the rooting of Rhododendron explants. All parameters of rooting were strongly inhibited as the concentration of ABA increased.

At 1 and 10mg/ml ABA stimulates IAA - induced rooting of 15 days old tomato cuttings additively and that of IBA synergistically (Basu, Roy and Bose, 1970). Rajagopal, Rao and Rao (1971) have used nitrogen deficient 40 days old tomato shoot cuttings, which have superior rootability, to evaluate the effect of IAA on rooting. ABA at 1 and 5mg/l enhances rooting and at 10mg/ml inhibition sets in. In the concentration used, ABA appears to act antagonistically to IAA - induced rooting. These two contradicting reports may be attributed to the

age and nutritional status of the plant material used.

To overcome this conflicting evidence, some authors proposed taking the IAA: ABA ratio as a measure of rooting potential rather than ABA level alone. Increasing ABA and decreasing IAA levels through the Winter months reduced the IAA: ABA ratios account for the difficulty of rooting (Howard, 1966, 1977; Fadl and Hartmann, 1967a; Nesterov, 1968; Howard and Nahlawi,1969; Doud and Carlson, 1972).

As with other hormones, ABA might exert its effect on nucleic acid metabolism (Villiens, 1968).

1.2.4.5 ETHYLENE

In the 1930s it was known that ethylene gas could stimulate development of root or stem of herbaceous plants, such as tomatoes, Zimmerman and Hitchcock (1933) established the promotory activity of several unsaturated hydrocarbon gases especially ethlylene, acetylene and propylene on adventitious root initiation.

Kawase (1971) demonstrated that centrifugation and submerging of cutting in water, caused an injection of water into the cuttings and will trigger ethylene production which in turn stimulates root initiation. Several workers share the idea of soaking cutting in water to improve rootings, Zimmerman and Hitchcock, 1933; Mullins, 1970; Fernquvist, 1966; Khan and Hall, 1954 . The promotory effect of soaking could well be a consequence of ethylene production (Kawase, 1971) and/or the leaching of inhibitors (Khan and Hall, 1954; Spiegel, 1954; Tyce, 1957; Tizio, Moyano and Morales, 1968).

Mullins (1970) reported that ethylene promotes the emergence of latent root priordia but inhibits

adventitious root formation in mung bean hypocotyl cuttings and rooting is promoted only when the rates of ethylene production are low relative to auxin concentration. Like Krishnamoorthy (1970) he has inferred ethylene production is a feedback mechanism for auxin effects by regulating endogenous auxin.

Further work by Batten and Mullins (1975) concluded that ethylene was not directly involved in auxin-induced root formation.

1.2.5 ROLE OF CO-FACTORS

Many workers had failed to correlate the failure of cuttings to root with any morphological factors, endogenous hormonal level or nutritional factors. In such cases, the alternative proposal of rooting cofactors is often employed to explain the situation.

Pea cuttings would not root even in auxin rich medium (Went, 1934). Further investigation showed that a certain rooting stimulating substance called 'Rhizocaline' in the cotyledon, leaves and buds can be isolated (Bouillenne and Went 1933; Went 1938).

Cooper (1935, 1936) reported that auxin plus another factor synthesized in lemon leaves were necessary for root formation. Simular conclusion were drawn by Greogory and Van Overbeek (1945) and Van Overbeek and Gregory (1945). However in 1946, Van Overbeek et al., demonstrated that the main function of the Hibiscus leaf was a nutrional one, and that sugar and nitrogenous compounds can replace these compounds. No evidence of specific 'Rhizocaline' compound could be found.

Until 1957, the classical work of Hess firmly

established the role of co-factor in the process of rhizogenesis. Hess (1957) successfully isolated 4 'rooting cofactors' from Hedera helix as determined by their activity in the mung bean rooting bioassay. And the difference between the rootability of easy-and difficult-to-root varieties of Hedera helix, Hibiscus rosasinensis and Chrysanthemum, which could not be accounted by the level of endogenous promoters or inhibitors, can always be attributed to the level of the rooting cofactor present. Several other studies also agree with this (Hess 1957, 1961, 1962b, 1965, 1968; Gregory and Overbeek, 1945; Girouard and Hess 1966; Girouard, 1969; Hackett, 1969; Foong 1977). The relative activity of these cofactors is cofactor 4 (Rf 0.8)> cofactor 3 (Rf 0.6)>cofactor 1 (Rf 0.1)>cofactor 2 (Rf 0.3).

Some workers attributed rootability to the mobility of cofactor. Lee (1969) found that rooting cofactors vary quantitatively and qualitatively over the growth seasons but their presence and amounts in stems and leaves are always more extensive in the easier rooting clones than the difficult-rooting clones of Rhododendron. It has been obsevered that the leaves and stems of the easy to root variety have equivalent cofactor content whereas the leaves have higher cofactor content than the stems in the intermediate and difficult to root varieties, suggesting that rooting cofactors may be less mobilizable from leaves to stems in the latter.

Centrifugation promotes root formation in cuttings of Salix acutifolia Wild, Salix fragilis L, Populus alba L., and Populus canadensis Moech, as well as many woody ornamentals viz. Cotoneaster, Euonymus, holly, honeysuckle, sweet leaf, Viburnum and yew by the facilitated basipetal transport of rooting cofactors (Kawase, 1964, 1970, 1971).

Rooting cofactors have been demonstrated in

many plant species viz. apple and plum (Challenger, Lacey and Howard, 1964; Quamme and Nelson, 1965; Ashiru and Carlson, 1968), Pear (Fadl and Hartmann 1967b), Rhododendron (Lee 1969), Willow (Kawase 1964), Dahlia (Biran and Halevy, 1973), and Prunus pseudocerasus (Feucht and Dausend;1976).

But in some plant species, rooting cofactors have been identified but no correlation exists between rooting potential and the content of these rooting cofactors (Biran and Halevy, 1973; Taylor and Odom, 1970; Lipecki and Dennis, 1972; Tustin, 1976).

Lanphear and Meahl (1963) suggested that the cofactor content may reflect the rooting potential of the cuttings but other factors had a mediating effect on the formation of roots.

1.2.6 NUTRITION

The regeneration of adventitious roots involve active protein synthesis and its mediation through the multiplication of DNA or production of MRNA or both (Jain and Nanda, 1972; Overbeek, Gordon and Gregory, 1946; Cameron and Rook, 1974), further demonstrated that in Pinus rediata stem, during rooting the respiratory rate rises by 40% above that at the time of severence. Since root formation is such a metabollically active process, it therefore requires the supply of carbohydrate and nitrogenous materials for the synthesis of biological blocks (Thimann and Poutasse, 1941). Both the nutritional state of the cuttings and that of the stock plants affect subsequent adventitious root formation and enhances the ability of cuttings to respond to exogenous hormone treatment (Pearse,1943).

1.2.6.1 CARBOHYDRATE

Carbohydrate provides energy and as an important carbon source for the biosynthesis of some enzyme proteins that are required for the regeneration of roots (Greenwood and Greenwood, 1970; Nanda, Jain, 1972).

Early in 1918, Kraybill observed that tomato cuttings with yellow stems, high in carbohydrates but low in nitrogen, produced many roots but only feeble shoots, the reverse is true for those with greenish stem. Considerable rootable shoot apices of Picea mariana are characterized by high carbohydrate and protein contents as shown by histochemical means (Riding, 1976). A positive correlation has been established between starch content and the root number of Hydrangea macrophylla cuttings (Molnar and Lacroix, 1972) and total carbohydrate and rooting of Chrysenthenum (Stoltz, 1968).

The difference between the rootability of the easy-to-root and the difficult-to-root varieties in many plant species can be attributed to the difference of nutritional factors (Overbeek and Gregory, 1945, Stoltz, 1968). For Macadamia integrifolia, easy-to-root cultivars yield good rooting regardless of carbohydrate levels while shy-rooters are best rooted when carbohydrate levels are high in Winter. Unlike the above, Ali and Westmood (1968) reported that the rooting behaviour of three Pyrus spp. do not conform to any logical relationship to the carbohydrate and nitrogen fractions. Easy rootability of the juvenile forms is attributed to a specific factor which is reduced or absent in adult cuttings.

Treatments like exogenous application of sucrose, illumination, lengthened photoperiod, etiolation and girdling all lead to higher carbohydrate level in the cuttings, and were commonly employed to increase rooting.

Exogenous application of glucose promotes rooting in Salix tetrasperma (Nanda and jain, 1971), Rhododendron stem cuttings and bean epicotyls (Olieman, Van der Meer, Pierik and Roest, 1971), adult Hedera (Stoutemyer and Britt 1962). Sugar treatment improves rooting for hop cuttings rooted at low light intensity (Howard and Sykes 1956), similar evidence been reported in Sinapis alba and Raphanus sativus. These differences in response are presumably due to the variability in endogenous carbohydrate levels under difference light condition (Lovell, Cobb and Moore, 1971; Moore and Lovell, 1972; Lovell, Ilisley and Moore, 1972 and 1973; Moore, Cobb and Lovell, 1972).

Auxin treatment alone had no effect on rooting of leafless etiolated Populus nigra stem segments, but auxin plus glucose is promotory (Nanda, Jain and Malhotra, 1971). And the activity of hydrolytic enzymes was enhanced (Nanda and Jain, 1972).

Girdling and etiolation were usually applied to improve rooting by increasing the total sugars, starch, total carbohydrates and nitrogen content (Taylor and Odom, 1969; Stolta and Hess, 1966; Cameron and Rook, 1974).

Increasing day length of stock plants has been shown to correlate positively with root initiation an cuttings (Stoltz, 1968). Promotion of rooting by sucrose in the presence of auxin is implicated in stem segments of Populus nigra under varying period of dark treatment (Nanda, Purohit and Mahrotra, 1968).

Nanda and Anand (1970) have found that a low activity of hydrolytic enzymes is associated with poor rooting of Populus nigra cuttings.

1.2.6.2 NITROGEN

Since the process of root regeneration involves active protein synthesis (Jain and Nanda, 1972). The involvement of nitrogen is inevitable as demonstrated by Bala, Anand and Nanda, (1979), in Bryophyllum tubiflorum and Dalbergia sissco cuttings, total and soluable nitrogen increase during early root induction and decreased subsequently showing the degradation of proteins and the utilization of soluble nitrogen for the rhizogenic process.

Girdling and etiolation of Pecan softwood cuttings increase the nitrogen content and its rootability (Taylor and Odom, 1969 and 1970). Various nitrogenous compounds were found to improve rooting, Potassium nitrate and adenine (Thimann and Poutasse, 1941). Adenine sulphate (Menhenett, 1970). Doak (1940) also found 30 different organic and inorganic forms of nitrogen increase the rooting of Rhododendron cuttings.

There is some evidence that the effect of nitrogen may be mediated through a regulation of endogenous auxin levels. In Justica gendarussa Linn cuttings, the auxin content is higher in stems treated with normal nitrogen level (N) and lowest with highest nitrogen (3N) (sen and Basu, 1960). On the other hand, auxin treatments have also been shown to affect nitrogen metabolism (Strydom and Hartmann, 1960; Burzynski, 1975).

However, there is some conflicting evidence concerning the effect of nitrogen level in the stock plants on rooting behaviour of cuttings taken from them.

Pearse (1943,1946) and reported that stock plants with reduced nitrogen level root better than that from full nutrient plants. Contrary to this, Samish and Spiegel, (1957) however found that fertilization of grape

stock plants does not decrease rooting but instead increases it.

Ammonium sulphate adversely affects the rooting of apical fragments of mustard cuttings (Saxena, 1976). Asparagine and glutamic acid are inhibitory in rooting of Justicia gendarussa Linn. cuttings (Sen, Sen and Basu, 1965).

This wide diversity of response of plant species to nitrogenous treatment reported can be due to the different qualitative and quantitative nitrogen content of the cuttings concerned.

Instead of regarding the nitrogen level of soluble nitrogenous materials with respect to the carbohydrate level to be taken as a measure for the rooting potential of cuttings (Schrader, 1924; Sen and Basu, 1960; Sen, Sen and Basu, 1965; Hynn, 1968; Hartmann and Kester, 1975).

There are others who disputed the hypothesis of C/N ratio, and suggested that it should be the combination of an optimum level of certain (Qualitative) type of carbon and nitrogen substances that generate the active state of adventitious root formation (Samish and Spiegel, 1957; Haissig, 1974c).

1.2.6.3 OTHER NUTRIENTS

Zinc treatment of stock vineyards promotes the rooting of cuttings through an accumulation of tryptophane and thereby increases auxin synthesis (Samish and Spiegel, 1957).

Boron appears to be required for the

elongation phase of root formation. Root elongation is ultimatly dependent on cell division of meristematic cells and their subsequent enlargement. In the absence of boron these processes are arrested (Albert, 1975). Many workers reported the promotory effect of boron which interacts with auxin (Weiser 1959; Sen, Bose and Bose, 1959; Weiser and Blaney, 1960; Tichnor and Roberts, 1968; Coorts, 1969; Bojarczuk and Jankiewicz, 1975; Bojarczuk, 1975).

Calcium and magnesium depress rooting (Thimann and Poutasse, 1941; Cormack, 1965; Cormack and Lenay, 1966; Hartmann and Kester, 1975).

Vitamins such as vitamin A, vitamin B, thiamine, pyridoxine, pantothenic acid, ascorbic acid, riboflavine and niacin, are beneficial in the rooting of cuttings (Hemberg, 1953; Aung, 1972; Bojarczuk, 1975; Bojarczuk and Jankiewicz, 1975).

From experimental evidence, it seems that auxin stimulates the formation of roots by an interaction involving organic materials in the cuttings, particularly carbohydrate and nitrogenous materials. This interaction apparently controls the basic step pf morphological defferentiation at the cellular level. This rooting response is both a qualitative and quantitative one. Therefore nutrient materials are of importance in rooting not only in relation to their ratio with auxin, but also in terms of the amount of substrate present for the actual growth of roots. And that the most effective organic form of nutrients varies from one species of plant to another. (Nanda, Jain and Malhotra, 1971).

1.2.7 TYPE OF WOOD SELECTED FOR CUTTINGS

There is a wide range of material for cuttings

from the very succulent terminal shoots of current growth to large hardwood cuttings several years old. It is rather impossible to pin point any ideal cuttings for all plants, but generally what is true for certain species often can be extended to other related species.

In rooting cuttings taken from individual plants of a species which ordinarily is propagated by seed, experience has shown that wide differences may exist among individuals in the ease with which cuttings taken from them from roots. Differences in rooting ability of clones had been reported in several fruit trees (Hartmann and Hansen, 1955; Hartmann, Griggs and Hansen, 1963; Higdon and Westwood, 1963; Kender, 1965; Kester and Sartori, 1966; Sinha and Vyvyan, 1943). Similar phenomenon has been demonstrated in some ornamental plants (Childers, and Snyder 1957; Gregory and Overbeek, 1945; Hess, 1962b, 1963).

In white pine and Norway spruce, both with and without auxin treatments, gave higher percentages of rooted cuttings than did terminal shoots (Denber, 1940; Farrar and Grace, 1942). In pear, horizontally oriented medium size cuttings root better than large or small ones (Higdon and Westwood, 1963). Young soft cuttings from lateral shoots of Rhododendron root better than those from vigorous, strong terminal shoots (Pierik, 1969; Hartmann and Kester, 1975). Lateral shoot cuttings from the lower half of spruce root better than the terminal ones (Girouard, 1974). The youngest part of a shoot containing the apex and with wood not yet fully lignified show the best rooting potential (Bojarczuk and Jankiewicz, 1975).

1.3 ROOT CUTTINGS

Compared to stem cuttings, root cuttings are

retatively unpopular as a means of commercial plant propagation. In fact, root cuttings are suitable for a wide range of woody plant. (Stoutemyer, 1968). Wobst (1968) published a long list of plants that can be propagated by root cuttings. He considered that the majority of species of the following families would root well from root cutting: Rosaceae, Apocynaceae, Asclepiadaceae, Papaveraceae, Leguminosae, Bignoniaceae, Geraniaceae, Passifloraceae, Campanulaceae and Rubiaceae. There is a number of other extensive lists of plants successfully grown from root cuttings published by various horticultual authorities; Lindsay, (1877), Saul (1847); Stoutemyer (1968); Donovan (1976); Hartmann and Kester (1975); Pikem (1972).

Like stem cuttings, seasonal fluctuation in shoot forming capacity of root cutting is prominent. Some species regenerate readily from root cuttings at any time of the year, whereas cuttings of other species show a well-marked seasonal fluctuations in capacity to grow. In the latter a high proportion of success is possible only if root pieces are taken from young stock plants in late winter or early spring when the roots are well supplied with stored foods but before new growth starts, (Lek, 1934; Graham, 1936; Denber and Farrar, 1939; Hudson, 1955; Robinson and Schwabe, 1977; Hartmann and Kester, 1975).

Buds on roots of certain species are reported to possess a pre-formed link with the parent cambium (Kormanik and Brown, 1967; Schier, 1973). While others found spontaneous and random origin of adventitous buds in undifferentiated cortex tissue, (Siegler and Bowman, 1939; MacDamels, 1953; Baldini and Moose, 1956 and Robinson and Schwabe, 1977).

While planting root cuttings, it is always advisable to maintain the root polarity, with the proximal

end of the root piece facing upward. Usually, the proximal end produce shoot and the other produce roots only. When tissue segments are cut, the physiological unity is disturbed. This probably causes a redistribution of some substance, probably auxin, thus accounting for the different responses observed at previously adjacent surfaces. The correlation of polarity of shoot regeneration with auxin movement has been noted in several instances (Warmke, and Warmke, 1950).

Bud initiation on root pieces is stimulated by cytokinin (Danckwardt - Lillestrom, 1957; Robinson and Schwabe, 1977), whereas auxin inhibits shoot formation on root cuttings.

CHAPTER 2

RATIONALE OF THE PRESENT WORK

The Kiwi fruit or Chinese gooseberry, Actinida chinensis (Planch) may be propagated by various methods viz. grafting, stem cuttings, root cuttings, seedlings and tissue culture (Fletcher, 1976; Harada, 1975).

Budding or grafting desired cultivars on seedling rootstocks is the most common practice. Owing to rootstock variability and the longer time required to obtain planting stocks, many propagators desire to grow kiwi fruit from stem cuttings. However, as a fairly new commercial crop, so far limited work has been carried out on this plant. It is therefore felt necessary that an investigation of some of the propagation methods and problems for this crop will be adventageous and such information may be extrapolated to other plants.

CHAPTER 3

GENERAL MATERIALS AND METHODS

3.1 PLANT MATERIALS

All cuttings were taken from 8 years old Actinidia chinensis (Planch) grown at the Horticulture Research Centre, Levin, New Zealand. The plants were being pruned back twice a year to provide juvenile shoots for cutting wood, diameters of 0.5 - 1.0 cm. were used.

3.2 COLLECTION AND PREPARATION OF CUTTING MATERIALS

Vigorous adventitious shoots 0.5 - 1.0 cm. in diameter of current season's growth were selected for use as cutting material, collection always being carried out between 9.00 - 11.00 a.m.

3.3 TREATMENT AND PLANTING OF CUTTING

3.3.1 WOUNDING AND ROOTING CONDITION

Cuttings of one node length were made by trimming the basal end of the shoots to just above an axillary bud and the leaves were shortened back to facilitate handling. A slice of bark about 1 cm. long was removed from the base of the cutting at the side directly below the bud. The cuttings could then be treated with auxin, air dried and planted into coarse river washed sand. Basal temperature of 23°C ± 2°C was maintained by means of a thermostatically controlled electric cable, intermittent mist was provided too. Air temperature in the glasshouse was maintained at 22°C ± 2°C.

Cuttings remained under these conditions for 40 days, after which cuttings were lifted. The numbers of cuttings rooted and number of roots and weight of root per cuttings were recorded.

3.3.2 INDOLE BUTYRIC ACID TREATMENT

Various concentrations of IBA were prepared in 50% ethanol solution. Dipping was done by the 10 second quick-dip method recommented by DR. B.H. Howard (East Malling) and was done to a depth of 1 cm. This was achieved by having a petri dish with sides 1 cm. high within a large dish so that upon addition of fluid, the petri dish was completely filled and any excess solution, when added or when stocks were immersed, was displaced into the large dish.

Dipping dish apparatus

This apparatus ensured all stocks were dipped to the same depth. After dipping the stocks were stood in beaker and left to drain and dry.

For usual IBA powder treatment, this consisted of dipping the base of the cutting into a mixture of 0.8% IBA in talc. All excess powder should be shaken from the cuttings to prevent possible toxic effects. The cuttings was then planted immediately, taking care not to rub off the thin layer of adhering powder. To this end, a stick of 0.5 cm. thick is used to make a trench in the media before cuttings were inserted.

3.3.3 APPLICATION OF INDOLE-ACETIC-ACID

The application of IAA was done by application of lanolin impregnated with IAA. Unlike the water sprays, lanolin provides a fairly continuous supply of auxin to the treated plant part. Woolley (1971) and Patrick and Wareing(1974) showed that 0.1% IAA in lanolin maintained auxin characteristics in the top node of dwarf bean which were similar to those in intact plants, but suggested that lanolin caps should be replaced every 24 hours. IAA also appeared to be transported at a rate which fell within the range of reported velocities and so could be regarded as being normal.

Pastes were prepared containing 0.1% IAA. The required amount of IAA was dissolved in a minimum quantity of ethanol and warm, semi-molten lanolin (35 - 40^{o}C) was added until the correct proportion was obtained. The liquid mixture was stirred vigorously to ensure complete homogeneity and then smeared thinly around the wall of a beaker and left in the dark at room temperature over night for ethanol to evaporate. The paste was then dispensed via a disposable plastic syringe into small tinfoil capsule. The capsules were applied firmly to the cutting pieces and covered with the tin foil capsule.

The IAA-lanolin treatment was replaced every third day and 1mm slice removed from the top of each cutting each week, to ensure a continuing supply of IAA to the cutting tissue.

3.4 PLASTIC TENT

The use of plastic tents for propagation was a possible means of keeping the cost of production down.

A dome shaped tunnel plastic tent was

constructed using galvanized wire, with polythene film spread on it forming an airtight tunnel. Propagation beds were heated by electric cable. Air temperature in the tenp were around 22 ± 5°C. Humidity was maintained as high as 80-90%.

3.5 SAMPLING OF MATERIAL FOR HORMONE EXTRACTION

When the hormonal status of cutting material was to be determined, extra stem tissue was harvested and used as samples for extraction.

Tissue samples were frozen in dry ice immediately after collection and then freeze dried for 60 hours. Once dried, the tissue samples were pulverized to a fine powder and stored in sealed jars at - 15°C until required.

The stem tissues used for hormone extraction were taken from plant material considered suitable for use as cuttings. Buds were avoided in the collection.

3.6 EXTRACTION OF PLANT MATERIAL FOR HORMONE ANALYSIS

3.6.1 INITIAL EXTRACTION FOR AUXIN AND INHIBITORS

Determination of endogenous levels of auxins, Abscisic acid and rooting cofactors level were made in conjuction with field experiments.

By comparisions with freeze dried stem tissue, it was found that 5 grams of dried tissue were equivalent to 10 grams fresh weight.

Organic solvents for all purposes were redistilled.

5 gram samples of lyophilized freeze dried stem tissue were homogenized at 4°C with 100 ml of chilled 80% aqueous, redistilled methanol containing 0.02% sodium diethyldithiocarbamate, giving a 10:1 V/W ratio of solvent to plant fresh weight. The homogenate was kept in refrigeration at 1°C for 18 hours in the dark with continuous shaking. After 18 hours, the extract was filtered and the residue extracted twice more in 5 volume of 80% methanol for 4 hours each time, giving a final volume of 200 mls.

The combined filtrate was reduced to 25 mls of the aqueous phase, using a rotary evaporator (Buchii Rotavapor - R), at 25°C under vacuum and shielded from direct light. The aqueous phase was transferred to a 250 ml centrifuge tube and made up to 50 ml with successive rinsings of distilled water from the rotary evaporating flask. The aqueous residue was then placed in the refrigerator at 1°C for 12 hours to precipitate chlorophyll and lipid material, and was then centrifuged at 23,000 xg for 40 minutes at 1°C.

After centrifugation, the clean aqueous phase was adjusted to pH 2.5 with 50%HCL. This was then shaken vigorously with 3 separate, equal volumes of redistilled diethylether for 5 minutes each.

The ether fraction was then extracted twice with 50 ml volumes of 50% sodium bicarbonate (pH 8.5) by shaking for 5 minutes each. The ether fraction was retained (Neutral Ether Phase) for assaying for neutral auxins and inhibitors.

The bulked bicarbonate fraction (100 mls) was acidified to pH 2.5 with 50% HCL and extracted 3 times

by shaking with equal volumes of diethyl ether. The ether extracts were combined (Acidic Ether Phase) and retained for assaying for acidic auxins and abscissic acid. The remaining aqueous fraction was discarded.

The neutral and acidic ether fractions were dried over anhydrous sodium sulphate at 1°C for 2 hours, then filtered and evaporated to dryness in the rotary evaporator. The residue was dissolved in several mls of absolute methanol and transferred to a sample vial, dried under vacuum in darkness, capped and stored at -15°C until required.

A summary of extraction procedure is presented in Figure 3.6.1.

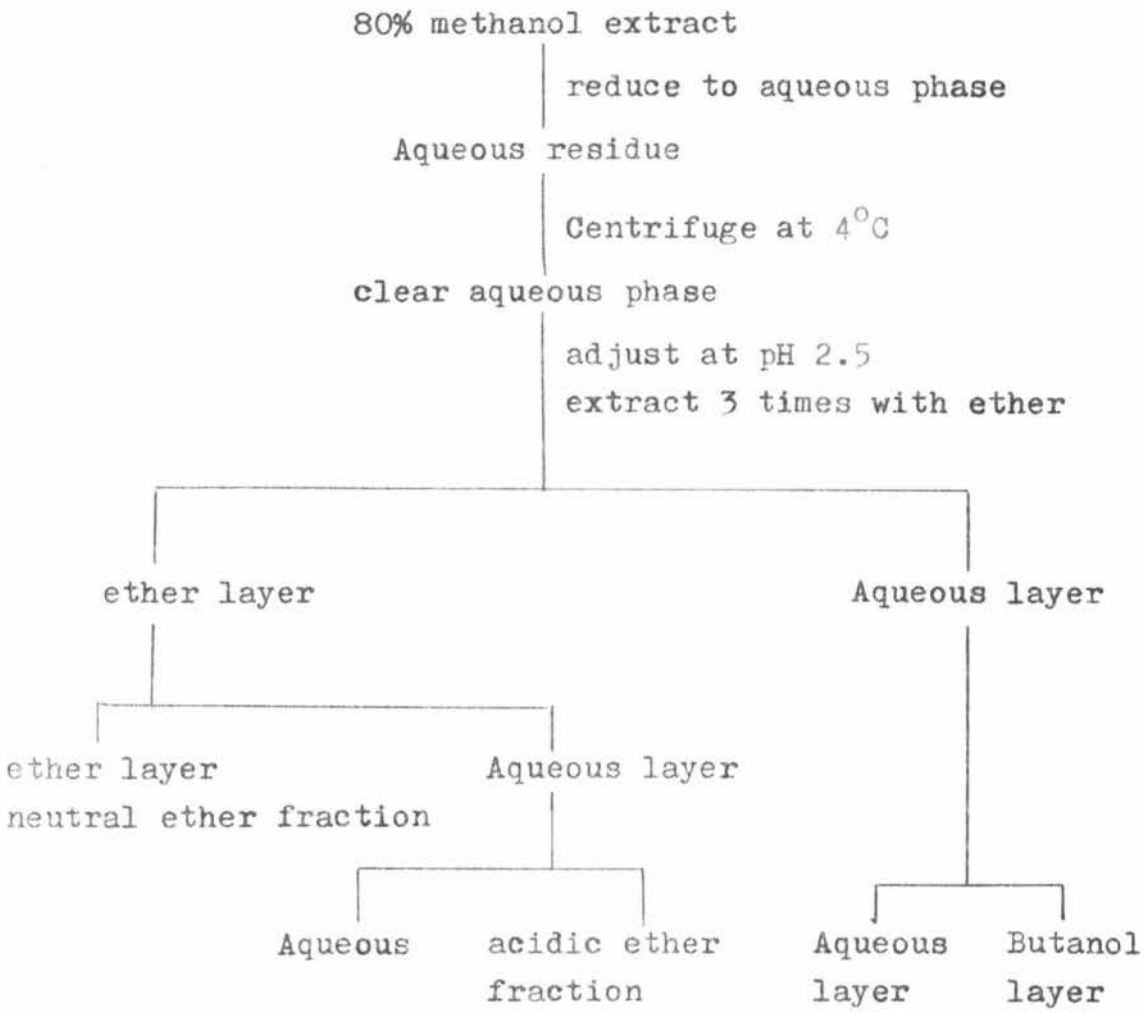


Fig. 3.6.1. Summary of hormone extraction procedure

3.6.2 EXTRACTION OF PLANT MATERIAL FOR ROOTING COFACTORS

0.5g of the lyophilized stem tissue was extracted with 3 changes of 50 ml chilled 80% redistilled methanol for 24 hours with constant agitation under 4°C. The combined extract was dried in rotary evaporator and taken up in 2 mls of methanol. There was no further purification undertaken, as rooting cofactors have been described as being phenolic in nature. Purification procedure used for other growth substance removed most of the phenolic content of the extract.

3.7 CHROMATOGRAPHY OF EXTRACTS FOR HORMONE ANALYSIS

3.7.1 PAPER CHROMATOGRAPHY OF AUXIN AND INHIBITORS

Further purification, immediately prior to bioassaying, was achieved by use of paper chromatagraphy of purified tissue samples. The residues of column chromatographed acidic and neutral ether fractions were dissolved in 0.5 ml acetone: methanol (1:1 V/V). further 0.25 ml was used to rinse the sample tube and was taken up in the same pipette. The samples were streak-loaded, 15 cm wide, on to pre-washed Whatmann No. 1 chromatography paper. All papers used for chromatography were pre-run in the developing solvent to be used. Marker spots of synthetic plant hormones relevant to the extracts were run concurrently with the developing chromatogram. The chromatograms were developed by descending chromatography using 10:1:1 V/V isopropanol:ammonia:water. Development was carried out in darkness at room temperature and the solvent allowed to run for approximately 20 cm. from the base line. When development was completed, the chromatograms were air dried for one hour then air dried under vacuum for a further 12 hours. The samples were now

ready for measurement of activity in the respective bioassays.

3.7.2 PAPER CHROMATOGRAPHY OF ROOTING COFACTORS

Extracts were chromatographed using the same method as for auxins and inhibitors.

Aqueous extracts were dissolved in 0.5 ml distilled water and the vials were rinsed in a further 0.25 ml of distilled water. The samples were steak loaded on to Whatmann No. 3 mm., prewashed, chromatography paper, in a band 15 cm. wide. Development of the chromatograms was achieved in the same manner as for auxins and inhibitors using the same solvent system.

3.8 BIOASSAY PROCEDURES

3.8.1 TRITICUM COLEOPTILE BIOASSAY

The procedure described by Nitsh and Nitsch (1956) was used.

Seed of the wheat variety "Aotea" was soaked in tap water for 2 hours and then placed on moist filter paper in plastic trays. Glass covers were placed over the trays and the wheat was germinated in the dark at 25°C. On the 3rd day after sowing, the coleoptiled were 2 to 3 cm. long and ready for use. 10mm. coleoptile sections, 3 mm. below the tip, were cut using a precision, double-bladed guillotine. All work was done in the dark using a green safe light. The cut coleoptiles were floated in distilled water until loaded into the bioassay vials.

The paper chromatograms were cut into 10 equal transverse strips, plus a control strip taken from above the base line. Each strip was lightly rolled and placed inside a 40 X 25mm. glass vial, so that the paper was in contact with the wall of the vial. 2 ml of phosphate citrate Buffer was added to each vial (see appendix I) 10 coleoptiles were introduced into each vial, under the green safe light and each vial was capped with a needle hole in the centre. The vials were then placed on a turntable, which rotated at approximately 1 r.p.m. for 20 hours. The vials were removed and 2 ml of 10% methanol were added to each vial to kill the coleoptiles. The coleoptiles were then measured on a photographic enlarger at 3X magnification.

With each bioassay, a standard series of ABA solutions from 0.001 mg/ml to 10 mg/ml in a log. dilution series, was run concurrently with strips of washed chromatography paper included with each standard vial to compensate for any effect of the paper on the bioassay. A typical standard curve is shown in figure 3.8.1.

3.8.2 AVENA COLEOPTILE BIOASSAY

The procedure described by Tustin (1976) was used.

Seeds of the oat cultivar "Brighton" were soaked for 15 hours in 0.1% hydrogen peroxide. They were then washed and spread evenly on moist filter paper in a plastic tray. A glass cover was placed over the tray, which was then placed in the dark room under red light for 24 hours at 25°C. The red light was turned off and the seed left for a further 2 days until the coleoptiles were 2 to 3 cm. long and ready to use. 5 mm. sections were cut 3 mm. below the coleoptile tip, using a precision double

bladed guillotine. The coleoptile sections were soaked in Burstrom's basal solution (see appendix I) for 1 hour prior to use. (Burstrom, 1973)

FIGURE 3.8.1

STANDARD CURVE FOR WHEAT COLEOPTILE RESPONSE TO ABA

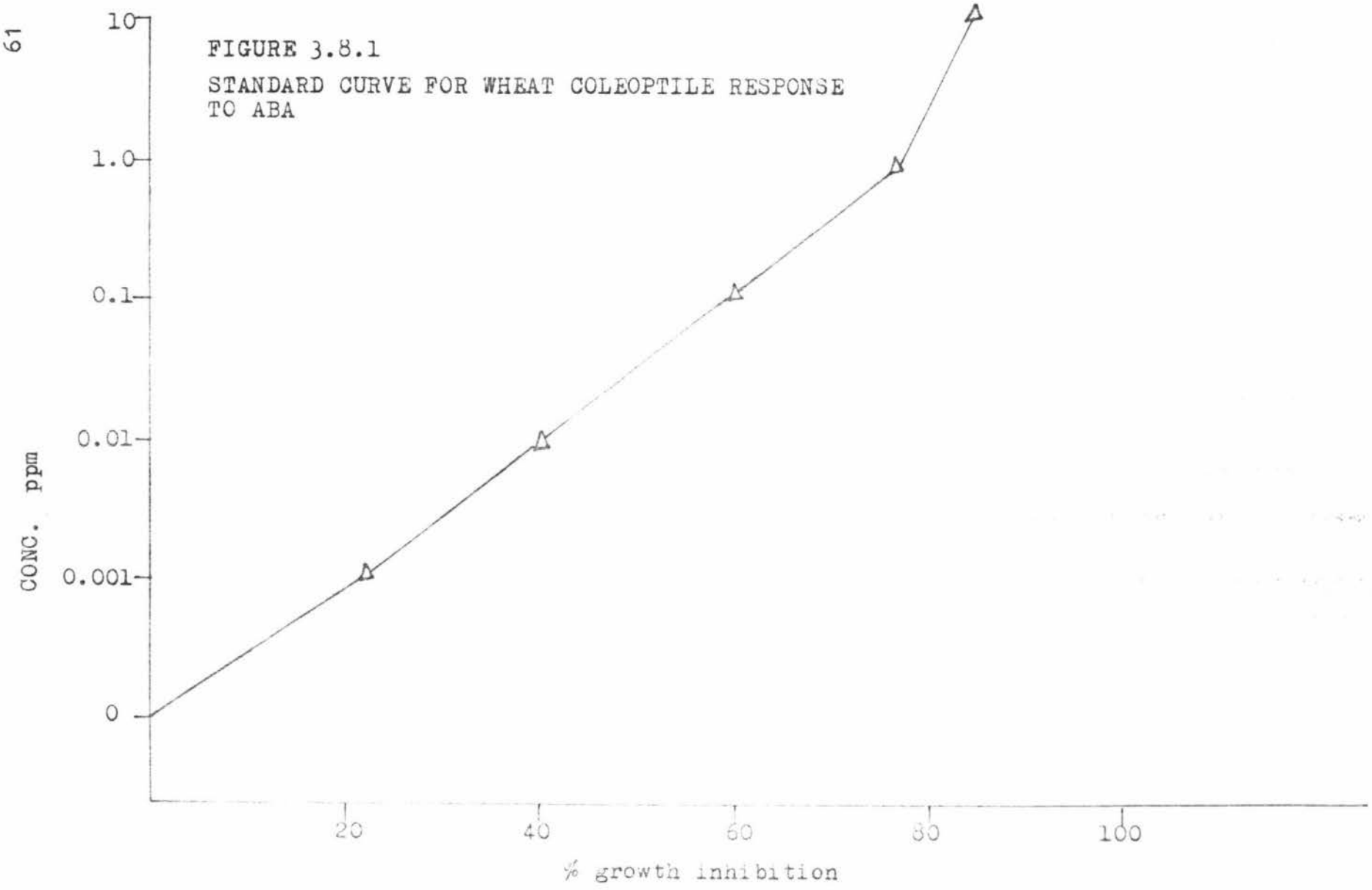


FIGURE 3.8.2

STANDARD CURVE FOR OAT COLEOPTILE RESPONSE TO IAA

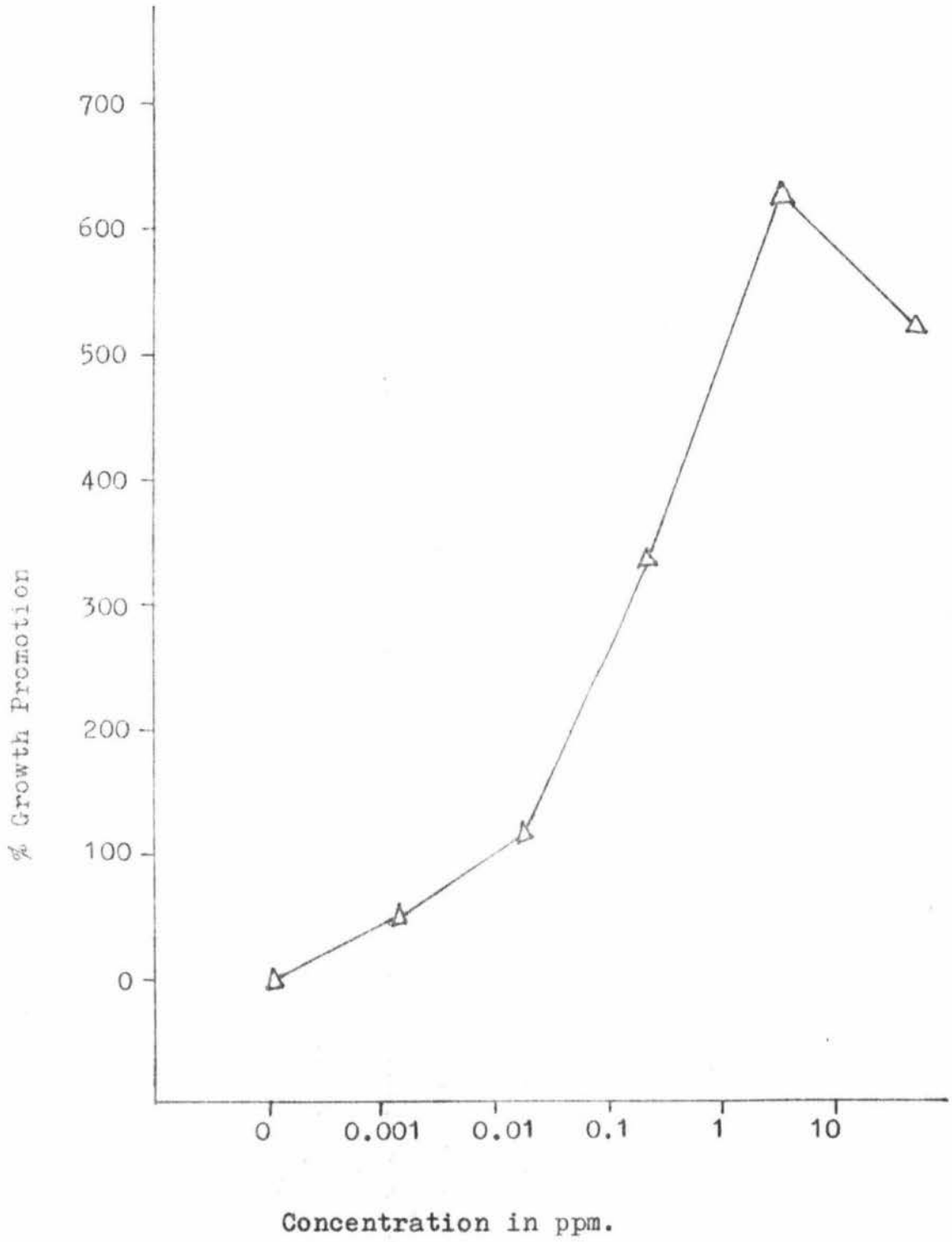
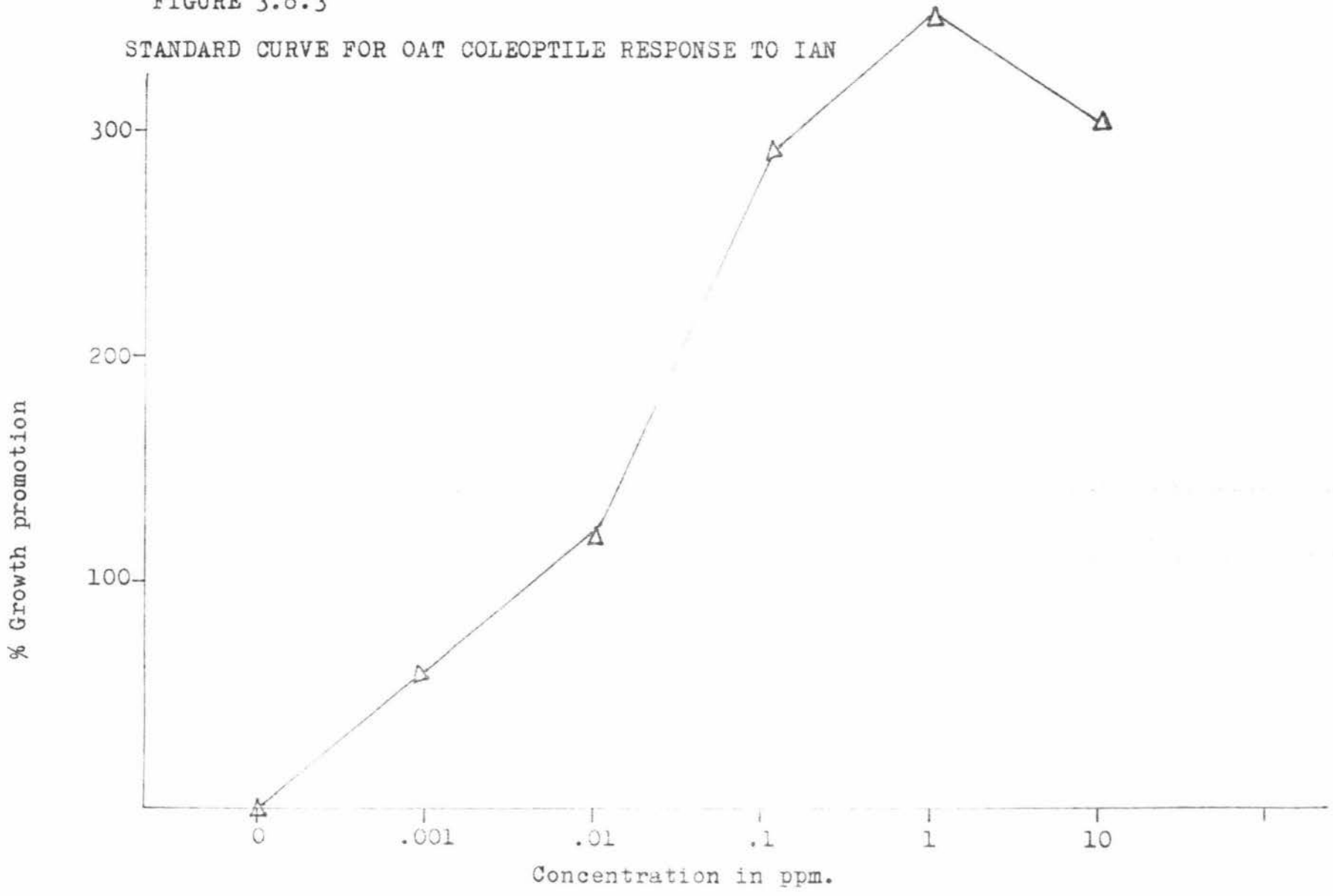


FIGURE 3.8.3

STANDARD CURVE FOR OAT COLEOPTILE RESPONSE TO IAN



The bioassay procedure was the same as described in the Triticum coleoptile bioassay. But Burstroms basal solution was used instead of Phosphate-citrate Buffer.

Standards of IAA and IAN was run as for ABA. Standard curves for IAA and IAN responses are shown in figure 3.8.2 and 3.8.3.

3.8.3 MUNG BEAN ROOT INITIATION BIOASSAY

A bioassay for endogenous substances which promote rooting in cutting was developed by Hess (1957). A criticism of this standard mung bean bioassay is the variable response among individual cuttings. Bassuk (1975) suggested removing the cotyledons at an early stage, or the leaves at a later stage of cutting preparation thereby removing the masking effects of rooting factors already present in the cutting, thus to reduce the associated variance.

Preliminary experiments now seem to support Bassuk's (1975) suggestion. The effects upon rooting variability of removing cotyledons and leaves were shown in Table 3.8.1. Standard deviations diminished as cotyledons or leaves were removed.

Seeds of Mung bean, (Phaseolus aureus) were sieved to grade out large and small seeds, retaining the middle grade for use in the bioassay.

30 cm^3 of "Taiwan No. 1" seed were washed in 1:16 (V:V) Janola:water solution for 5 minutes then soaked in running tap for 24 hours. The seeds were sown in damp pumice, in 2 trays, each 30 cm. X 46 cm. X 7.6 cm. The trays were placed in a bioassay cabinet under artifical

light and maintained at 27°C and a relative humidity of 80 -90%. The light was derived from a mixture of 3 white fluorescent tubes and two 60 watt light bulbs to give a light intensity approximately 800 feet candles at plant level.

After 2 days uniform seedlings were selected for bioassay. Uniformity was judged mainly on leaf area which had been shown to affect adventitious root formation (Overbeek, Gordon and Gregory, 1946; Girouard and Hess, 1964; Altman, 1972; Altman and Wareing, 1975). Cuttings were harvested at ground level, cotyledons removed and leached in distilled water for 6 hours (Zabkiewicz and Steels, 1974) to remove any exudate that was emitted from the cut base of the cuttings.

Chromatograms of stem extracts were cut into 10 equal transverse strips plus a control strip taken above the base line. Each strip was rolled and placed in a dark brown 40 X 20 mm. glass vial with 6 ml. of 1 mg/l IAA solution.

Cuttings of similar size and vigour were selected and cut 3 cm. below the cotyledonary node. Cotyledons were removed and 10 cuttings were added to each vial and the vials placed in the high humidity cabinet.

The test solutions were taken up in 24 hours, at which time the vials were filled to a level equal to the cotyledonary nodes of the cuttings with distilled water. Distilled water was maintained at this level until the root on the cuttings had developed sufficiently to allow counting. This took 4 days from the time the cuttings were placed in the test solutions.

Effects were recorded by counting the number of roots initiated on each cutting.

3.9 ROOT CUTTING

At Massey University, Vines raised originally from rooted cuttings were established in an experimental orchard during 1966/1967 and these trees were capable of yielding an adequate supply of roots for experimental purposes.

Abbott vines of 5 years age were used. Roots, from 0.5 to 1.5 cm. in diameter were excavated to a depth of 0.5 m before removal. To avoid planting the cuttings upside down, the proximal end was made with a straight cut and the distal end with a slanting cut, And for cuttings that were planted upright, the proximal end of the root pieces should always be up. For cuttings that were inserted horizontally, they were cut into 15 cm. lengths end laid horizontally 1 cm. into the sand, watered to maintain a moist root surface without water logging.

The cuttings were left under intermittent mist and basal heat same as that for stem cuttings. After 60 days the root pieces were lifted and number of roots forming shoots, and number of shoots per root cutting were recorded.

CHAPTER 4

PROPAGATION OF ACTINIDIA CHINENSIS (PLANCH.) BY STEM CUTTING

4.1 THE EFFECT OF WOUNDING ON ROOTING A. CHINENSIS (PLANCH.)

4.1.1 INTRODUCTION :

In a number of plant species, root production on the stem cuttings may be promoted by wounding the base of the cutting. Cuttings of Juniper, arborvitae, Rhododendron, maple, magnolia and holly species are reported to be helped by basal wounding (Hartmann and Kester 1975).

4.1.2 METHODS

160 cuttings of Hayward were collected on 5th of May 1977, and subjected to the following treatments:

Treatment 1	+ wound	no IBA
Treatment 2	+ wound	+ IBA
Treatment 3	- wound	- IBA
Treatment 4	- wound	- IBA

There were 40 cuttings for each treatment subdivided into 4 replicates of 10 cuttings for each treatment.

IBA used was 0.8% in talc. Details of the method is described in Section 3.1, 3.2 and 3.3. (Materials and Methods).

4.1.3 RESULTS

Results are presented in Table (4.1.1) and the details of the analysis of variance is shown in appendix 2.

Table 4.1.1

THE PERCENTAGE OF ROOTING AND CALLUSING OF HAYWARD CUTTINGS TREATED WITH IBA AND WOUNDING

Treatment	% rooting		% Callused	
+ wound - IBA	15	c	47.5	b
- wound - IBA	0	d	52.5	b
+ wound + IBA	62.5	a	60	a
- wound + IBA	25	b	65	a

Treatment denoted by the same letter are not significantly different when analysed by Duncan's Multiple Range Test ($P < 0.05$).

The results indicate that both wounding and IBA improve rooting significantly ($P < 0.01$). For the greatest benefit, cuttings should be wounded and treated with 0.8% IBA in talc, though the data do not show any significant interaction between IBA and wounding.

There is a significant difference in percentage callusing of cuttings treated with IBA and those without. But no significant distinction between wounding and no wounding.

Cuttings treated without wounding or IBA do not root at all, although it callused considerably. Wounding alone improves rooting by 15%.

4.1.4 CONCLUSION

It was found that a lengthwise cut through the bark at the base of cuttings will stimulate rooting of Hayward cuttings. Following wounding, callus production and root development frequently are much heavier along the margins of the wounds, thus expanding the region where the roots originate. Evidently in such cases wounded cells, or adjacent ones near the base of the cutting, are stimulated into cell division and production of root primordia. This is due, perhaps, to a natural accummlation of hormones and carbohydrates in the wounded area and an increase in respiration (Hartmann and Kester, 1975). The other commonly held view is that increased uptake of growth substance and water was achieved through a basal wound (Day, 1933). This hypothesis is evidenced in this experiment by the fact that wounded cuttings that are treated with IBA perform better than those without.

In stem tissues of some species, there is a ring of tough sclerenchyma cells in the cortex external to the point of the origin of adventitious roots. Roots may have difficulty in penetrating this band of cells (Beakbane, 1961; Ciampi and Gellini, 1958). Wounding per se was also investigated by Howard (1968a). Wounding was found to give a big stimulus to rooting similar to disbudding and also a combination of the two, on both Myrobalan plum and M26 apple rootstock cuttings.

Recently some workers have detected certain high molecular weight phenolic compound, which they called 'Auxin protector' (Literature Review) Pr - I (Molecular weight 8000g/mole) and A - II (molecular weight 2000g/mole) were found to be abundant in wounded tissues, juvenile tissues and regenerating tissues of stem cuttings in Japanese morning glory (Stonier and Yoneda, 1967; Yoneda and Stonier, 1967). Wounding have postulated to be

responsible for bringing enzymes and modifiers to the site of IAA oxidation (Stonier, Hudek, Vande - Stoume and Yang, 1970; Stonier and Yang, 1973).

Wounding has been used traditionally in propagation especially by hardwood cuttings, but the reasons for doing so are probably not fully understood yet.

4.2 VARIETAL AND SEXUAL DIFFERENCES IN THE PROPAGATION OF ACTINIDIA CHINENSIS (PLANCH.)

4.2.1 INTRODUCTION

Investigation has found that certain inherent characters of plants influence their rootability. Among the characters are inherited clonal variation and sexual differences.

There have been extensive reports that differences exist between varieties and between species of a general in respect to their ability to form roots on stem cuttings. This has been especially true in fruit trees (Hartmann and Hansen, 1955; Hartmann, Griggs and Hansen, 1963; Higdon and Westwood, 1963; Kender, 1965; Kester and Sartori, 1966; Sinha and Vyvyan, 1943; Tustin, 1976) where a wide range of clones may exist in a particular species, but also been demonstrated in some ornamental plants (Childers and snyder, 1957; Gregory and Overbeek, 1945; Hess, 1962b; 1963).

The influence of sex in the propagation of plants by cuttings has received very little attention (Snow, 1942; Neal and Pease, 1954; Edgerton, 1944).

In Actinidia chinensis (Planch.) so far there is no substantial report about any varietal or sexual

differences concerning rooting ability. Fletcher (1976) commented that Abbott is prone to excessive callusing and therefore more difficult to raise satisfactorily from cuttings than other varieties such as Hayward and Bruno.

The following experiment was carried out to test substantiate Fletcher's comment.

4.2.2 METHOD

60 cuttings of each Hayward , Abbott and Matua were collected on two harvest dates 5/5 and 25/1. The cuttings were prepared and wounded at the base as described in Chapter 3. All the cuttings were then treated with 0.8% IBA in talc, and arranged in randomised block design of 6 blocks of ten. After 40 days, the cuttings were lifted and percentage rooting recorded.

4.2.3 RESULTS

The results were listed in Table 4.2.1, and the analysis of variance presented in Appendix 2.

	% rooting	
Varieties	5/5/77	25/1/78
Hayward	51.7	90
Abbott	56.7	85
Matva	51.7	85

Table 4.2.1 THE PERCENTAGE ROOTING OF HAYWARD, ABBOTT AND MATUA PROPAGATED UNDER STANDARD CONDITIONS

There was no significance difference in the percentage rooting between the three varieties at either harvesting date.

4.2.4 DISCUSSION

Results of the cuttings experiment fail to show any varietal difference between the three varieties - Hayward, Abbott and Matua for their ability to develop roots in the stem cuttings. Thus the data failed to confirm the comment of Fletcher (1976). However, it is observed that Abbott does form considerably more massive callus than the other 2 varieties especially on the 25/1/78 harvest.

The influence of sex on the propagation of plants by cutting had been reported in several plant varieties. Snow (1942) reported that cuttings selected from male trees of the red maple rooted better than female ones. Neal and pease (1954) found that in Ilex verticellata male cuttings perform better than comparable female cuttings. Edgerton (1944) and Neal and Pease (1954) evidenced that the difference in the rooting of the sexes was related to carbohydrate content of the cuttings. They suggested that the cutting from female plant might have a lower carbohydrate supply because it was used in fruit production. However, it seems that there is no such sexual differences in Actinidia chinensis (Planch), as far as present data show.

4.3 PROPAGATION OF LEAFY CUTTING UNDER POLYTHENE - TENT

4.3.1 INTRODUCTION

The methods of rooting leafy cuttings and

grafts under polythene are well known and widely practised by nurserymen (Dewerth, 1963; Warner, 1963). It is a much cheaper set up than the mist propagation bed.

The experiment below is to investigate the feasibility pf using a polythene tent in propagating leafy cuttings of Actinidia chinensis (Planch.)

4.3.2 METHODS

Hayward stem cuttings of 1 node length were collected on 26th January 1978. The cuttings were prepared and wounded at the base as described in Chapter 3, Materials and Method these cuttings were subjected to the following treatments : -

Treatment 1	0.8% IBA	under mist
Treatment 2	No IBA	under mist
Treatment 3	0.8% IBA	under polythene
Treatment 4	No IBA	under polythene

There were 80 cuttings in each treatment, divided into 8 blocks of 10.

The cuttings were watered heavily when embedded into sand, with subsequent watering being carried out once every week. Air temperature around the cuttings was recorded at a weekly intervals during mid-noon, and the mean air temperature was recorded.

After 40 days, the cuttings were lifted and the following data collected: -

1) % rooting
2) % leaf rot
3) % leaf burn

4) mean air temperature

4.3.3 RESULTS

The results are shown in Table 4.3.1.

Table 4.3.1 RESULTS FOR LEAFY HAYWARD CUTTING PROPAGATED UNDER MIST OR POLYTHENE TENT:

	% rooting		% leaf rot	% leaf burn	mean air temp.
+IBA + mist	88	a	0	3.8	23°C
-IBA + mist	2.5	c	0	2.5	23°C
+IBA + poly.tent	67.5	b	8.8	0	27°C
-IBA + poly.tent	2.5	d	6.3	0	27°C

Treatments denoted by the same letter are not significantly different when analysed by Duncan's Multiple Range Test. Details of the Analysis of Variance is in Appendix 2.

There was a very significant difference ($P < 0.01$) in the percentage rooting between IBA and no IBA treatment. There is no significant superiority for mist over polythene tent. However, a positive synergistic interaction between IBA and mist was detected.

The mean air temperature in the polythene tent is about 4°C higher than that on the mist bench. Cuttings under mist had a higher % leaf burn because the mist did not reach the edge of the experimental block, and due to shading effects of the leaves, resulting in some of the leaves becoming dried and burnt. There was no leaf burn on cuttings under the polythene tent, instead a low

percentage of the leaf became mouldy and rotted. The rot was due to Botrytis.

4.3.4 DISCUSSION

There was no significance difference between mist and polythene treatment. However, a highly significance ($P < 0.01$) interaction was evidenced between misting and IBA application. This indicates that mist probably provides a better condition for auxin activity which in turn stimulates rooting.

The air temperarure around the leaf of cuttings under mist was found to be $4^{o}C$ lower than that of polythene tent. Mist can maintain a film of water on the leaves resulting in a pretty constant high water vapour pressure surrounding the leaf, and lowering the leaf temperature, all these tend to decrease the transpiration (Hartmann and Kester, 1975). Whereas in a polythene tent system, the rate of transpiration depends on humidification rather than mist. Thus the rate of transpiration varies with the relative humidity around the leaf and increases or decreases the water vapour pressure around the leaf. Since the leaf had been known to be an active hormone producer (Zimmerman and Hitchcock, 1933; Altman, 1972; Bilan, 1974; Ohta and Furukawa, 1975; Porlingis and Therios, 1976), source of rhizocaline (Cooper, 1936, 1938; Went and Thimann, 1937; Went, 1938; Hartmann and Kester, 1975), supplier of thiamine (Bonner, 1942) and pyridoxine (Bonner and Dorlund, 1943) necessary for root formation, therefore, it is likely that any change in leaf temperature, vapour pressure, transpiration rate due to mist or polythene tent would in some way affect the buds of some of these growth regulators thus reducing or increasing the rooting potentiality.

Another alternative explanation for this

mist-auxin interaction was put forward by Lee and Tukey, (1971). During the propagation of Euonymus alatus 'conpactus' dormant cuttings under intermittent mist, these author found increases in substances such as carbohydrates, phenolics, flavonoid compounds, auxin-like substance and rooting cofactors, presumably due to the leaching of certain inhibitory substances present.

Schultz (1963) reported that in the polythene tent system, excessive condensation may result in water vapour dripping on to the plants below and causing adverse effects to the plant such as defoliation or a higher tendency to rot. In the present experiment, rotting under polythene tent was 7.5% in contrast to 0% under mist. On the other hand, about 3.2% of the cuttings got leaf burn under mist due to edge effects of the mist bench, and shading effects of the leaves. Therefore, the problem of leaf rot under polythene was not a major draw back for advocating the use of this system, rather it was the beneficial of mist - IBA interaction that should be capitalized on, and more resesrch should be looking into this area.

4.4.1 THE INFLUENCE OF IBA AND BASAL TEMPERATURE ON THE ROOTING OF ACTINIDIA CHINENSIS (PLANCH.)

4.4.1.1 INTRODUCTION

The benefits are derived from treating cuttings with a synthetic root-promoting substance (Hatcher and Garner, 1950) and of providing a low level of basal temperature (Hatcher and Garner, 1957) have been shown. Howard (1968b) found that increasing the concentration of IBA to an optimum will improve rooting in terms of percentage rooted cuttings, number of roots per cutting and dry weight of root system. Increasing temperature of

the rooting medium to an optimum was found (Howard, 1968b) to increase percentage rooting and root number.

This experiment is to test the significance of such practice in the case of Actinidia chinensis.

4.4.1.2 METHODS

128 cuttings of 1 node length were obtained from 8 years old Hayward variety to provide 8 replicates of 4 cuttings in each of the 4 treatments.

Treatment 1	+ IBA	+ bottom heat
Treatment 2	+ IBA	- bottom heat
Treatment 3	- IBA	+ bottom heat
Treatment 4	- IBA	- bottom heat

These were planted in May, August and January, 1977 - 1978.

IBA was applied to the basal 1 cm. of the cuttings by dipping into 0.8% IBA in talc, and bottom heat was supplied to the rooting media by thermostatically controlled low voltage heating wires. A thermometer was inserted in the rooting medium to the level of the base of the cutting and checked at an weekly intervals, ensuring the basal temperature is maintained at $22^{o}C \pm 2^{o}$.

4.4.1.3 RESULTS SHOWING THE EFFECT OF BOTTOM HEAT AND IBA ON HAYWARD STEM CUTTING

(1) 5/5 Table 4.4.1.1

Treatments	% rooting	% callusing	% rotted
+ Heat + IBA	50 a	75	28.3
+ Heat - IBA	2.5 c	90	7
- Heat + IBA	22.5 b	98	10
- Heat - IBA	0 d	91	1

(2) 9/8 Table 4.4.1.2

Treatments	% rooting	% rotted
+ Heat + IBA	0	80
+ Heat - IBA	0	46
- Heat + IBA	0	72.5
- Heat - IBA	0	20.7

(3) 26/1 Table 4.4.1.3

Treatments	% rooting	% rotted
+ Heat + IBA	90 a	14
+ Heat - IBA	2.5 c	11.7
- Heat + IBA	42.5 b	25
- Heat - IBA	0	2.2

4.4.1.3 RESULTS

Without IBA treatment few cuttings rooted, but a significant response was obtained at 0.8% IBA, except in August.

Both IBA and bottom heat contributed significant ($P < 0.001$) (Appendix 2) improvement in rooting. There was also a strong synergistic effect between IBA and bottom heat.

Basal rot was highest when conditions were not favourable for rapid callus and root development. This occured predominantly in August.

The percentage of cuttings with rots was increased by IBA and basal temperature.

In August hardwood cutting, both IBA and basal heat did not improve rooting, and rather more severe rotting resulted.

The response of the cuttings to heat and IBA varies according to time of harvest. In May + Heat + IBA gives 50% improvement over -Heat -IBA, In Aug. 0% improvement, in Jan. 90% improvement.

4.4.2 THE EFFECT OF VARIOUS CONCENTRATION OF IBA ON ROOT REGENERATION OF ACTINIDIA CHINENSIS (PLANCH.) STEM CUTTING

4.4.2.1 INTRODUCTION

The use of IBA is important and widespread in propagation. It is therefore essential to define critically the optimum levels of IBA in terms of commercial propagation. An investigation in this issue would hopfully enable improvement of existing treatments using exogenous rooting promoters.

4.4.2.2 METHODS

Abbott cuttings of 1 node length were prepared and wounded at the base. Solutions of IBA at various concentrations were prepared in 50% ethanol solutions. Cuttings were treated accordingly, and planted on a heated bed under intermittent mist in the glass house.

The range of IBA treatments were 5 seconds quick dip in 5000 ppm, 2500 ppm, 1000 ppm, 500 ppm, 0 ppm (50% ethanol), 0.8% IBA in talc; 24 hours soak in 100 ppm and 24 hours soak in 0 ppm (50% ethanol).

Each IBA concentration treatment was replicated 8 times with cuttings selected randomly from at least 6 different plants. The cuttings were arranged in randomized blocks, 4 cuttings for each replicate block.

Rooting was assessed 40 days after planting. The percentage of cuttings rooted and the number of roots per cuttings were recorded.

The experiment was repeated in other seasons. May, Nov. , Aug. and Jan., with slight alteration in the IBA concentration.

4.4.2.3 RESULTS

Without IBA treatment, there was no rooting at all, but a significant ($P < 0.01$) response was obtained at 100 ppm in May, November and January. Higher concentration of IBA gave higher percentage strike and increasing number of roots per cutting/or increasing mean weight of roots per cuttings, except in Winter.

Long periods (24 hours) of soaking the cuttings in dilute (100 ppm) IBA solution did not improve

rooting in May.

In August the cuttings do not respond to IBA at all even at 2% IBA treatment.

Table 4.4.2.1 Tables showing the effect of different IBA concentration on the rooting of Abbott stem cuttings

(a) 5th of May

IBA conc.	% rooted		no. roots/cutting	
5000 ppm	65.6	a	8.47	a
2500 ppm	50	b	6.6	b
1000 ppm	18.75	e	3.5	c
500 ppm	12.5	d	0.25	e
0.8% IBA	37.5	c	2	d
24 hrs. soak in 100 ppm	0	f	0	f
24 hrs. soak in 50% Ethanol	0	f	0	f
Ethanol	0	f	0	f

(b) 9th of August

IBA conc.	% rooted
0	0
0.8%	0
2%	0

FIGURE 4.4.2.1

PERCENTAGE ROOTING OF ABBOTT CUTTING

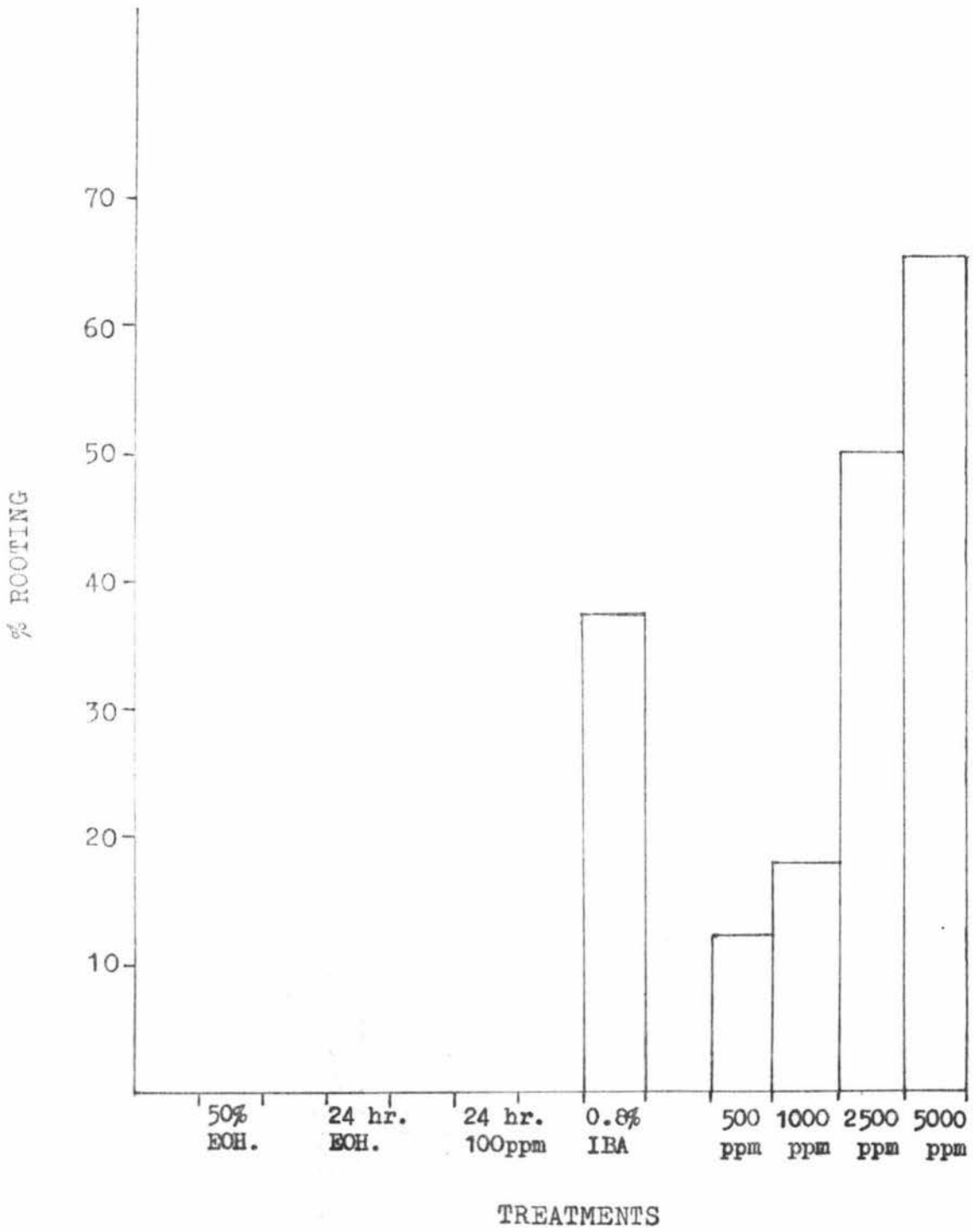
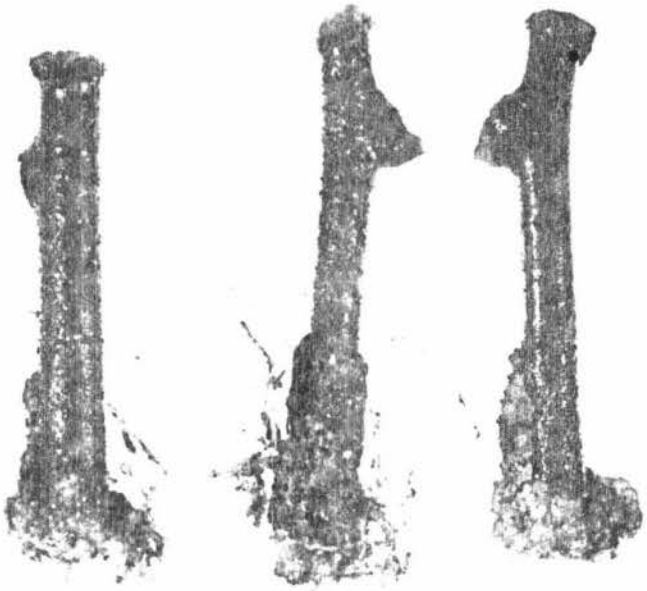


FIGURE 4.4.2.2

THE EFFECT OF VARIOUS CONCENTRATION OF IAA ON ROOT REGENERATION OF ACTINIDIA CHINENSIS



IBA 1000 PPM

IBA 2500 PPM

IBA 5000 PPM

SERADIX NO 3

IBA 100 PPM
20 HRS SOAK

CONTROL
20 HRS SOAK

(c) 25th of November

Treatment	% rooting		mean wt.of root/ cutting
0	0	b	-
0.5%	38.9	a	0.45 g
0.8%	66.7	a	1.62 g

(d) 25th of January

IBA	% rooting		roots mean wt./ cutting
50% Ethanol	0	d	0
0.8% IBA	90	b	2.232
1000 ppm	74	c	0.766
5000 ppm	96.3	a	3.413

Non-rooted cuttings, were included in the calculation of the average number of roots per cutting and average weight of roots per cutting for purpose of statistical analysis, which was by Analysis of variance and Duncan Multiple Range Test.

Treatments denoted by the same letter are not significantly different when analysed by Dubcan's Multiple Range Test.

4.4.2.4 DISCUSSION

While the effect of wounding or root intiation was elucidated, another critical treatment is high temperature storage of the bases of cuttings.

It is noted that by maintaining a basal temperature of 22°C during storage, after an IBA treatment, cuttings root much more readily. The effect is most noticeable on material collected in the Summer (25/1/78). Hatcher and Garner (1956); Hartmann (1955) also noted that by providing a bottom heat (45°C - 50°C), after an IBA treatment, provided the rooting of cuttings.

A temperature range of 21°C - 27°C is sufficent for the rooting of most cuttings (Esper and Roof, 1931; Hartmann, 1946; Howard, 1965; Pierik, 1969; Bhella and Roberts, 1974; Hartmann and Kester, 1975).

Although basal heat improves the rooting of cuttings through the enhanced utilization of Carbohydrate, its extensive use may result in reduced establidhment (Cheffins, 1975). A high temperature of 25°C has been found to increase the rooting of hardy hybrid Rhododendron cultivars but rooting was prominent (Whalley and Loach, 1976). A similar phenomenon has been observed in this experiment. Bottom heat and IBA improve rooting of Hayward cuttings, but also increase percentage rot, especially in late Winter when conditions are most favourable for rapid callus and root development. It is likely that IBA is not the primary cause for basal rot, but the activated tissue following treatment is more susceptible to break down.

The present results confirmed (Howard 1966, 1968b, 1971) that treatment of cutting with high bottom heat promotes root initiation. But from the data especially the August harvest, it seems that the degree of promotion by IBA and bottom heat treatment is limited by some factors, which shall be investigated later.

Increasing concentration of IBA treatment, is found to be beneficial to rooting. Concentration up to 5000 ppm result in 96.3% rooting in Summer (25/1/78). In comparision the commercial usage of 0.8% IBA in talc

gave 90% rooting, which can be considered as near optimum. This improvement of rooting percentage with increasing IBA concentration will not be a infinite trend, there will be a stage when the super-optimum level is reach and toxicity occur as been reported by Tustin (1976).

The response of the cuttings to auxin treatments seems to vary quite considerably with the date of harvesting. Response is best in Summer and Spring, but drops dramatically in Winter. This phenomenon will be further reported on in later Chapters.

4.5 SEASONAL CHANGES IN ROOT INITIATION OF *ACTINIDIA CHINENSIS* (PLANCH.)

4.5.1 INTRODUCTION AND METHODS

It has been well documented that cuttings from most species root better at certain times of the year than at others. To enable the study of seasonal changes in the ability of *Actinidia chinensis* (Planch.) cuttings to form adventitious roots, juvenile plant material was used (section 3.1, Materials and Methods).

Cuttings of Abbott and Hayward varieties were harvested throughout the year in 1977-78. Cuttings consisted of 1 node each, wounded at the base (section 3.3, Materials and Methods) and treated by dipping in 0.8% IBA in talc. 6 replicates of 10 cuttings each, of each variety were planted in the heated bed (Section 3.3, Materials and Methods). Another 6 blocks of 10 cuttings of Hayward and Abbott were treated in a similar fashion, except that they were not treated with hormone.

Cuttings were planted in washed river sand, on

a heated bench for 40 days and then lifted carefully and the percentage of rooting recorded. Each trial was terminated at this point and unrooted cuttings were discarded.

Table 4.5.1

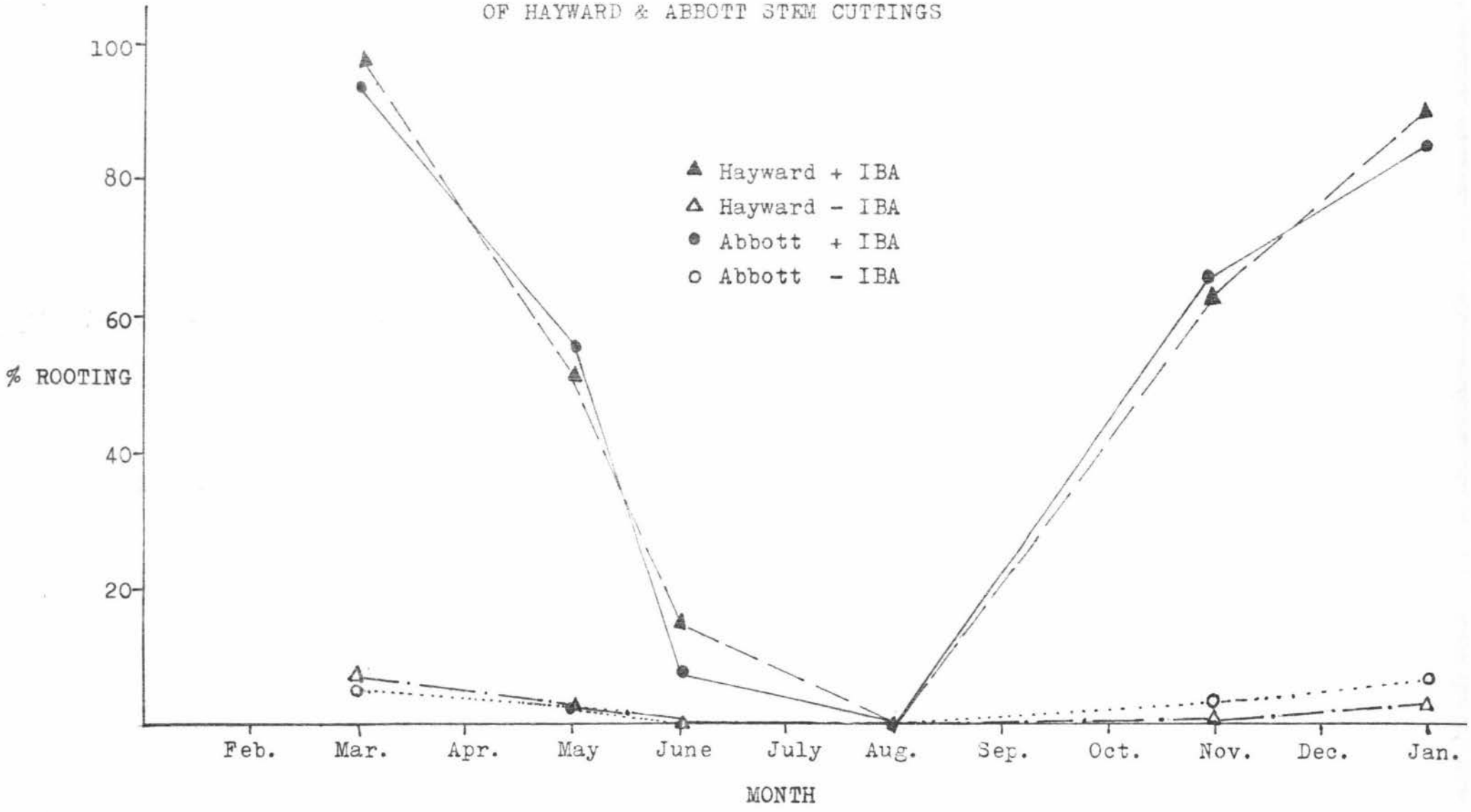
Percentage rooting on successive harvest date

Cuttings/Date		23/3	5/5	17/6	9/8	25/11	25/1
Hayward	+ IBA	96.6 a	50 c	15 d	0 e	63.3 b	90 a
	- IBA	6.7	3.3	0	0	1.7	3.3
Abbott	+ IBA	93.3 a	55 b	5 c	0 c	66 b	85 a
	- IBA	5 ab	3.3 b	0 b	0 b	3.3 b	6.7 a

Treatment denoted by the same letter are not significantly different when analysed by Duncan's Multiple Range Test (5%).

FIGURE 4.5.1

SEASONAL FLUCTUATION IN THE ROOTING ABILITY OF HAYWARD & ABBOTT STEM CUTTINGS



4.5.2 RESULTS

The results are presented in Table 4.1.5.1. Details of analyses of variance are recorded in Appendix 2.

The results clearly show that there is no difference in the ability of Hayward and Abbott to initiate roots.

Without any auxin treatments, both Abbott and Hayward cuttings show very low rooting percentage throughout the year. In the Winter months June and August there is no rooting at all.

Treating cuttings with IBA, give a highly significant improvement in rooting in all seasons except in August; During which, 66% of the axillary bud burst on the cuttings shortly after been placed on the heated bed. These data reaffirm those of Section 4.3.

Cuttings treated with IBA exhibit a distinctive seasonal rhythm in their ability to regenerate adventitious roots. Cuttings perform best (over 90%) in the Summer months when the wood is relatively soft.

4.5.3 DISCUSSION

The effect of IBA has been discussed in 4.1.3 and 4.1.7.

The time at which cuttings are collected has been found to be quite critical in terms of the expected successful propagation of Hayward and Abbott varieties. Several authors recommended taking softwood cuttings during the active season (Smith, 1973; Optiz and Beutel, 1975; Fletcher, 1976) for Actinidia chinensis (Planch.).

Commercial producers in New Zealand also preferred taking cutting from October to March, while at other times of the year the plant is deciduous and difficult to root (Duncan and Davies, 1977).

Periodicity in the capacity of cuttings to regenerate is well known in many other crops. Cuttings of apple, peach, pear, cherry and apricot cultivars root better in Autumn than Winter (Howard, 1966; Doud and Carlson, 1972). A rapid rise in rooting ability occured in Spring. Barker and Link (1963) reported that many woody ornamental species also shown seasonal fluctation in rooting ability. Actively growing softwood cuttings frequently show optimum rooting in mid-summer whereas dormant hardwood cuttings are best taken in Autumn (Smith and Wareing, 1972a & b).

During August, 66% of the cuttings were found to have bud burst shortly after being placed on the heated bed. This corresponds to the period of lowest rooting ability. This phenomenon is contradictory to many reports that treatments which induce bud burst will enhance root initiation (Fadl and Hartmann, 1967a; Smith and Wareing, 1972a, b; Roberts et al, 1974; Whitehill and Schwabe, 1975). However, Cahlahjah and Nekrasova (1962 cited by Biran and Halevy, 1973) have shown that peach cuttings bearing sprouting buds do not root, while those with dormant buds root satisfactorily. Snyder (1955) found no correlation between rooting response and the degree of lateral bud activity in Taxus cuspidata. Further investigation of bud effect on root initiation will be reported in Section 4.1.6.

In August rooting ability is lowest, and cuttings failed to respond to IBA. This is in accordance to the common finding that hard-to-root cuttings usually do not respond to auxin (Audus, 1963). Since no treatment factor was varied there may be some endogenous factor which varies with the season, having been altered. Root

initiation is determined by the physiological condition of the cutting material, and that response to exogenous auxin was governed by the endogenous auxin status in the cuttings, which fluctuates with season (Spiegel, 1955). In this case, it might be that in the Winter the endogenous auxin level is much too low and that the exogenous auxin applied is not enough to raise the level of auxin to an optimum level to facilitate rooting.

Hess (1959) found that auxin is only effective when it is reacting with some the other compound or compounds to stimulate root formation. Similarly Cooper's (1938) experiments showed that auxin treatments appeared to react with some other substance, and when this substance was depleted auxin treatments had no further effects upon root formation.

Of course the lack of IBA response during this phase of development (August) may also be the result of catabolic metabolism, storage, inactivation or use for other purposes.

Haissig (1973) postulates that lack of root initiation in response to applied auxin (or even to native auxin) may be due to one or more of the following:

1) a lack of necessary enzymes to synthesize the root inducing auxin-phenol conjugates.

2) lack of enzyme activators.

3) presence of enzyme inhibitors.

4) lack of substrate phenolics or

5) physical separation of enzyme reactants due to cellular compartmentalization.

4.6 THE RELATIONSHIP BETWEEN BUD DORMANCY AND ROOT INITIATION

4.6.1.1 INTRODUCTION

The role of bud dormancy in the control of adventitious root regeneration from cuttings is both obscure and complex. Conflicting reports (see Literature Review) have been made, pertaining to the influence of dormant buds on inhibition root initiation of cuttings (Fadl and Hartmann, 1967a; Howard, 1968a).

The experiments reported here were designed to detect any effect of bud activity on the rooting of Actinidia chinensis (Planch.). The experiment is of two parts : -

Part 1, To measure the intensity of bud dormancy in Actinidia chinensis (Planch.) using as criteria the number of days required for bud break on cuttings at various time of the year. Dormancy curves thus established were then compared with those previously established for rooting of cuttings to verify any correlative relationships.

Part 2, Buds were physically removed to see their effect on rooting at various time of the year.

4.6.2.1 METHODS

At each harvest date, 10 cuttings of Abbott were taken and placed in distilled water under continuous light at 27°C. The method of Hewett and Wareing (1973) was used to record the time to bud break.

The intensity of bud dormancy at time of shift to growth chamber was expressed as maximum percentage bud

break was the percentage of total cuttings that eventually burst open and showed green leaves. Days to bud break was time from moving the plants into the controlled environment until 50% of the buds that finally burst were showing green leaves.

The recording of bud burst was terminated when there was no further bud burst for 10 successive days.

4.6.2.2 RESULTS

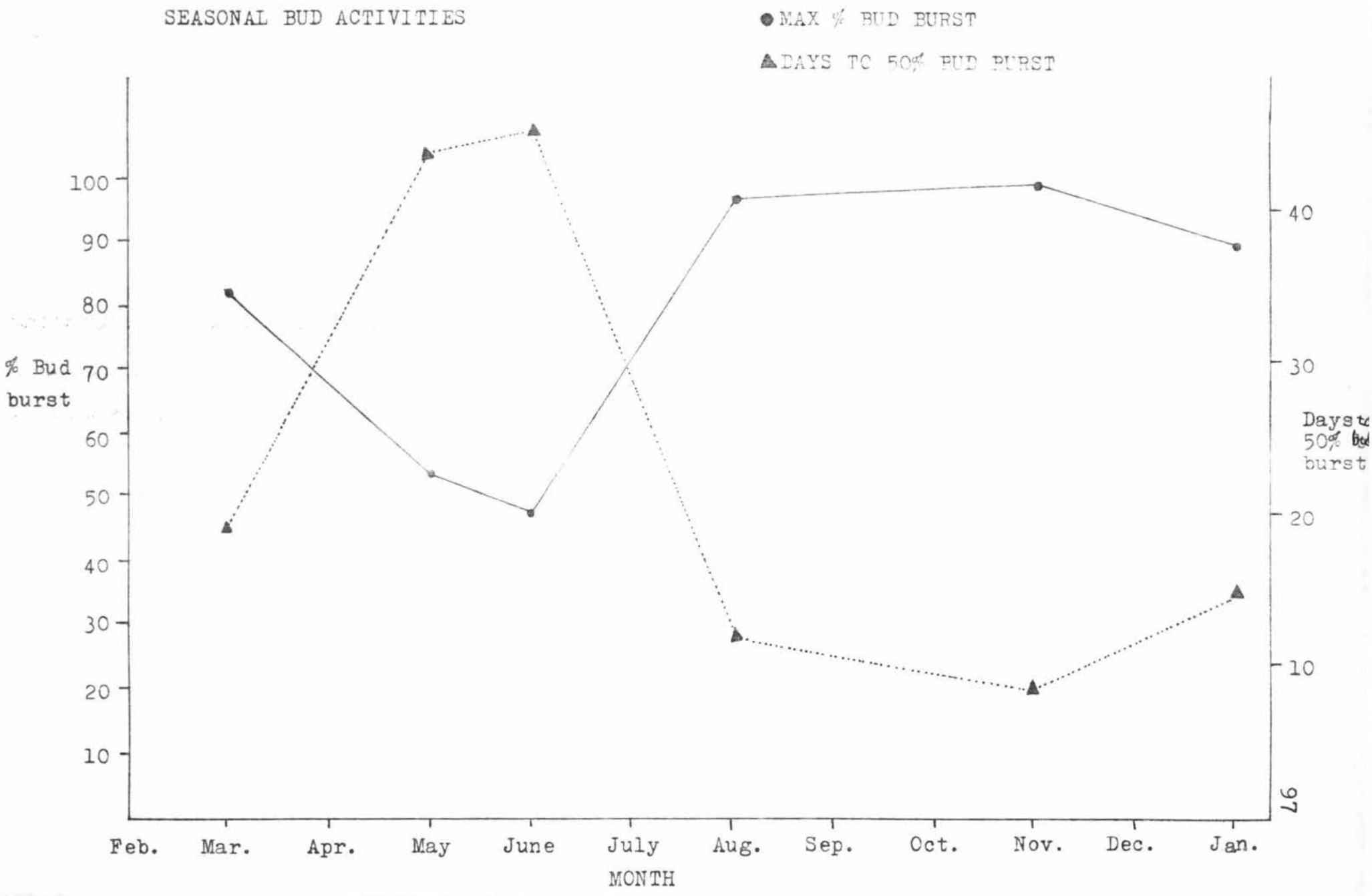
The results are presented in Table 4.6.2.1

Table 4.6.2.1 Percentage bud break and days to 50% bud break of cuttings of Abbott variety

HARVESTING DATE	23/3	5/5	17/6	9/8	25/11	25/1
Days to 50% bud burst	19	44	46	12	8	14
Max. % bud burst	82	53	47	95	98	89

FIGURE 4.6.2.1

SEASONAL BUD ACTIVITIES



The pattern and degree of bud dormancy, as evidenced by the number of buds burst (maximum % bud burst) was comparable to that of the speed of bud burst (days to 50% bud burst).

Bud dormancy was most severe in May and June. In August bud activity quickly increased to its near maximum, and activity starts to decline after December.

4.6.3.1 METHOD

Abbott stem cuttings were collected during various time of the year, namely 6/5, 17/6, 9/8, 25/11, and 25/1. All leaves were physically removed, to prevent any masking effect of the leaves over the bud effect. The defoliated cuttings were then subjected to the following treatments: -

Treatment 1	with Buds	+ IBA
Treatment 2	with Buds	- IBA
Treatment 3	without Buds	+ IBA
Treatment 4	without Buds	- IBA.

There were 45 cuttings in each treatment, divided into 9 blocks of 45.

For treatment 3 and 4, the buds were excised by scalpe. Where treatments with IBA were used and this consisted of dipping the base of the cutting into a mixture of 0.8% indo yl - butyric acid in talc.

The cuttings were arranged in a randomised block design on a heated bench with mist propagation equipment. After 40 days, cuttings were lifted and rooting percentage recorded.

4.6.3.2 RESULTS

Results are presented in Table 4.6.3.1. For the analysis of variance refer Appendix 2.

Table 4.6.3.1

% Rooting of bud and budless Abbott cuttings treated with/without IBA at various harvests

Harvesting Date / Treatment	6/5	17/6	8/8	25/11	26/1
+ Bud + IBA	5.6	5	0	27.8	2.67
+ Bud - IBA	0	0	0	0	0
- Bud + IBA	0	0	0	22.2	3.2
- Bud - IBA	0	0	0	0	0

There were no significance effects from either bud or IBA treatments, except in November and January where there were strong IBA and IBA-Bud interactions.

There was no significant fluctuation in rooting ability at the various harvesting dates. Rooting percentage was generally very low throughout the year even with the help of IBA treatment.

4.6.4 DISCUSSION

The idea that bud activity may influence root regeneration of cuttings has been suggested by several authors (Van der Lek, 1925; Went, 1929; Harada and Narkayama, 1957; Fadl and Hartmann, 1967a; Roberts et al, 1974). The presence of active buds on a cutting has a strong promotive effect on rooting. (Lek, 1925; Went, 1929; Fadl and Hartmann, 1967; Whitehill and Schwabe, 1975).

In this experiment it would seem that seasonal change in root formation of Abbott were not related to the degree of bud activity of the cuttings, an opinion shared by Howard (1968a) from studies on M.26 apple rootstock and Myrobalan B plum rootstock. Tustin (1975) also draw a similar conclusion for MM106 and EMXII cuttings.

The bud activity of Abbott followed an expected trend of increasing from June to November and then declined until bud dormancy in June, no positive or negative relationship to rooting ability of the cuttings was evident.

Physical removal of the bud did not affect rooting in any of the 5 harvests. This confirmed the finding in Part I that bud activity had no influence on root regeneration. On the contrary, Lek (1925), Went (1929), Van der Lek (1934) reported that removal of active buds from cuttings or if all the buds were dormant will stop root regeneration.

Howard (1968a) also found that buds have no effect on rooting, but disbudding in effect produces a phenomenon similar to wounding in stimulating rooting. In the present experiment, all cuttings were wounded at the base, and the additional wounding due to disbudding on some of the cuttings did not produce any improvement in rooting at all.

In general the bud does not show any positive or negative effect on the rooting of Abbott cuttings. This result is in line with Snyder's (1955) report that there is no correlation between rooting response and the degree of lateral bud activity in Taxus cuapidata.

4.7 SEASONAL CHANGES OF ENDOGENOUS GROWTH REGULATORS OF ACTINIDIA CHINENSIS (PLANCH.)

4.7.1 INTRODUCTION

A correlation between the level of various endogenous growth regulators in cuttings and their rooting has been reported for several plants (Hemberg, 1951; Heide, 1967; Fadl and Hartmann, 1967a; Hess, 1964; Stoltz and Hess, 1966). Whereas in other no such correlations could be found (Steponkus and Hogan, 1967; Stoltz, 1968).

An investigation to find if any correlation existed between the ability of cuttings to regenerate roots and the level of various endogenous growth regulators was undertaken.

4.7.2 METHODS

At each harvest date, stem tissue samples were taken and freeze dried prior to analysis for endogenous plant growth regulators.

Lyopholized samples were extracted in 80% methanol and purified by basic and acidic ether extraction (Section 3.6, Materials and Methods).

Measurement of hormonal activity was achieved by use of various bioassay systems relevant to the growth regulator being assayed. Auxins were assayed by using the wheat coleoptile bioassay; and rooting cofactors using the mung bean bioassay. Details of these procedures are presented in Section 3.1 - 3.3 of the Materials and Methods.

4.7.3 RESULTS

(a) AUXINS

An acidic growth promoter, similar to IAA was isolated chromatographically from Hayward stem cuttings. Marked promotory zones were observed at Rf 0.2 - 0.4, the same zone being covered by IAA marker spots on paper chromatograms developed in isopropanol: NH_3 : Water (10: 1 : 1 V/V).

Values of promoter concentration are expressed as gm equivalents per 5 gm. dry weight of plant stem tissue and are plotted in Fig 4.7.1.

Confidence limits of $P < 0.01$ were calculated by the Link and Wallace method for histograms derived from the oat coleoptile bioassay. Values below 0.001 mg/ml IAA (from standard run concurrently) were not significantly different from controls but are plotted in Fig 7, for comparative purposes. These are from the June and August harvests.

There is a general trend from spring until Winter, with higher Auxin levels in Spring and Summer, decreasing in Autumn and reaches its minimum in Winter. The trend coincides with that of the root regeneration ability shown in Figure 5, but no obvious relation with bud burst.

(b) IAN

A neutral growth promoter chromatographically similar to Indoleacetonitril (IAN) was isolated from Hayward cuttings. Considerable promotive activity in the oat coleoptile bioassay was observed in Rfs 0.7 - 0.9 from paper chromatograms develop in isopropanol : ammonia :

water (10 : 1 : 1 V/V).

Values of promoter concentration is expressed in a similar way as the IAA levels and are represented in Figure 4.7.2. The fluctuation in IAN level is in no way correlated with that of percentage rooting and bud bursting.

(c) ABSCISIC ACID

Marked inhibitory activity was observed in acidic ether extracts at Rf 0.5 - 0.7, the same zone described by Bennett-Clark and Kefford (1953). ABA marker spots run concurrently with extracts in isopropanol : ammonia : water (10 : 1 : 1 V/V) correspond to the inhibitory zone.

Values of inhibitor concentration are expressed as gm equivalents per 5 gm. dry weight of plant stem tissue and are plotted in Figure 4.7.3.

The seasonal trend in ABA levels show high correlation with that of rooting but not that of bud bursting.

FIGURE 4.7.1

SEASONAL CHANGES IN AN ACIDIC GROWTH PROMOTER, SIMILAR TO IAA, DETERMINED BY THE OAT COLEOPTILE BIOASSAY OF THE ACIDIC ETHER FRACTIONS OF STEM TISSUE SAMPLES OF HAYWARD.

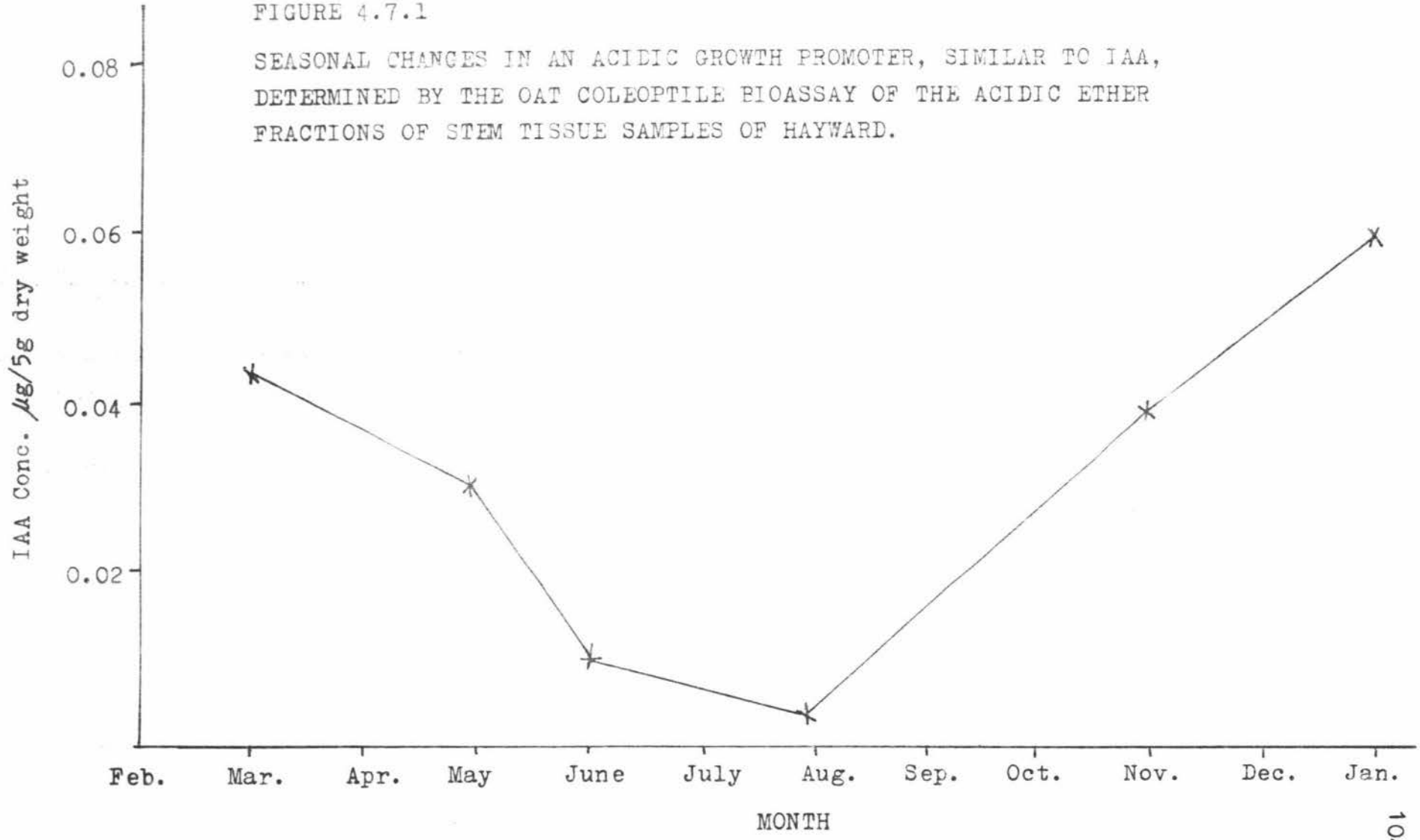


FIGURE 4.7.2

SEASONAL CHANGES OF A NEUTRAL GROWTH PROMOTER, SIMILAR TO IAN, DETERMINED BY THE OAT COLEOPTILE BIOASSAY OF NEUTRAL ETHER FRACTIONS OF STEM TISSUE SAMPLES OF HAYWARD.

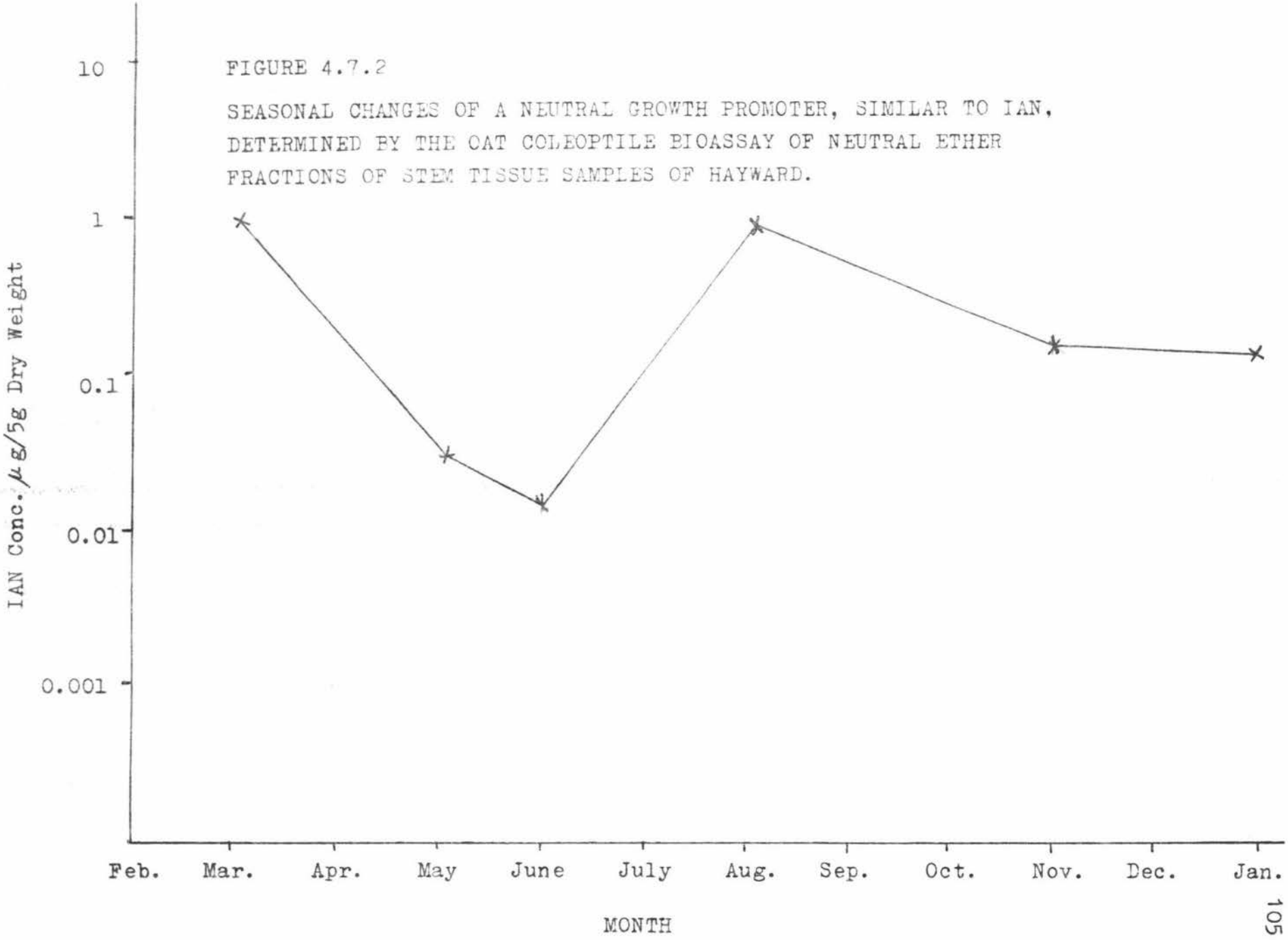


FIGURE 4.7.3

SEASONAL CHANGES OF AN ACIDIC GROWTH INHIBITOR, SIMILAR TO ABA, DETERMINED BY THE WHEAT COLEOPTILE BIOASSAY OF ACIDIC ETHER FRACTIONS OF STEM TISSUE SAMPLES OF HAYWARD.

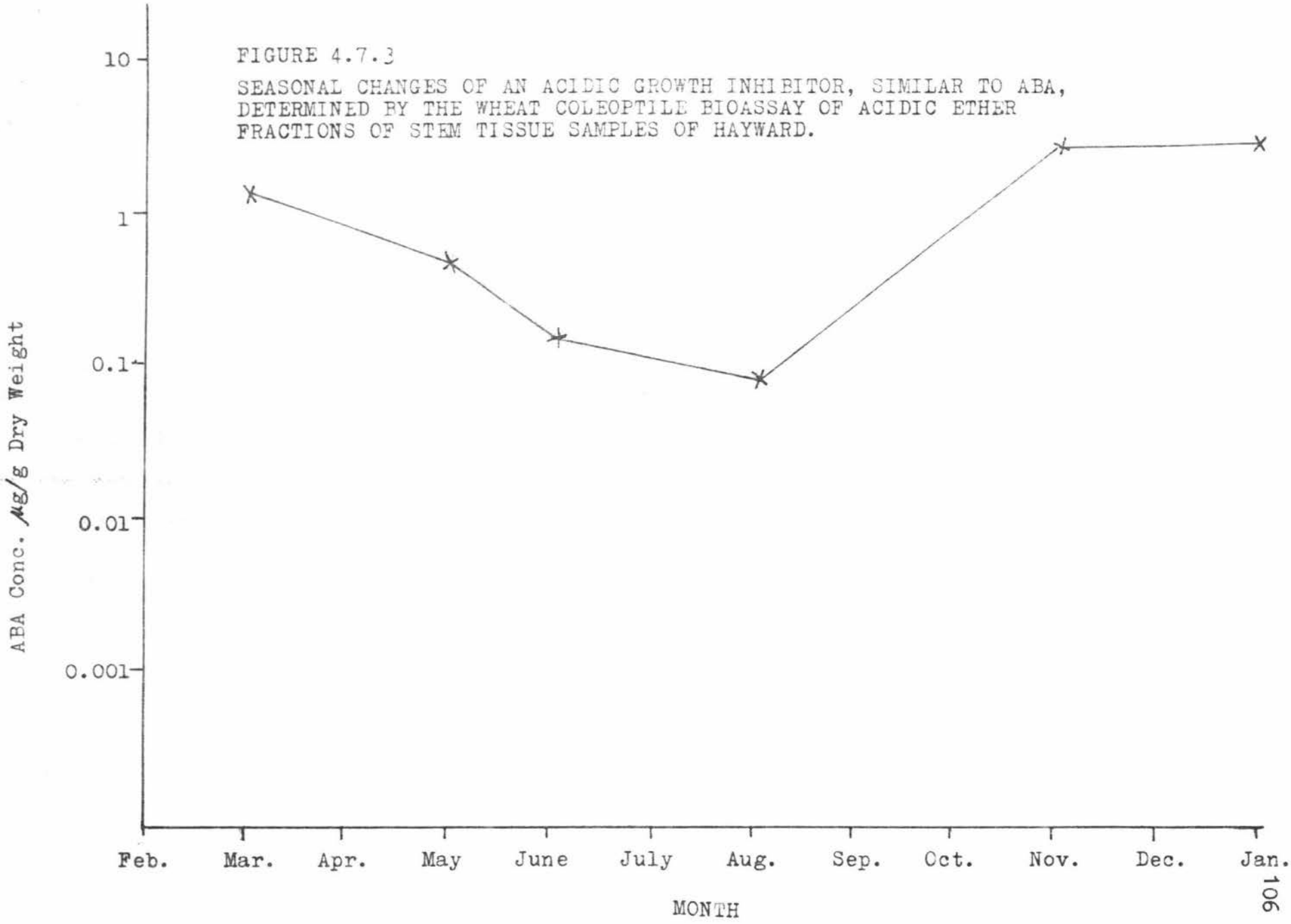
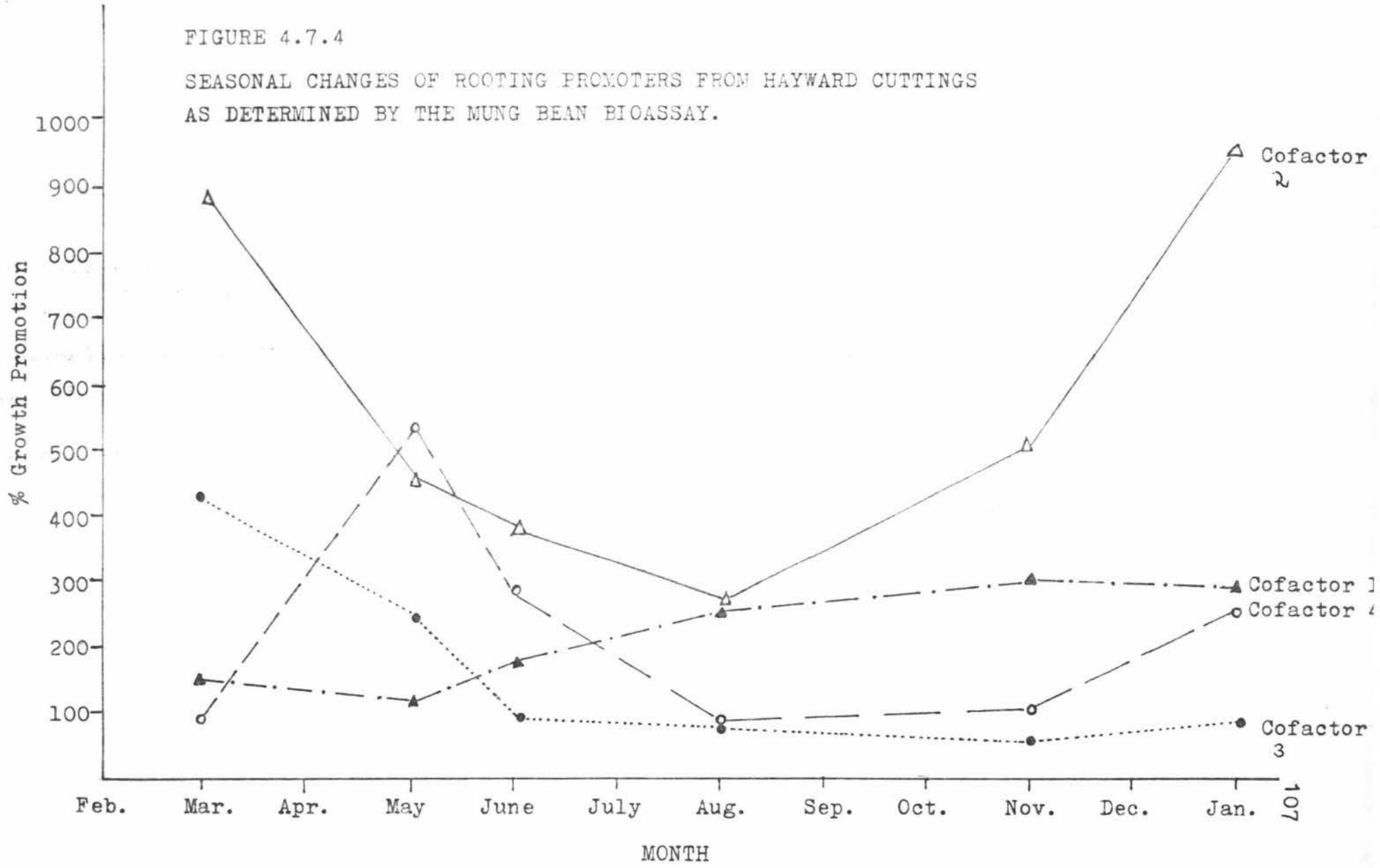


FIGURE 4.7.4

SEASONAL CHANGES OF ROOTING PROMOTERS FROM HAYWARD CUTTINGS AS DETERMINED BY THE MUNG BEAN BIOASSAY.



(d) ROOTING COFACTORS

Results are presented in Figure 4.7.4. Rooting promotory zones were detected in the histograms of the crude extracts, namely cofactor 1 (Rf 0 - 012), cofactor 2 (Rf 0.3 - 0.5), cofactor 3 (Rf 0.5 - 0.7) and cofactor 4 (Rf 0.7 - 1.0).

The relative activity of these cofactors in decreasing order is as follow: cofactor 2 cofactor 1 cofactor 4 cofactor 3.

It was noted that while root initiation on mung bean cuttings was dramatically increased by the aqueous promoter, root elongation was retarded.

No significance inhibition of rooting was recorded at any harvest.

4.7.4 DISCUSSION

The importance of IAA in promoting adventitious foot formation is now widely accepted and changes in the pattern of root formation have often been related to changes in endogenous auxin levels, (Warmke and Warmke, 1950; Spiegel, 1955: Odom and Carpenter, 1965; Kachecheba, 1975, 1976). In the present study, the seasonal changes in root formation of Hayward cuttings followed the seasonal changes in endogenous IAA levels for each harvest date. Promotion of rooting with IBA was most spectacular on cuttings with a higher endogenous IAA level and seemed dependant on an optimum level of endogenous IAA. In August, cuttings do not root at all, not even with the help of IBA treatment, this corresponds to the very low level of endogenous IAA in the August harvest. It is therefore conceivable that there is certain critical minimum level of

the endogenous IAA, below which rooting is impossible. From the present data, the critical level of endogenous IAA which appeared necessary to promote rooting for Hayward cutting was approximately 0.005 mg/5 gm. dry weight of stem tissue. Only cuttings with auxin level at or above this concentration exibited a potential to root when treated with IBA. Early in 1955, Spiegel had concluded that root initiation was determined by the physiological condition of the cutting material, and that any response to exogenous auxin was governed by the endogenous auxin status of the cuttings, which fluctuate with season. He suggested that cuttings will respond to exogenous auxin treatment positively when the endogenous auxin level is sub-optimal, on the other hand auxin will inhibit rooting if endogenous auxin is at supra-optimal levels. Many other workers also reported that easy-to-root cuttings usually respond to auxin treatment, whereas difficult-to-root varieties do not (Audus, 1963). Cuttings retaining auxin levels at or above certain critical minimum level of endogenous auxin exibited a high potential to root when treated with IBA (Tustin, 1976).

From available literature and the findings of present investigation, it can be appreciated that there may be a lower and upper limit, namely the critical minimum level (Tustin, 1976) and the optimum level (Spiegel, 1955) respectively. Within these two limits cuttings will response positively to exogenous auxin treatment. From the present data collected, the upper and lower limits for Hayward are approximately 0.06 and 0.005mg/5 gm dry weight respectively.

Many workers found high correlation between bud activity, and seasonal rhythm of the rooting capacity of cuttings. Seasonal variability in rootability of Populus nigra, Hibiscus rosa-sinensis and Ficus infectoria has been related to bud activity, auxin content and the mobilization of starch (Nanda, Anand and Kumar, 1970).

The root initiation on Oldman pear hardwood cuttings was best in Autumn and was correlated with the ability of the buds to shoot readily (Fadl and Hartmann, 1967a). It was also postulated that hormone like substances formed in the developing buds and were transported through the phloem to the base of the cuttings where they stimulated rooting (O'Rourke, 1942). Contrary to those reports, the present experiment found no rooting rhythm correlation between bud activity and, seasonal auxin level. The correlation of bud burst with root initiation is well documented (Spiegel, 1955; Lanphear and Meahl, 1963; Howard, 1965; Fadl and Hartmann, 1967; Roberts et al, 1974) and treatments which induce bud burst have been known to enhance root initiation (Fadl and Hartmann, 1967; Smith and Wareing, 1977a and b; Bhella and Roberts, 1974; Whitehill and Schwabe, 1975). In contrast, during August sample the Hayward cuttings sprouted readily on the heated bed, also the bud bursting data were high (95%) but rooting was poorest of all harvest and endogenous auxin is at its lowest level. This may be considered as a negative correlation but in November a positive correlation seemed to hold. Thus no simple correlation can be drawn between bud activity, auxin level and seasonal rooting capacity. As indicated by this experiment the promotive role of active bud is not a general rule some contrary evidence has also been reported in Taxus cuspidata (Snyder, 1955), in plum (Howard, 1968a) and Hartmann and kester (1975) also pointed out that cuttings made from shoots with preformed root initials, seem to be little influenced by bud activity during rooting.

Auxin activity, chromatographically similar to IAN was observed in Hayward cuttings at all harvesting dates. The role of IAN in promoting root formation is relatively unknown although its physiological activity may depend on being converted to IAA, a process which has been shown to occur in apple rootstocks (Gur and Samish, 1968). Sin and Sung (1968) and Sung (1969) found that in the absence of IAA, IAN appeared to be an active rooting

promoter in brachyblast cuttings of pine. IAN is active in root initiation through conversion to IAA in Coleus Species and Chrysanthemun Species (Odom and Carpenter, 1965), and in grain coleoptiles.

From data collected, the seasonal changes in IAN levels in Hayward cuttings did not resemble the seasonal pattern in root initiation, or the bud activity.

Abscisic acid has been found to promote root initiation on cuttings of some plant species (Chin and Beever, 1969; Basu, Roy and Bose, 1970; Thorpe and Meier, 1973; Bojarczuk and Jenkiewicz, 1975; Foong, 1977), but many reports noted the inhibition of root inhibition by ABA (Heide, 1968; Eliason, 1969; Pierek and Stegmans, 1975). The present results fall into the first option. It was found that the acidic ABA level of Hayward were low in Autumn (May) and Winter (August and June) and were higher in Spring and Summer. This seasonal rhythm of ABA level is highly comparable with the seasonal periodicity in rooting capacity.

The ABA fluctuating cycle also has a good correlation with the IAA seasonal cycle. And it is highly possible that ABA not only promotes rooting, but may have some synergistic or additive effects with IAA. Basu, Roy and Bose (1970) reported that at low concentration. (10 and 20 mg/l) ABA have an additive effect with IAA (8.76 mg/ml) in promoting rooting of mung bean hypocotyl cuttings.

There is a growing group of workers taking IAA: ABA ratio as a measure of rooting potential (Howard, 1966, 1971; Fadl and Hartmann, 1967a; Nesterov, 1968; Howard and Nahlawi, 1969; Doud and Carlson, 1972). Increasing ABA and decreasing IAA levels would reduce the IAA: ABA ratio and rooting is expected to be poor (Howard, 1966, 1971). But present data show no indication that ABA

is a potent competitive rooting inhibitor at all.

Other workers suggested ABA exibits promotory effects through antagonising the inhibitory effect of Gibberellin level in adventitious root formation (Chin, Meyer and Beevers, 1969; Thorpe and Meier, 1973; Foong, 1977). Present experiments cannot confirm this, but it is suggested that further work is required.

Four rooting cofactors of Hayward were found to have similar Rf's as those characterised by Hess (1957) in juvenile Hedera helix, namely, cofactor 1 (Rf 0 - 0.2), cofactor 2 (Rf 0.3 - 0.5), cofactor 3 (Rf 0.5 - 0.7) and cofactor 4 (Rf 0.7 - 1.0).

Cofactor 2 was the most active promoter in Hayward cuttings. In Spring and Summer cofactor 2 was higher and declined towards Winter. As for Hedera helix 'cofactor 4' was the most active promoter (Hess, 1957), Foong (1977) also reported that cofactor 4 was the most active cofactor found in Rhododendron ponticum and R. briltania, and its activity was highest in Spring and slowly declined towards Winter.

The seasonal fluctuation of cofactor 2 coincides well with the seasonal oscillation of the rooting potentiality of Hayward cuttings. Correlations have been established between the amounts of rooting cofactors and rooting ability (Hess, 1957, 1961, 1962(b), 1965 and 1968; Girouard and Hess, 1966; Girouard, 1969; Hackett, 1969; Lee, 1969; Foong, 1971). However, some studies fail to give a correlation between cofactors and rooting response (Lipecke and Dennis, 1972; Biran and Halevy, 1973; Taylor and Odom, 1970; Tustin, 1976) and it has not been demonstrated that extractable co-factors have any causal effect in rooting.

There were also seasonal fluctuations in the

other cofactor contents (Figure 4.7.4), but no obvious relation ship can be drawn when compared with rooting potentiality of cuttings.

As a whole such crude extracts may provide some insight of the amounts of promoters and inhibitors present, they are not totally reliable. However, there are possibilities of overlapping of promoter(s) and inhibitor(s) in a certain chromatographic regions such that their opposing effects may cancel each other out. There are possibilities of concentration effects also. Certain compounds may be promotory at low concentrations but inhibitory at high concentrations or vice versa. Such as effect has been found to be the case for the Avena straight growth test (Vieltez, et al 1967). A concentration effect is evident from the mung bean rooting data of Pinus radiata (Zabkiewicz and Steele, 1974).

4.8 EXAMINATION OF THE ROLE OF IAA AND IBA IN ROOT FORMATION OF BRUNO CUTTINGS

4.8.1 INTRODUCTION

Since the use of IBA is so important and widespread in propagation, elucidation of the role of action of IBA would enable improvement of existing treatments using exogeneous rooting promoters.

The hypothesis that IAA alone directly promotes root initiation in Actinidia chinensis cutting and that IBA can only promote rooting in the presence of a threshold level of IAA, will be evaluated.

4.8.2 METHODS

Bruno cuttings of 1 node length were harvested in June. Leaves and buds were known to be an active growth regulator producer, here to avoid interactions which might obscure the effect of the treatments, all leaves and buds were removed during preparation of cuttings. The cuttings were then treated as follow : -

Table 4.8.1 Treatment for Bruno Cuttings

	+ 0.1% IAA		No IAA	
IBA Conc.	Centrifuged	Non-centrif.	Centrif.	Non-centrif.
0 ppm	1	7	13	19
1000 ppm	2	8	14	20
5000 ppm	3	9	15	21
10000 ppm	4	10	16	22
0.8% ppm	5	11	17	23
20,000 ppm	6	12	18	24

Numbers 1-24 denote treatment numbers.

IBA was applied basally as a quick dip in 50% ethanol, except for 0.8% IBA in talc. Cuttings not treated with IBA, received a quick dip in 50% ethanol, IAA was provided as a continous supply by apical application of IAA-lanolin paste (0.1%). Lanolin was applied to cuttings not receiving any IAA treatment. The IAA-lanolin and pure lanolin were replaced every 3rd day and a 1 mm. slice removed from the top of each cutting each week, to ensure a continuing and constant supply of IAA to the cutting tissue.

Centrifugation was at 200 g for 2 hours at 15°C base down in 20 ml. of water and after this the basal 2 cm. of each cutting was removed. The centrifugate was

retained and acidifed and partitioned 3X with diethyl ether. Both the ether and aqueous fractions were reduced to dryness and stored for analysis of growth regulators.

The excised bases and remaining cuttings were vacuum dried, homogenized and extracted in chilled 8% methanol, and this solution was then purified and then tested.

The centrifugation procedure adopted was designed to reduce the levels of endogenous rooting promoters within the cuttings by extending treatments used by Kawase (1964) and Fadl and Hartmann (1969) over a greatertime period. Removal of the cutting base was to ensure reduced growth factor levels.

The randomized experimental design was of 24 different treatments of 3 replicates of 9 cuttings each.

Table 4.8.2

Percentage Rooting of Bruno Cuttings treated with IAA, IBA and Centrifugation singly or in combination

	+ 0.1% IAA		No IAA	
IBA	Centrifuged	Non-centr.	Centrifuged	Non-centr.
0 ppm	14.8	25.9	0	0
1000 ppm	22.2	25.9	0	0
5000 ppm	25.9	40.7	0	0
10000ppm	29.6	40.7	0	3.7
0.8% in talc.	33.3	40.7	18.5	29.6
20000ppm	40.7	37.0	7.4	7.4

LSD 0.05 = 2.06
LSD 0.01 = 2.795
LSD 0.005 = 3.619

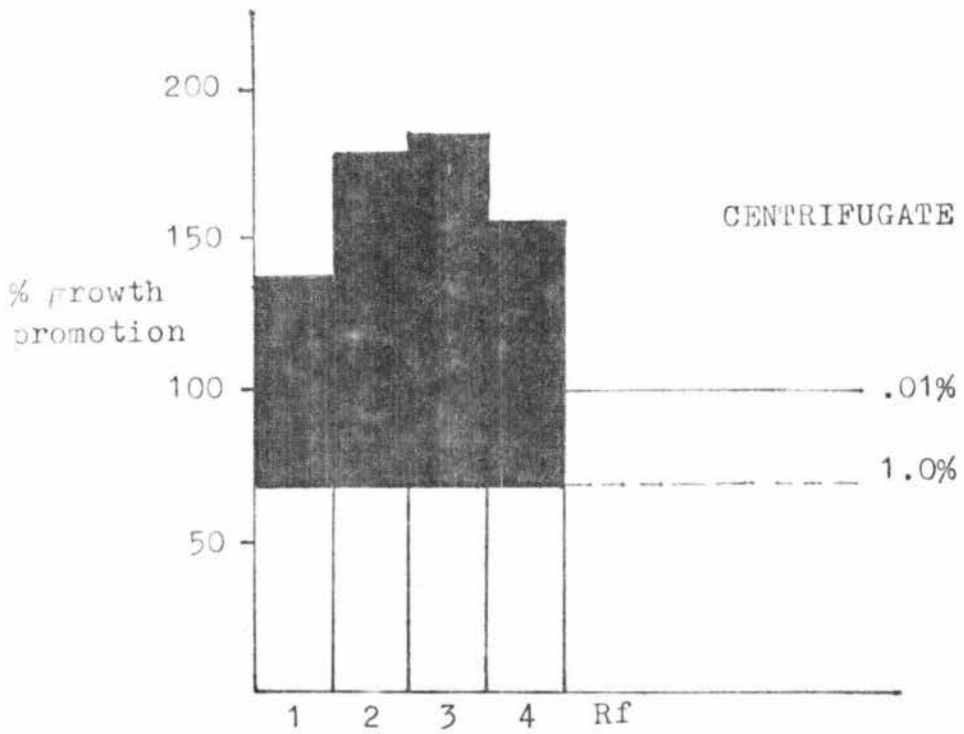
4.8.3 RESULTS

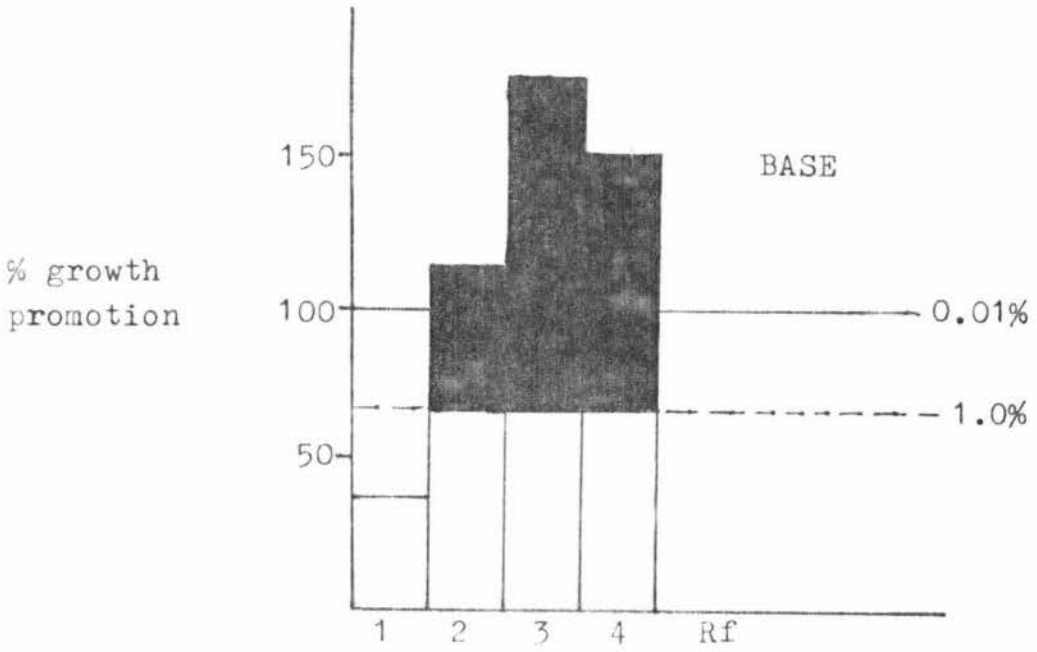
In this June collection, cuttings failed to root completely without IBA or IAA treatment.

IBA was found to improve significantly ($P < 0.05$) the rooting percentage (Appendix 2). However, in the absence of IAA, even high concentrations (10,000 ppm) of IBA have only a small promoting effect, but low IBA is ineffective. Some toxic effect was observed in the -IAA +centrifugation, -IAA -centrifugation and +IAA -centrifugation treatments at 20,000 ppm IBA. The damage was in the form of stunted, twisted roots and a significant drop ($P < 0.001$) in the percentage rooting compared to that of 10,000 ppm and 0.8% IBA treatment.

FIGURE 4.8.1

HISROGRAMS OF IAA-LIKE GROWTH PROMOTERS FROM STEM TISSUE & CENTRIFUGATE OF BRUNO




% growth promotion
150
100
50
BASE
0.01%
1.0%
1
2
3
4
Rf

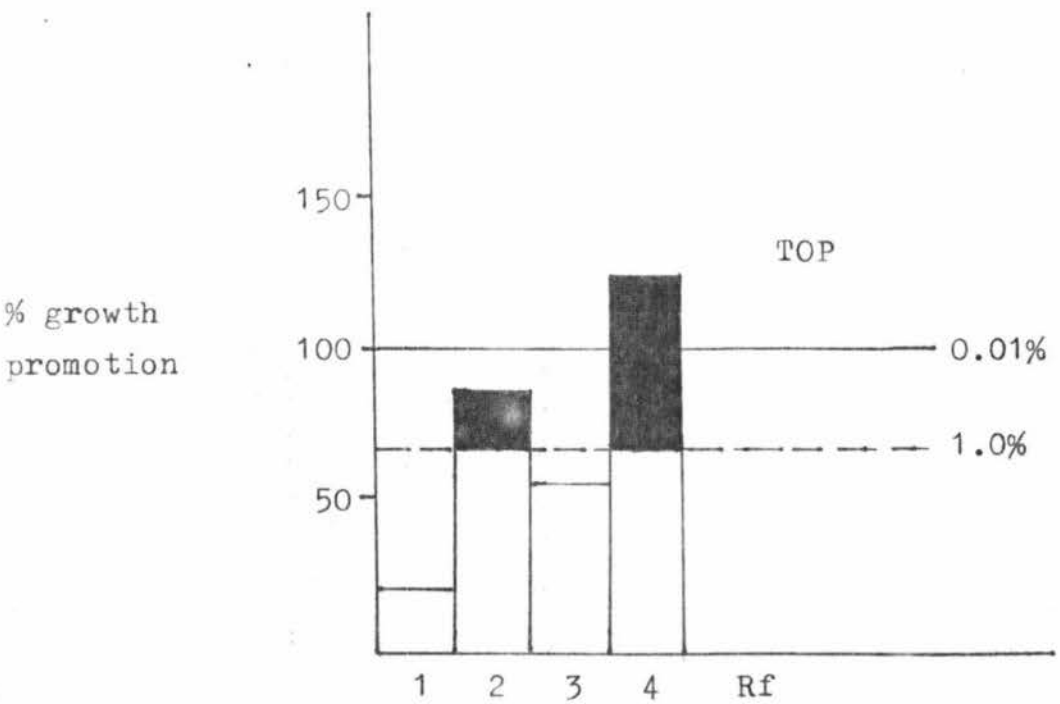
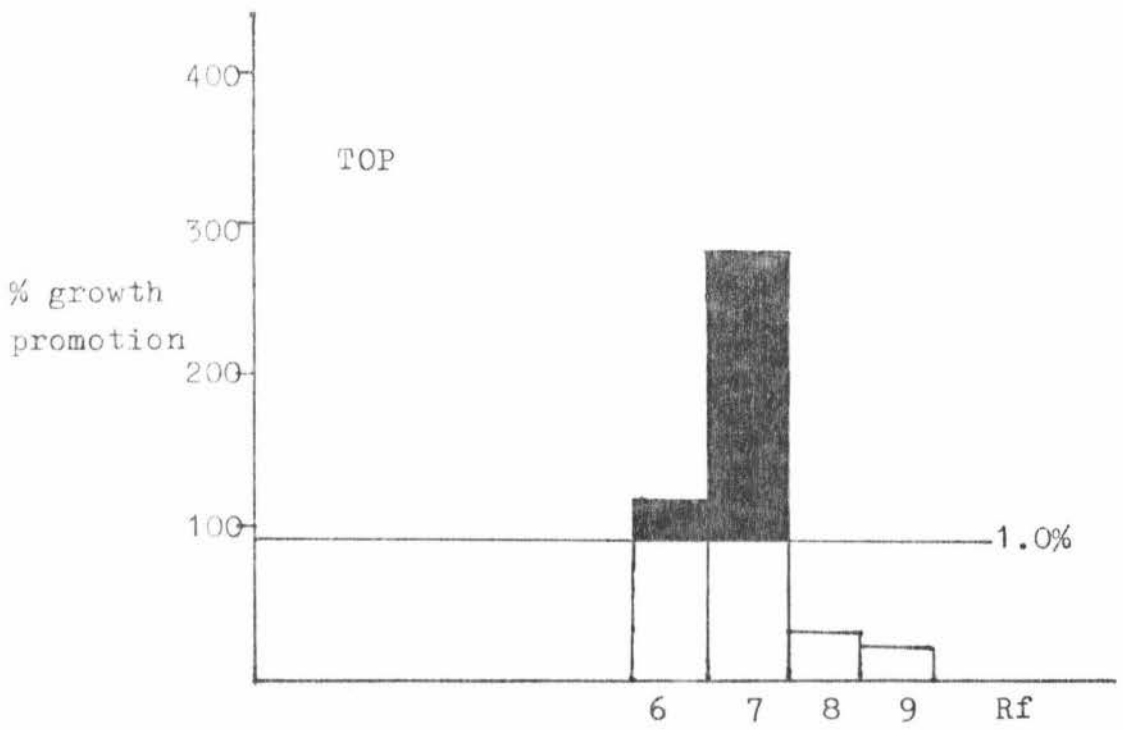
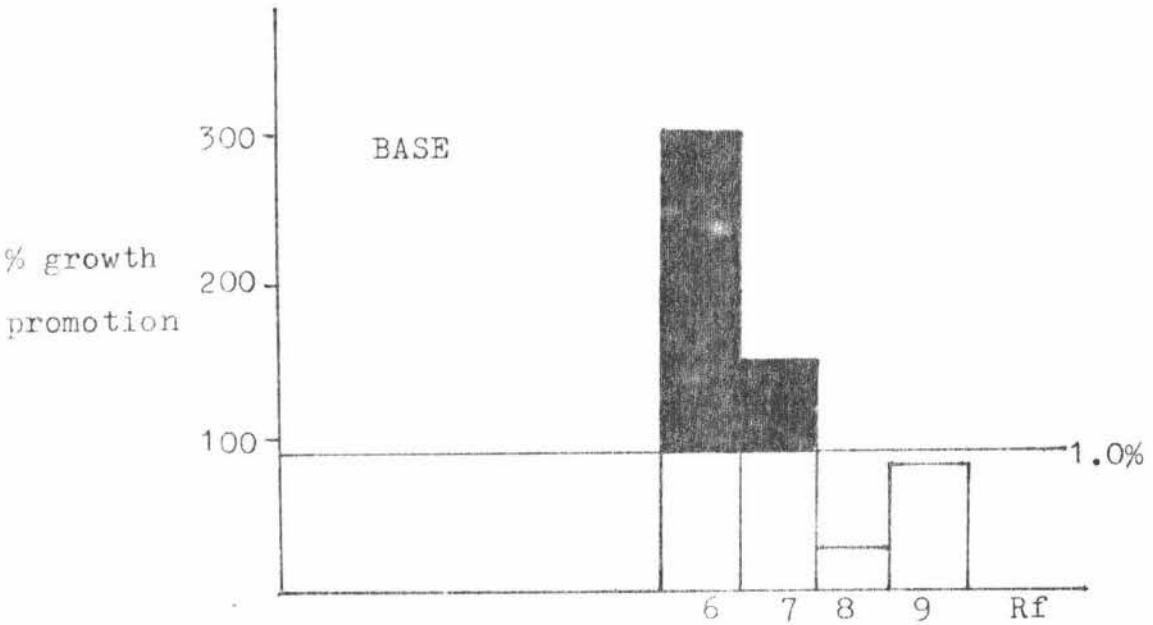

% growth promotion
150
100
50
TOP
0.01%
1.0%
1
2
3
4
Rf

FIGURE 4.8.2

HISTOGRAMS OF IAN EXTRACTED FROM STEM TISSUE & CENTRIFUGATE OF BRUNO




BASE
% growth promotion
300
200
100
1.0%
6
7
8
9
Rf

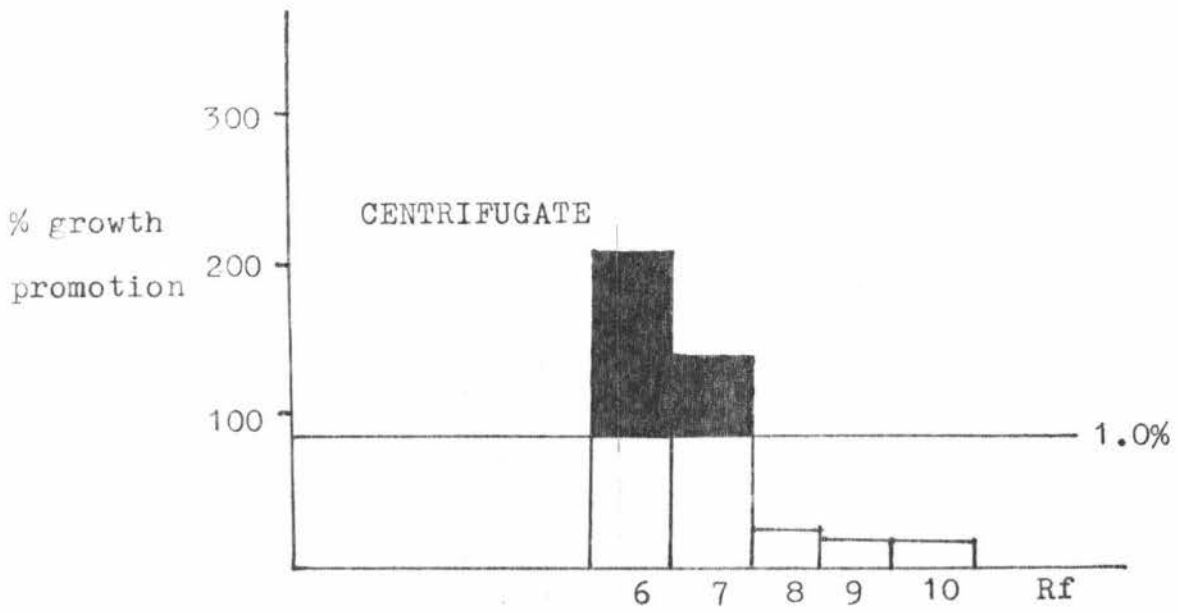
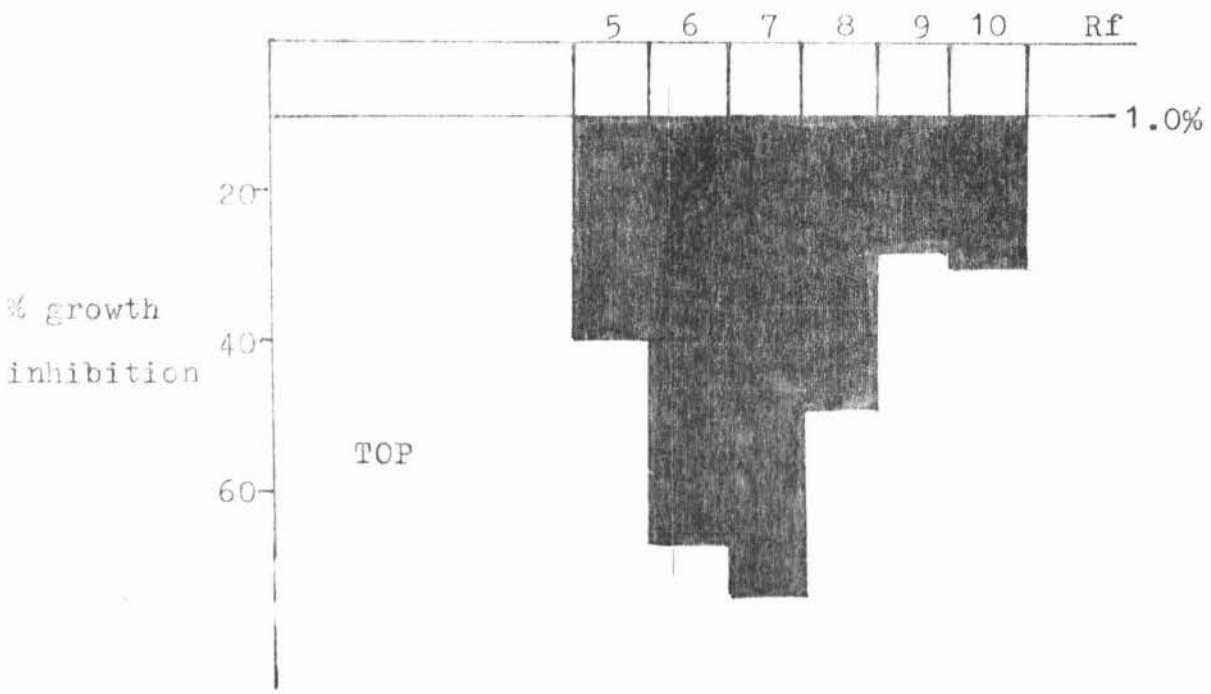
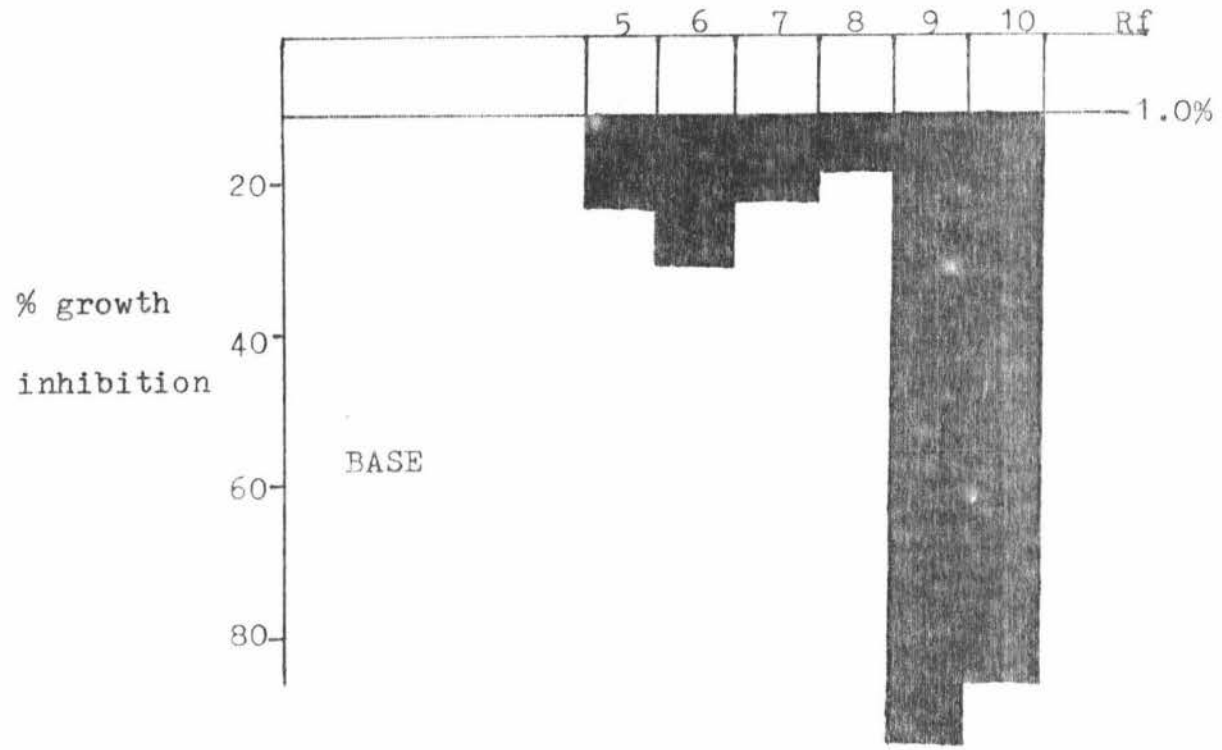

CENTRIFUGATE
% growth promotion
300
200
100
1.0%
6
7
8
9
10
Rf

FIGURE 4.8.3

HISTOGRAMS OF ABA EXTRACTED FROM STEM TISSUES OF CENTRIFUGATE OF BRUNO




5
6
7
8
9
10
Rf
1.0%
20
40
60
80
% growth
inhibition
BASE

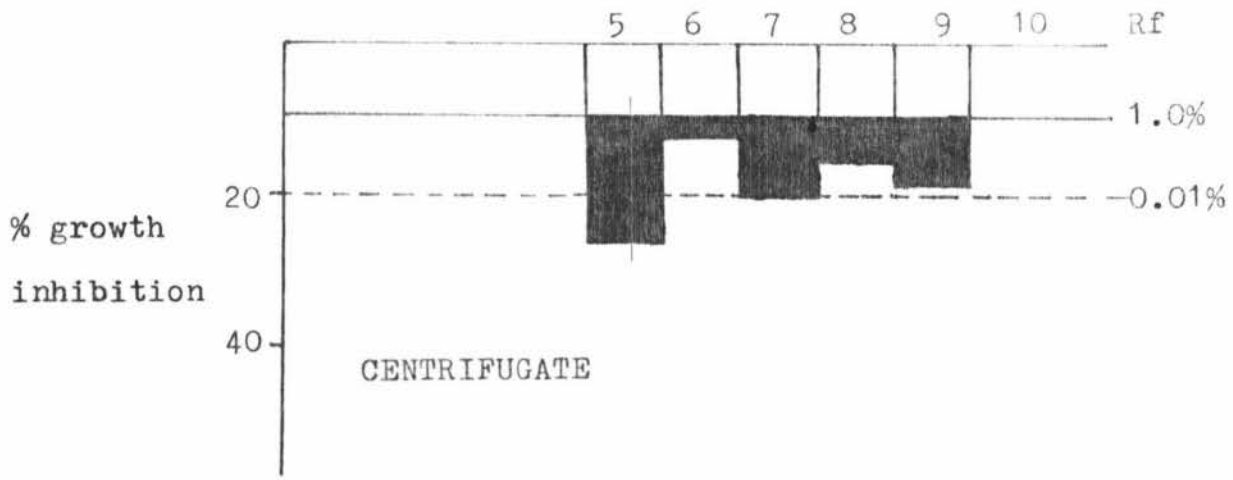
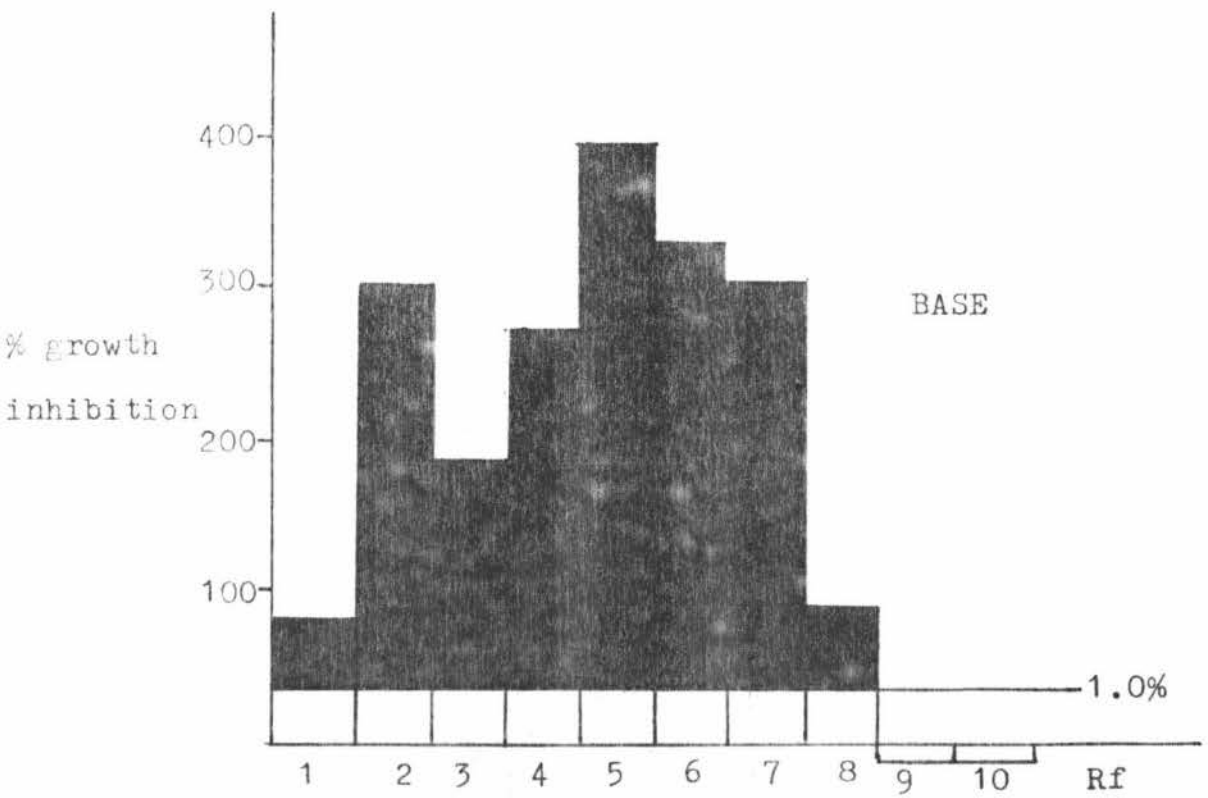
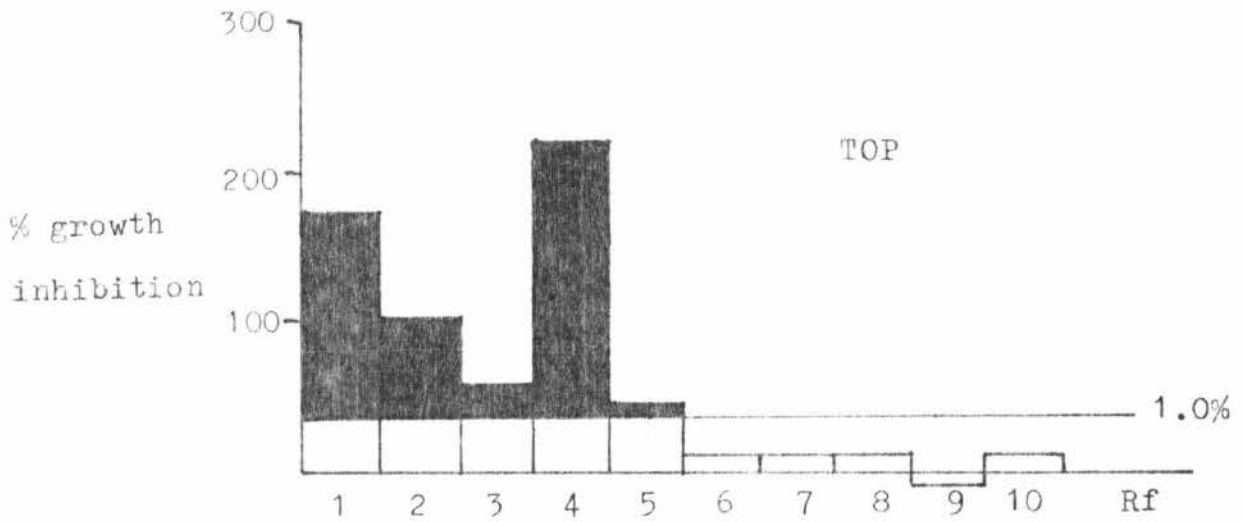
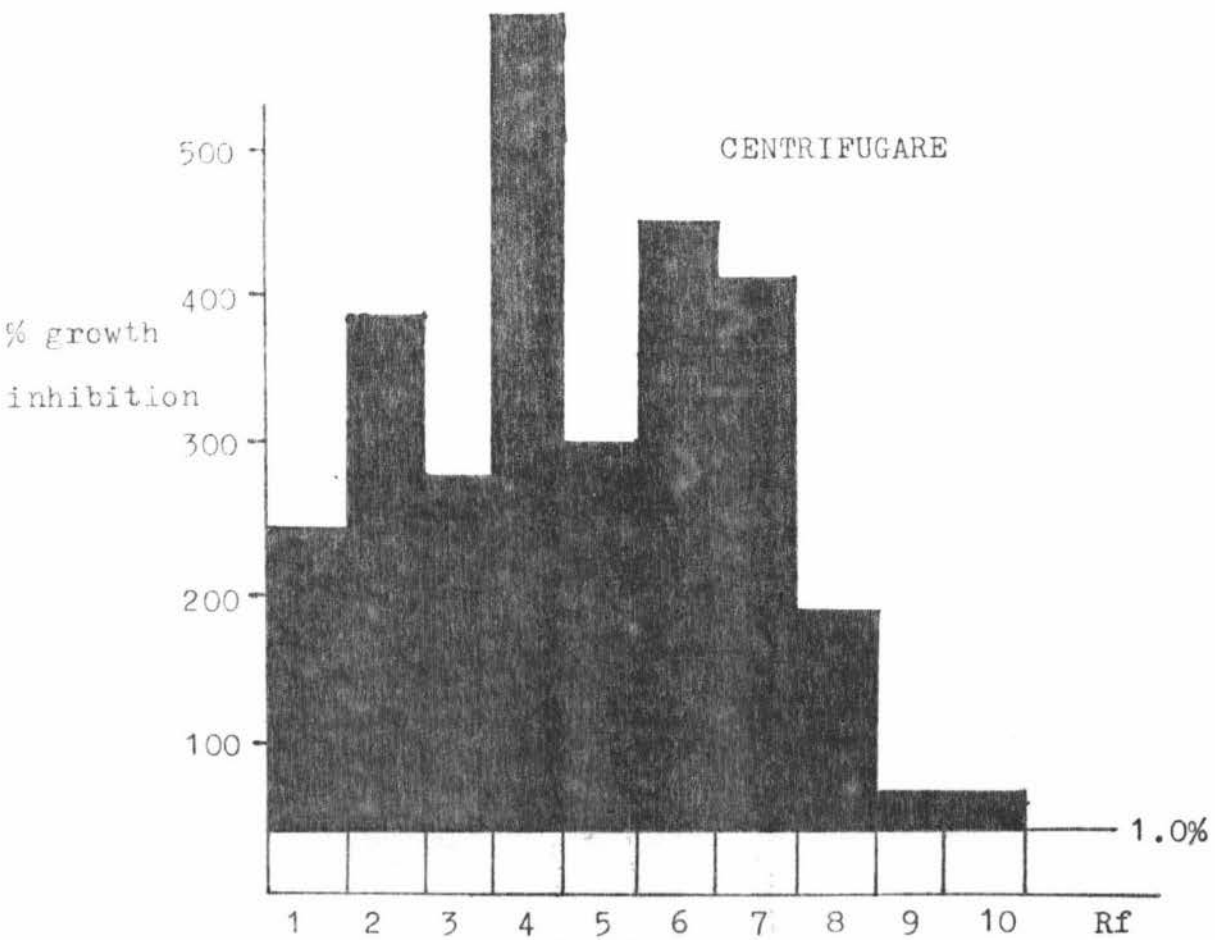

5
6
7
8
9
10
Rf
1.0%
20
0.01%
40
% growth
inhibition
CENTRIFUGATE

FIGURE 4.8.4

ROOTING COFACTORS EXTRACTED FROM STEM TISSUES OF BRUNO




300
200
100
% growth
inhibition
TOP
1.0%
1 2 3 4 5 6 7 8 9 10 Rf


500
400
300
200
100
% growth
inhibition
CENTRIFUGARE
1.0%
1 2 3 4 5 6 7 8 9 10 Rf

The promotive effect of IAA was also highly significant ($P < 0.001$). With the presence of IAA, the IBA treatments were found to be more effective even at very low concentration of IBA. In fact, just the application of 0.1% IAA alone would improve rooting by 25.0% of the leafless cutting. An additive effect between IAA and IBA was evidenced, though it was not statistically significant.

Centrifugation generally gave only a small reduction in the already low percentage rooting. It reduced the effect if IBA and IAA, though the analysis of variance shows no significant effect of centrifugation, which suggests only small amounts of endogenous root promoting factors were present in the cutting. An examination of the centrifugate, excised bases and remaining cuttings would indicate the nature of the extractable growth promoter.

An examination of the endogenous IAA, IAN, ABA and rooting cofactors is presented as histograms in Figures 4.8.1, 4.8.2, 4.8.3 and 4.8.4 respectively.

Table 4.8.3 Extractable Level of endogenous growth regulators from the excised bases, cuttings and centrifugate of Bruno after centrifugation

	IAA	IAN	ABA	Cof 1	Cof 2	Cof 3	Cof 4
Top	+	++	++	++	++	+	+
base	++	++	+	++	++	++	+
centrifugate	+++	+	+	+++	+++	+++	++

Table 4.8.3 is a summary of the extractable growth regulators from the excised bases, cuttings and centrifugate of Bruno after centrifugation.

Considerable reductions in endogenous IAA, cofactor 1, 2, 3, and 4 were achieved by centrifugation. The centrifugate contained the highest level of IAA, and the 4 cofactors. The basal extract contained the next highest concentration, and the cuttings retained the lowest concentration of IAA and cofactors. This indicates that IAA and the 4 cofactors were removed from the cuttings by centrifugation.

Other growth regulators examined were IAN and ABA. It was evident that centrifugation had only a small effect on the mobilization of the IAN and ABA. For IAN, although a considerable level is present in the bases which were excised, the cuttings still retained a high level of the promoter.

4.8.4 DISCUSSION

Tables 4.8.2 and Figure 4.8.1 showe that improved rooting on Bruno cuttings in June was due to the high levels or increases in endogenous IAA levels. This result reaffirms that of Section 4.7.

It was also clear that the promotion of rooting by IBA can be enhanced by high auxin levels. Combined addition of IAA and IBA produced a greater promotive effect on rooting when compared with either auxin applied singly.

Centrifugation reduced the rooting ability and this reduction of root initiation seems likely to be a result of depleted endogenous IAA (Figure 4.8.1), a situation comparable to that of Malus sylvestris M. (Tustin, 1976). Partial restoration of the rooting ability was possible by the addition of exogenous applied auxins.

It is evidenced that IAA is one of the fundamental promoters of root initiation in Bruno cuttings.

Many recent research papers show that root initiation was an IAA-specific mechanism and that IAA influenced the first "Initiation of root meristems" phase of adventitious root regeneration (Haissig, 1970, 1973; Eriksen,and Mohammed, 1974; Mohammed and Eriksen, 1974) and that, the initiation phase is dependent upon a continuous supply of IAA rather than its availability at one time in high concentration (Haissig, 1970, 1973; Eriksen and Mohammed, 1974; Mohammed and Eriksen, 1974; Ryugo and Breen, 1974; Greenwood et al, 1974; Mohammed, 1975). Present results suggest that root initiation in Bruno is controlled primarily by endogenous IAA levels.

The traditional interpretation of the role of exogenous auxin application as been to increase the total auxin pool to a level which will promote root initiation, but the present results show that rooting was promoted by endogenous IAA and that IBA could only further promote root initiation in the presence of a threshold level of IAA. Similar situation was reported by Lanphear and Meahl (1963), Tustin (1976).

In the absence of IAA, IBA is effective only at high concentration (>10,000 ppm), but this promotive effect is quickly saturated at 20,000 ppm IBA and toxicity occurs. In contrast, IAA is effective even without IBA treatment (Table 4.8.2), however, increasing concentration of IBA will further enhance the promotive effect of IAA. Also it was noticed that changes in IBA concentration from 10,000 to 20,000 ppm in the - centrifugation + IAA treatment did not result in toxicity and reduction of rooting percentage was found in that of - centrifugation - IAA; + centrifugation - IAA; + centrifugation + IAA; treatments. As one could postulate that IAA level in the latter treatments is lower than that of the former. It is therefore considered that IAA is the primary factor in the promoting of rooting, and that the portulation that IBA promotes rooting by the same mechanism as IAA does not

seem tenable. An effect on some supporting mechanism of IAA induced root initiation appeared more likely to be the role of IBA in promoting root formation.

A suggestion that IBA acts by undergoing B-oxidation to form IAA as found by Fawcett et al (1958) does not seems likely since some of the reported effects of using IBA for propagation are the persistance, poor translocating ability and resistance to auxin - degrading enzymes (Weacer, 1972.)

By studying the metabolism of C^{14} -IAA in the cutting base, Tustin (1976) proposed that IBA exerts its promotive effect by protecting the endogenous IAA from enzymic degradation and conjugation (both inactivation processes). In another words IBA protects and ensures the persistence of free IAA in the basal tissue. Ryugo and Breen (1974) also found that IBA inhibited IAA - oxidase activity as measured by the rate of $14CO_2$ evolution in plum cuttings.

Present evidence shows that IBA is not acting on the same root-promoting process as IAA. Rather, IBA can be seen as promoting root initiation by some supporting reaction to that of IAA. Inhibition of the IAA - inactivation systems in Bruno could account for the observed promotion of root initiation in these cuttings. In cuttings with a high endogenous IAA level, any inhibitor of IAA inactivation would allow optimum levels of IAA to be reached more rapidly in the cutting base thus facilitating greater induction of root primordia, the auxin sensitive phase of root initiation (Haissig, 1970; Greenwood et al, 1974; Mohammed and Eriksen, 1974). This would be reflected in a promotion of root initiation. In August with a low IAA status, application of IBA may not promote rooting, since insufficient IAA may have been synthesised even though IAA inactivation systems were inhibited. If IBA was applied and the endogenous IAA level increased

simultaneously, (eg. in Spring and Summer or by exogenous IAA - lanolin application) the IAA would be protected from inactivation and could then induce root initiation. The protective action of IBA would allow endogenous IAA levels at the base of the cutting to increase to a concentration nearer the optimum for initiation of roots. This was the most likely mode of action in centrifuged Bruno cuttings treated with IBA/IAA. Odom and Carpenter (1965) found that the greatest response to synthetic root - promoting substences was from cuttings which exhibited a slowly accumulated acidic auxin content.

Thus IAA appeared to be the fundamental promoter of root initiation in cuttings, but this does not exclude the possibility of the involvement of other growth regulators. The fact that exogenous application of 0.1% IAA and IBA could only partially restore the rooting ability in the June collection lead to two conclusions (a) even with the application of 0.1% IAA, the total IAA level is still sub-optimum. (b) other rooting regulator(s) is/are limiting. The second option is strongly supported by the fact that centrifugation removed a considerable amount of rooting cofactors (Figure 4.8.4) which may be part of the reason for the relatively low rooting percentage after centrifugation, and that only partial restoration was possible by addition of exogenous auxins.

The mung bean bioassays of the centrifugates and tissues samples indicate a reduction in the rooting cofactors level within the cutting which corresponds with the reduction in rooting ability after centrifugation. This observation supports the conclusion drawn in Section 4.7 that cofactors play a role in root formation. Further reference to cofactors is included in General discussion 6.

Only a small portion of the ABA was extracted by centrifugation, yet a significant ($P < 0.5$) drop in rooting percentage was observed after centrifugation.

To be consistent with the conclusion (4.7) that ABA promotes rooting, the only feasible explanation for the present observation is that, the initial levels of ABA in the cuttings were marginal, thus even a slight reduction in ABA due to centrifugation would reduced the ABA level so as to become sub-optimal, consequently resulting in a significance drop in rooting percentage.

4.9 THE EFFECT OF LEAF

4.9.1 INTRODUCTION

Plant propagators are well aware the loss of leaves from cuttings greatly reduces the chances for successful rooting (Duncan and Davis, 1977; Hartmann and Kester, 1975). However, the mechanism of this promotive effect of the leaves is still a debatable issue. Many researchers suggested that the role of leaves is mainly a nutritive one (Zimmerman and Hitchcock, 1933; Altman, 1972; Bilan, 1974; Ohta and Furukawa, 1975; Porlingis and Therios, 1976). Hartmann and Kester (1975) stated that the root promotive effects of leaves and buds are due chiefly to auxin production. There is a third group advocating the rhizocaline hypothesis (Cooper, 1938; Went, 1938; Bouillenne and Bouillenne-Wartand, 1955). The following study attempts to evaluate these hypotheses with repect to the role of leaves in Abbott cuttings.

4.9.1.2 METHOD

Cuttings of Abbott variety were harvested on 26.1.78, and subjected to the following treatments.

	No IAA		+ IAA (0.1%)	
	leaf	No leaf	leaf	No leaf
bud	1	2	5	6
No bud	3	4	7	8

Numbers 1-8 denote treatment number.

There were 40 cuttings for each treatment with 8 replications arranged randomly.

Bud and leaf were removed with a scalpel. In cuttings with the leaf retained, half of this leaf was cut off to facilitate handling.

Table 4.9.1 Percentage rooting of Abbott leafy and leafless cuttings in response to IAA

	No IAA		+ IAA	
	leaf	- leaf	leaf	- leaf
bud	13.3	0	80	23.33
No bud	8.3	0	81.7	23.67

Table 4.9.1 shows the results of the experiment. Details of the analysis of variance is presented in Appendix 2.

The effects of IAA and leaves on rooting percentage were highly significant ($P<0.01$), their interaction effect also being highly significant ($P<0.01$). The bud does not exert any significant effect either alone or with auxin.

Cuttings not treated with IAA but with their leaves removed, failed completely to root. Auxin treatment could not replace the function of the leaves completely but rather it produces a synergistic effect with the leaves.

4.9.2.1 INTRODUCTION

In the previous section, leaf was found to exibit synergistic effect with IAA. And many workers had attributed such promotive effect of the leaf to a nutrition especially carbohydrates (Zimmerman and Hitchcock, 1933; Girouard and Hess, 1964; Altman, 1972; Bilan, 1974; Altman and Wareing, 1975; Porlingis and Therios, 1976). A classical method used to verify this hypothesis is by external application of IBA, sucrose and a suitable source of nitrogen to test whether it replaced the root forming effect of leaves (Van Overbeek, 1945; Ohta and Furukawa, 1975).

4.9.2.2 METHOD

Abbott cuttings were taken on 26.6.78, a time when rooting was expected to be very low, therefore any positive result of the nutrition treatment would then strongly illustrate the hypothesis.

The treatments were as follows : -

	+IBA		-IBA	
	+leaves	-leaves	+leaves	-leaves
+ NH_4SO_4 + Sucrose	1	3	5	7
- NH_4SO_4 + Sucrose	2	4	6	8

Numbers 1-8 denote treatment number

The carbohydrate used was 4% sucrose solution, with the nitrogen source as 1000 mgl^{-1} NH_4SO_4, while the auxin treatment was 0.8% IBA in talc. There were 10 cuttings in each treatment replicated 5 times. Cuttings were dipped in the respective solutions for 24 hours and then planted in sand according to a randomised design.

4.9.2.3 RESULTS

Cuttings which originally sustained a leaf dropped them shortly after being planted on the heated bed, a phenomenon corresponding to that of the plant in the field.

None of the cuttings succeeded in forming roots. Cuttings treated with Auxin and nutrient did not show any rooting either.

4.9.3 DISCUSSION

The results in Table 4.9.1 show that leaves play a very significant role in root formation of Abbott stem cuttings. This promotive effect of leaves on root

formation has been reported in hop cuttings (Howard, 1965) Justicia gendarussa Linn cuttings (Sen and Basu, 1960), olive cuttings (Porlingis and Therios, 1976), Pinus radiata (Cameron and Rook, 1974), Phaseolus vulgaris cuttings (Altman and Wareing, 1975).

In the June collection cuttings that originally had leaves, lost them by abscission shortly after the cuttings were been placed in the heated bed, and none of the cuttings rooted. In fact plant propagators are well aware that the loss of leaves from cuttings greatly reduces chances of successful rooting (Duncan and Davies, 1977; Hartmann and Kester, 1975). Fletcher (1976), Opitz and Beutel (1975) also recommended taking cuttings with leaves in the propagation of Actinidia chinensis (Planch.). Many other workers have stressed the importance of leaves on cuttings, and the importance of retaining these leaves until after roots have been formed (Chaudary, Ullah and Ahmad, 1962; Cooper, 1938; Dubrovickaja and Krenke, 1958; Halma, 1931; Went, 1934). These reports indicate that some compound or compounds are produced in the leaves, and that these materials have an effect upon the formation of roots (Gregory, Van Overbeek, 1945; Van Overbeek and Gregory, 1945).

Leaves had been known to be powerful hormone producers (Bouillenne and Went, 1933; Bouillenne and Bouillenne - Warland, 1955; Girouard, Hess, 1964; Okoro and Grace, 1976). The IAA was a limiting factor of root formation since extenal application of IAA increased the percentage of rooting of leafless cutting by 23.7%. But the synergistic interaction effect of IAA and leaves suggests that IAA and leaves are probably not acting through the same mechanism in promotion of root formation, and that the poor rooting of leafless cutting may be due to factors other than auxin. This conclusion is in agreement with those of Zimmerman and Hitchcock (1923), Girouard and Hess (1964); Ohta and Furukawa (1975); Porlingis and Therios (1976).

The carbohydrate resulting from photosynthesis in the leaves, has also been shown to contribute to rooting (Zimmerman and Hitchcock, 1933; Bilan, 1974; Altman and Wareing, 1975; Porlingis and Therios, 1976). Haissig (1974c) further concluded that auxin stimulates the initiation of root formation, whereas nutritive materials like carbohydrates, nitrogenous compounds, vitamins etc are essential energy sources and act as the building blocks during root formation. This seems to account for the highly significant ($P < 0.001$) synergistic interaction between leaf and IAA in experiment 4.9.1. However, the data of experiment 4.9.2 rejected this leaf-carbohydrate hypothesis. Exogenous application of IBA, sucrose and ammonium sulphate, did not stimulate any root formation. This indicates that other substances must be present in the leaves to perform the synergistic interaction with auxin in experiment 4.10.1, a conclusion parallel to many others. Girouard and Hess (1964), found that in Hedera helix, the presence of leaves not only improves rooting by synthesis of photosynthates but also serving as a means of retaining intrinsic rooting substances which have a tendency of diffusing out in their absence. The leaves of the easy-rooting Hibiscus rosa-sinensis L. seem to supply a factor necessary for root induction by auxin (Overbeek and Gregory, 1945). Leaves do supply thiamine (Bonner, 1942) and pyridoxine (Bonner and Dorlund, 1943) necessary for root formation.

Some evidence about the nature of the substance produced by the leaves which interact with auxin to control rooting was produced by Cooper (1935) who found that auxin gave greater stimulation of root formation on leafy cuttings than in cuttings where the leaves had been removed and from which he concluded that some compound from the leaves was associated with the process of root formation. Later he found that, if the base of the cutting is treated with auxin, and the base then cut off, the cutting did not respond in the same way to retreatment with auxin. This

indicated the depletion of 'rooting substances' by auxins, and indicated the primary effect of root formation is not from auxin but 'Rhizocaline' (Cooper, 1936, 1938; Went and Thimann, 1937).

Further experiments by Went (1938) showed that both auxin and rhizocaline are essential for root formation in cuttings. Auxin causes redistribution and accumulation of the rhizocaline at the base of the cuttings. Rhizocaline was proposed to be considered as a complex of 3 components:-

1) A specific factor, translocated from the leaves and characterised chemically as an otho-dihydroxyphenol.

2) A non-specific factor (auxin) which is translocated downwards and found in biologically low concentrations.

3) A specific enzyme located in cells of certain tissues. (pericycle, phloem, cambium) which is probably of the phenol-oxidase type (Hartmann and Kester, 1975; Bouillenne and Bouillenne-Warland, 1955).

An overall evaluation of the effect of leaves on root formation based on present data, seems to reject the hypothesis that the role of leaf in root formation is mainly as an IAA or carbohydrate supplying agent. But several other hypotheses mentioned in the discussion can not be fully justified until further biochemical analysis is carried out to determine the substrated produced by the leaf.

CHAPTER 5

PROPAGATING ACTINIDIA CHINENSIS (PLANCH.) BY ROOT CUTTING

5.1 INTRODUCTION

The root cutting method of propagation is one of the least frequently used of all methods of vegetative propagation. The reason fot it is probably because it is inconvient to secure the cuttings from under the soil and many plants do not form adventitious buds on their roots or root parts.

Despite the difficulties in securing propagating material, root cutting propagation is by far the best method for increasing certain special plants which will not root from stem cuttings (Flemer, 1961).

Propagation from roots is also a most valuable tool for restoring a state of juvenility in certain plants where this is a most valuable condition for subsequent propagation.

The following experiments, attempt to assess the feasibility of propagating Actinidia chinensis by root cuttings.

5.2 THE SHOOT REGENERATION POTENTIAL OF ROOTS OF DIFFERENT DIAMETER

5.2.1 INTRODUCTION AND METHOD

This was a preliminery trial to see what type of root thickness was most suitable for shoot regeneration.

Abbott root cuttings were taken on 1/4/77.

Roots excavated were divided into 3 groups according to their root diameter : -

group A - less than 0.5 cm.
group B - 0.5 - 1.5 cm.
group C - 1.5 cm. to 2.5 cm.

There were 32 cuttings in each group and arranged into 8 blocks. The cuttings were inserted horizontally 1.0 cm. below river washed sand surface. All the cuttings were 15 cm. in length. After 60 days the percentage shoot regeneration and number of shoots per shooted cuttings were recorded.

5.2.2 RESULTS

Table 5.2.1 shows the summary of the result. For detailed analysis of variance refer Appendix 3.

Table 5.2.1

Table showing the percentage shoot regeneration and number of shoot per root cutting

Root diameter	% shooted	No. shoots per shooted cutting
0.5 cm.	12.5 c	2.0
0.5 - 1.5 cm.	53.125 a	2.05
1.5 - 2.5 cm.	43.75 b	2.4

Figures denoted by a different letter of the alphabet are significantly different when analysed by Least significant difference (0.005).

5.2.2 RESULTS AND DISCUSSION

About 7 days after planting, some rounded swellings formed in the root periderm. After about twenty days the swellings ruptured and green shoots emerged which could grow up to 10.0 cm. by 60 days after planting. These tall thin stems bore small round serrated leaves, regarded as typically juvenile features. The shoots usually emerged from the cut or wounded surface, at the proximal end of the root cuttings.

There was pronounced polarity along the root cuttings with the shoots arising in the proximal end. Callus was produced from both cut surfaces but predominantly at the distal end. Multiple shoot production from a single bud site was a common phenomenon.

Softwood shoots developing on root pieces were characterized by the complete absence of adventitious roots at their bases, only very rarely ($< 1\%$) does the shoot posses adventitious roots at its base. Detachment was shown to be essential for new root growth which then readily occurred under mist.

This preliminary experiment on planting method and root diameter (ranging from less than 0.5 cm. to more than 1.5 cm.) showed that roots in the size range from 0.5 - 1.5 cm. gave reliable shoot production. Shoot numbers increased significantly ($P < 0.01$), 2.05 for roots of diameter between 0.5 cm - 1.5 cm. and 2.4 for root of diameter greater than 1.5 cm. The percentage of roots producing one or more shoots in the 3 diameter classes were 12.5, 53.125, 43.75 respectively. In this experiment longitudinal polarity was strong, over 95% of all shoots being produced on the proximal polarity, (shoots originating both on the upper and lower sides.) Roots around 1.0 cm. in diameter could survive more than 4 months producing an average of 9.5 shoots each, if early shoots were removed.

For subsequent experiments, roots in the diameter range 0.5 - 1.5 cm. were selected and were planted horizontally 1.0 cm. below the surface.

5.3 ROOT LENGTH AND POLARITY

5.3.1 INTRODUCTION AND METHOD : -

The orientation and lengths of Abbott root cuttings were compared to evaluate the optimum for shoot regeneration. Abbott roots were collected on 11/7/77 and 31/9/77. The roots were cut into length of 15 cm. each and 5 cm. each. These cuttings were then planted in the sand horizontally, vertically up-right or vertically upside down as indicated in the table below : -

Treatments

root length	orientation
5 cm.	Vertically upright
5 cm.	Vertically upside down
5 cm.	Horizontal
15 cm.	Horizontal

The cuttings were propagated under mist and basal heat for 60 days and the percentage strike and number of shoots per cutting were recorded. The standard root length was 15 cm. and data from shorter (5 cm.) cuttings were converted to 15 cm. equivalent for analysis.

Root thickness was 0.5 - 1.0 cm.

FIGURE 5.2.1

PHOTO SHOWING THE SHOOT REGENERATION CAPACITY OF ROOTS OF DIFFERENT DIAMETER AND LENGTH

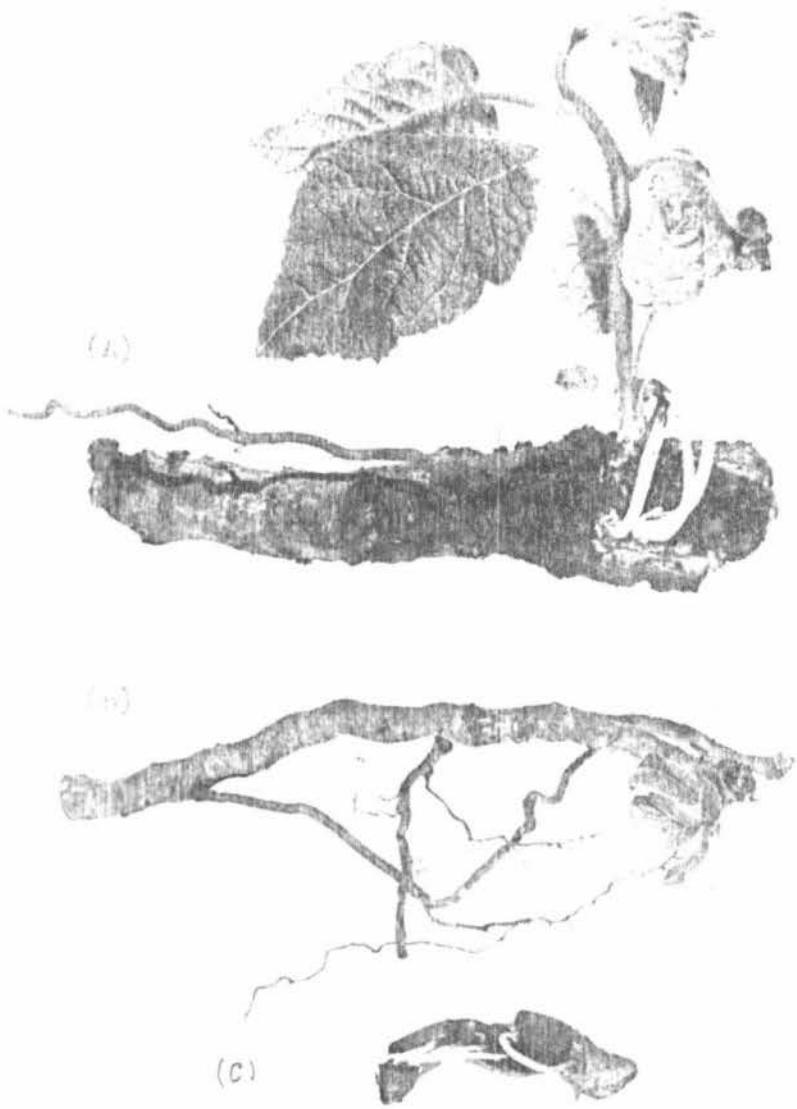


FIGURE 5.1.1

A) Length = 15cm., diameter = 2 cm.
B) Length = 15cm., diameter = 0.5 cm.
C) Length = 5 cm., diameter = 0.5 cm.

FIGURE 5.2.2

PHOTO SHOWING THE SHOOT REGENERATION CAPACITY OF ROOTS OF DIFFERENT DIAMETER

(A) Length = 5 cm., diameter = 2 cm.

(B & C) Length = 5 cm., diameter = 1 cm.

FIGURE 5.2.3

CLOSE-UP PHOTO SHOWING LOCATION OF SHOOT EMERGENCE FROM CORTEX OF CUT END.

5.3.2 RESULTS

Tables 5.3.2 and 5.3.3 showing the results on the two harvesting dates. Detail of the statistical analysis is presented in Appendix 3.

Table 5.3.2 Shoot regeneration capacity of Abbott root cuttings taken on 11/7/77

	% shooted	No. shoot/ cutting		No. shoot/ 15cm
long horizontal	100	4.3	a	4.3
short horizontal	100	3.6	b	10.8
short upright	100	2.4	c	7.2
short upside down	0	0	d	0

Table 5.3.3 Shoot regeneration capacity of Abbott root cuttings taken on 31/9/77

	% shooted	No. shoot/ cutting		No. shoot/ 15cm
long horizontal	100	4.6	a	4.6
short horizontal	80	1.4	b	5.2
short upright	50	0.8	c	2.4
short upside down	0	0	d	0

For July harvest, root cuttings were 100% shooted, except for cuttings that were planted upside down which did not form any shoot at all.

On 31/9/77, long cuttings appear to have a higher percentage strike compare with short pieces. But when considering the number of shoots produced per 15 cm. root length, short horizontal root pieces yield significantly ($P<0.01$) higher than that of long (15 cm) horizontal ones. While the short upright root cutting performed worse both in terms of percentage and number of

shoot per cutting.

A strong polarity is observed both in July and September collection. Cuttings which are planted upside down failed to shoot at all, and for those cuttings which stood upright or lay horizontally, the shoots always emerged only form proximal end (Fig. 5.2.3).

5.4 SEASONAL EFFECTS

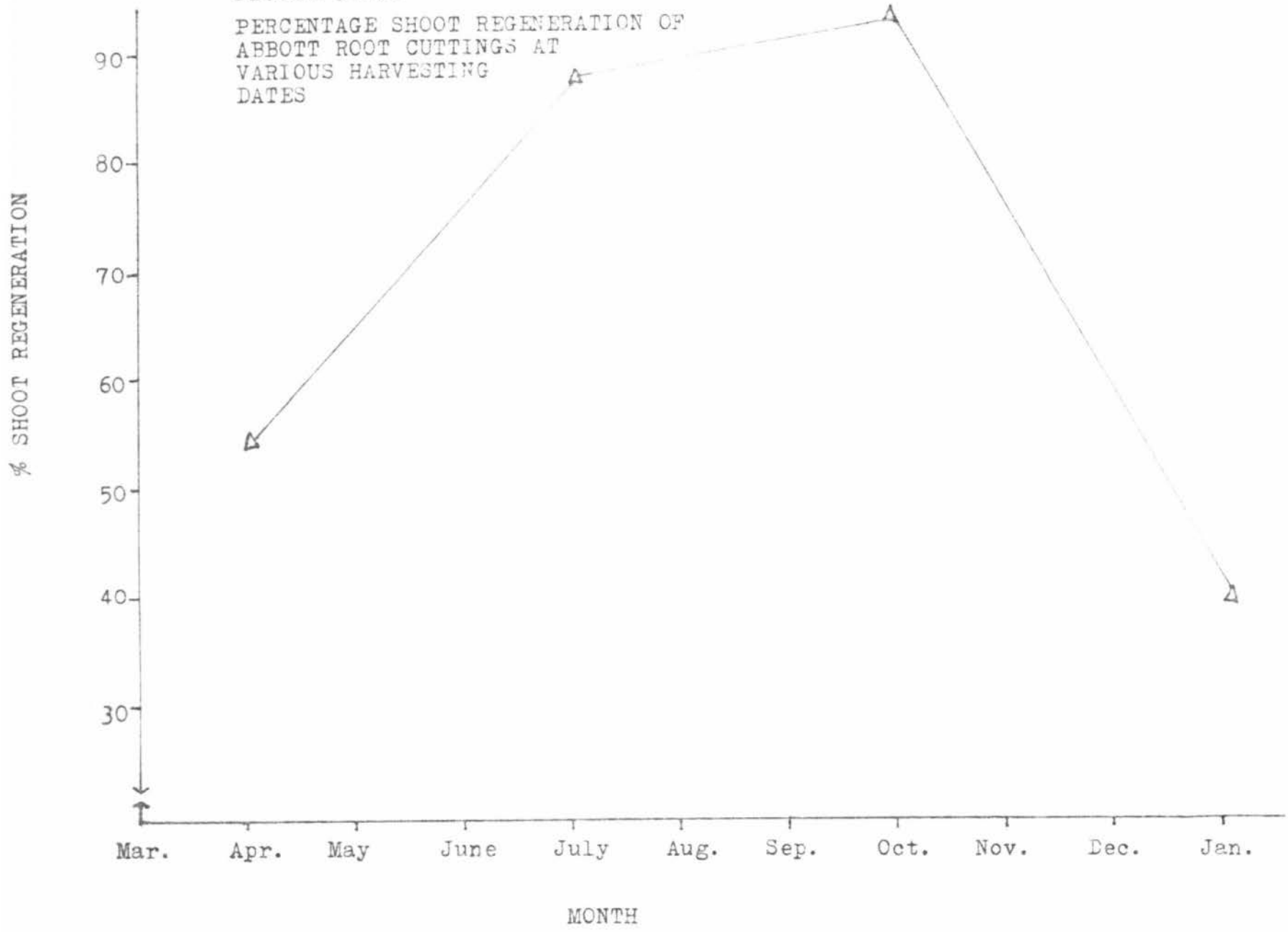
5.4.1 INTRODUCTION AND METHODS

Periodicity in the capacity of cuttings to regenerate is well known in horticulture. The seasonal fluctuation of stem cuttings is discussed in Chapter 5.4. In root cuttings, this seasonal cycle in shoot regenaration is well documented by Wenger (1953); Hudson (1954, 1955); Mackenzie (1957); Hartmann and Kester (1975) and Robinson and Schwabe (1977).

Experiments with Abbott root cuttings were carried out to assess their relative ability to regenerate at various time of the year namely Autumn (1/4/77), Winter (11/7/77), Spring (1/10/77) and Summer (8/1/78). 50 cuttings of 15 cm. diameter in length each were planted horizontally 1.0 cm. below sand surface and left on a heated bench and under mist for 60 days. After which, the percentage of shoot regeneration was recorded.

FIGURE 5.4.1

PERCENTAGE SHOOT REGENERATION OF ABBOTT ROOT CUTTINGS AT VARIOUS HARVESTING DATES



5.4.2 RESULTS

Table 5.4.1 Percentage of shoot regeneration in Abbott root cuttings at various harvest dates

	harvesting date			
	1/4/77	1/7/77	31/9/77	8/1/78
1	7	7	9	4
2	5	10	8	4
replicate 3	4	10	10	4
4	6	7	10	4
5	5	10	10	3
Total shooted	27	44	47	19
% shoot regeneration	54	88	94	38

Results are presented in Table 5.4.1. The detailed statistical analysis of the data is presented in Appendix 3.

The analysis of variance shows that there is a highly significant ($P < 0.01$) difference in the shoot regeneration capacity at various season, lowest in Summer, higher in Winter and early Spring.

5.5 APPLIED GROWTH REGULATORS

5.5.1 INTRODUCTION AND METHOD

Hormonal regulation of bud initiation on roots was suggested by the seasonal fluctuation of shoot forming ability, and by a pronounced polar distribution of buds suggesting a gradient of inhibition and promotion

along the cuttings. Evidence for such control was sought by applying auxin (150 mgl^{-1} IBA), gibberellin (50 mgl^{-1} GA_3), cytokinin (75 mgl^{-1} benzylaminopurine BAP) and sucrose to root cuttings to modify the hypothetical gradient.

Abbott root cuttings of 5cm. long were treated with various growth regulators and sucrose as shown in Table 6.5.1 and 6.5.2. There were 10 cuttings for each treatment replicated 4 times. Treated cuttings were then arranged according to radomised design and propagated under mist and bottom heat for 60 days. The treatment commenced on 1/4/77 when performance was expected to be poor for an untreated root cutting.

Table 5.5.1

Treatments of Various hormone on Abbott root cuttings

	Apply to distal end	Apply to proximal end
distill water	1	5
IBA (200mgl^{-1})	2	6
GA_3 (50 mgl^{-1})	3	7
BAP (75 mgl^{-1})	4	8

Numbers 1-8 denote the various treatments.

Table 5.5.2

Treatments of BAP & Sucrose on Abbott root cuttings

BAP mgl^{-1} Sucrose	0	10	40
5%	a	b	c
0%	d	e	f

Alphabets 1-f denote the various treatments.

The hormones like IBA, GA_3 and BAP were initially dissolved in ammonia solution and then vapourised to dryness under a rotary evaporator. This was then picked up in distilled water and to the concentration required. During the treatment, cuttings were soaked in the solution for 24 hours.

RESULT : Table 5.5.3

EFFECT OF IBA (150 mgl^{-1}) treatment on Abbott root cuttings

	% shooted	no shoots per cutting
proximal	10 XXX	2
distal	40	3
Etoh	42.5	3.5

Table 5.5.4

Effect OF GA (50 mgl^{-1}) treatment on Abbott root cuttings

	% shooted	No/cutting
proximal	42.5	2.3
distal	40.0	2
Etoh	42.5	1.5

Table 5.5.5

Effect of BAP (75 mgl^{-1}) treatment on Abbott root cuttings

	% shooted	No/cutting
proximal	85 XX	5
distal	65 XX	4.0
Etoh	40	1.5

Table 5.5.6

Effect of BAP and sucrose on shoot regeneration

	% with shoot	no. shoots/ cutting
5% Sucrose		
0 ppm BAP	50	2.2
10 ppm BAP	75	2.8
40 ppm BAP	87.5	3.2
0% Sucrose		
0 ppm BAP	32.5	1.8
10 ppm BAP	47.5	2.2
40 ppm BAP	67.5	2.0

The summary of the results is presented in Table 5.5.3 to 5.5.6, and details of the analysis of Variance are in Appendix 3. Proximal application of IBA significantly ($P < 0.01$) reduced the percentage of shoot regeneration, and the number of shoots per cutting. The 24 hours basal dip of the cutting in 200 mgl^{-1} IBA solution did not have any significant effect on shoot regeneration.

Treating the root cuttings with 50 mgl^{-1} of gibberellin for 24 hours, did not have any significant effect whether treatment was applied at proximal or distal end of the cuttings.

BAP improved shoot regeneration by 45% if applied at proximal end and 25% when applied at distal end. The number of shoots per cutting increased as well.

The demonstration of the effect of BAP and sucrose on shoot formation is shown in Table 5.5.6. Both BAP and sucrose increased the percentage shooting significantly ($P < 0.01$). Sucrose also increased the shoot

number per cutting, whereas BAP show no significant effects on shoot number.

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSION

6.1 STEM CUTTING

The formation of adventitious roots is a very complex process, governed by some anatomical, physiological and hormone parameters which in turn are influenced by a multitude of environmental factors.

Wounding, by a lengthwise cut through the bark at the base of the cuttings was shown to improve rooting, a phenomenon reported on many occasions (Hartmann and Kester, 1975; Day, 1933; Beakbane, 1961; Stonier and Yoneda, 1967; Stonier and Yang, 1973), and popularly practised by plant propagators for ages, but the mechanism is still not fully understood. Several hypothesis had been put forward to explain this phenomemon. Cells adjacent to the wound are stimulated into cell division and aid root initiation, due to a natural accumulation of hormones and carbohydrates around the wound, and also with an increase in respiration (Hartmann and Kester, 1975); wounding increased uptake of growth substances and water (Day, 1933); wounding helps to break through the tough fibre cells surrounding the point of root origin (Beakbane, 1961; Ciampi and Gellini, 1958); wounding brings enzyme and modifiers to the site of IAA oxidation (Stonier et al, 1970; Stonier and Yang, 1973).

The influence of sex in the propagation of plants by cuttings has received very little attention. Snow (1942), Edgerton (1944) and Neal and Pearse, 1954 had reported sexual differences in the rooting of various cuttings. In the present investigation, no significance difference between sex in Actinidia chinensis (Planch.) (Haywood and Abbott- female, Matua- male) was observed on two occassions - 5/3/77 and 25/1/78. Varietal differences

in rooting ability on stem cuttings has been discovered in fruit trees (Hartmann and Hansen, 1958; Higdon and Westwood, 1963; Kender, 1965; Kester and Sartori, 1966; Sinha and Vyuyan, 1943; Tustin, 1975) and several ornamental plants (Childers and Snyder, 1957; Gregory and Overbeek, 1945; Hess, 1962, 1963a, 1963b). In Actinidia chinensis (Planch.), Fletcher (1976) commented that Abbott is prone to excessive callusing and thus is more difficult to raise satisfactorily compared with other varieties such as Hayward and Bruno. However, present data fail to support this view, no significant differences were found in the 3 varieties tested, Hayward, Abbott and Bruno on the two occasions 5/3/77 and 25/1/78.

The trial set up to investigate the feasibility of using polythene tent, instead of the usual mist propagation, for propagating leafy cuttings of Actinidia chinensis (Planch.), showed no significant differences between treatments, however a significance ($P<0.01$) interaction is observed between mist and IBA treatment. This suggests that misting probably provides a better condition for auxin activity which in turn stimulates rooting. This can be attributed to one or several of the following explanations.

i) Mist provides cooling effect on leaf temperature (lower by $4^{\circ}C$), this may affect the activities of hormones and/or enzymes.

ii) In the polythene tent, humidity varies with water vapour pressure around the leaf, (Hartmann and Kester, 1975) this may also affect hormonal and enzymic activity.

iii) Mist may leach away certain inhibitory substances in the cuttings.

The beneficial effects of bottom heat reported by Hatcher and Garner (1957) and Howard (1968) were demonstrated in Hayward cuttings. A highly

significant ($P < 0.001$) IBA/bottom heat interaction was detected too. However, this promotive effect of IBA and bottom heat alone or in combination was found to be lost in the August harvest. Bottom heat and IBA improves rooting, but on the other hand the rotting percentage was increased also, especially in late Winter when conditions are not favourable for rapid callus and root development.

Increasing concentration of IBA was found to be beneficial to rooting up to a concentration as high as 5000 ppm. The present commercial usage of 0.8% IBA in talc can be considered as near optimum especially in the 25/1/78 collection. In other seasons, rooting response varies, which could be due to other factors which will be discussed latter.

Reports on the influence of the season of harvest on adventitious root formation of fruit tree stem cuttings indicated that Autumn and Spring planted cuttings rooted most readily with a definite falling-off of rooting ability occuring during the mid Winter (Howard, 1966; Fadl and Hartmann, 1967a; Nesterov, 1968; Howard and Nahlawi, 1969a; Doud and Carlson, 1972). This seasonal fluctuation in rooting ability was demonstrated in both IBA treated and untreated Hayward and Abbott cuttings. Cuttings perform better in Spring and Summer but decline in Autumn and Winter. It was also apparent that the promotion of rooting was most pronounced in cutting exhiting a higher rooting potential (that is in Summer). This seasonal periodicity in its rooting ability accounts for the usual preferences for taking leafy softwood cuttings during the actively growing season of Actinidia chinensis (Planch.) (Smith, 1973; Opitz and Beutel, 1975; Fletcher, 1976; Duncan and Davis, 1977).

It has been known for a long time that bud activity may influence root initiation (Van de lek, 1925; Went, 1929; Harada and Nakayama, 1959; Fadl and Hartmann,

1967a; Roberts et al, 1974). The presence of active buds has a promotive effect on rooting (Lek, 1925; Went, 1929; Fadl and Hartmann, 1967; Whitehill and Schwabe, 1975, while Snyder, 1955; Howard 1968(a); Tustin, 1975) found no correlation between rooting response to the degree of bud activity. In contrast, Cahlalijah and Nekrasova (1962, cited by Biran and Halevy, 1973) reported that dormant buds would facilitate rooting of peach cuttings. Seasonal changes in root formation of Abbott cuttings did not support any positive or negative correlation with bud activity. This controversial evidence was best explained by Biran and Halevy (1973). They proposed that growing buds affect rooting in two opposed directions, that is, they inhibit rooting, on the one hand by diverting certain metabolites away from the rooting zone, and on the other, they also promotes rooting by enhancing cambial activity and creating a fine balance of phytochromes favourable for rooting. Whichever influence is stronger will determine promotion or inhibition.

Among the many biochemical factors, IAA had been considered to be of greatest importance in respect to the formation of roots on cuttings, (Cooper, 1935; Overbeek and Gordon, 1945; Spiegel, 1954; Vieitez, et al, 1964; Smith and Wareing, 1972 ; Odom and Carpenter, 1965; Haissig, 1970; Tustin, 1976). The seasonal root forming capacity of Hayward seems to fluctuate with the endogenous IAA level.

Several reports in the literature suggest the presence of an optimum auxin level (Spiegel, 1955) and a critical minimum level (Tustin, 1976) for root initiation. From the present data, one could define roughly the upper and lower auxin threshold levels for Hayward cuttings as 0.06 ug/5g dry weight and 0.005 ug/5g dry weight respectively. Any level drop was evidenced between mid-Spring to mid-Autumn; whereas in Winter cuttings, auxin level falls well below the minimum critical level, and thus cuttings failed to respond to IBA treatment and no rooting

was possible. Many other workers also reported that easy-to-root cuttings usually respond to auxin treatment, whereas difficult to root varieties do not (Audus, 1963).

Treatments designed to overcome the various complicating factors were used to further illustrate the role of IAA. Centrifugation was used to remove any endogenous rooting regulators. Examination of cenrifugates and tissues samples indicated that a reduction in the endogenous IAA level within the cuttings accounted for the decreased rooting ability. Restoration of the rooting ability of the centrifuged Bruno cuttings was achieved by both IAA and IBA application, the combined treatment gave an additive promotion effect (Table 4.8.2). This observation again reaffirms the promotive role of auxin in root formation.

IBA and IAA were both found to have a significant effect on rooting promotion, and combined applications of IAA and IBA produced an additive promotion of rooting when compared with either auxin applied singly. This additive promotion of rooting in Bruno by IAA/IBA can not be explained satisfactorily by the traditional hypotheses that IBA promotes root formation by increasing the active auxin pool (Nanda and Anand, 1970) since

i) IBA was effective only when there is a continuing supply of IAA available eg. in leafy cuttings during Spring and Summer, with the presence of IAA supply from lanolin caps.
ii) increasing concentrations of IBA could not promote rooting further then was achieved with 10,000 ppm when applied alone.

There observations indicate that IBA is effective only with the presence of an optimum level of IAA and that this IAA must be in constant supply as pointed out by several contemprorary reseachers (Haissig, 1970, 1972; Mohammed and Eriksen, 1974; Mohammed,

1975). Thus it is unlikely that IBA is acting through the same metabolic pathways as IAA. Weaver,(1972) reported that the interpretation that IBA promotes root formation by increasing the active auxin pool seems unlikely when it is considered that IBA has weak auxin activity only. In the programme here reported it was observed that auxin-promoted growth by IBA in the oat coleoptile bioassay was only 1% of a similar concentration of IAA. An alternative suggestion that IBA acts by undergoing B-oxidation to form IAA was put forward by Fawcett et al, (1958) does not seem likely since some of the reported benefits of using IBA for propagation are the persistence, poor translocating ability and resistance to auxin - degrading enzymes (Weaver, 1972).

The proposal put forward by Tustin (1976) seems to be a more satisfactory explanation for the present results. IBA was postulated as exerting its promotive effect by protecting the endogenous IAA from enzymic degradation and conjugation - both are inactivation processes, thus ensuring the persistence of free IAA in the basal tissue. This proposal would explain the following phenomena :-

i) an increase in endogenous IAA resulting in improved rooting in Spring and Summer was the result of an IBA pretreatment prior to planting the cuttings. For cuttings not treated with IBA, the endogenous IAA would have been inactivated, thus resulting in poor rooting.

ii) in Winter, endogenous IAA is below critical level, thus a mere protection of already low level IAA by exogenous IBA did not produce any promotive effect.

iii) In Table 4.8.2 toxicity occured at 20,000 ppm IBA in treatment 12, 18 and 24, but not in treatment 6, this could be because in treatment 12, 18 and 24 IAA is limiting, thus by merely increasing IBA concentration would not improve rooting, whereas in treatment 6, IAA level is expected to be higher thus giving an

additional promotive effect. As a whole, it seems that IAA is playing a primary role in root promotion while IBA acts in an IAA - sparing role rather than boosting the total auxin (pool).

Auxin may be a limiting factor in root regeneration, but it is not necessarily so at the exclusion of other growth substances, this may be the reason why only a partial restoration of rooting potential is possible with the application of IBA and IAA in June.

Mung bean rooting histograms of crude extracts demonstrated the occurance of four rooting cofactor zone in Hayward stem tissues. All four promotory zones were subjected to seasonal fluctions of these cofactors but only cofactor 2 (Rf 0.3 - 0.5) seems to oscillate with the seasonal rooting cycle giving a similar correlation between rootability and cofactors level had been reported by Hess (1957, 1961, 1962(b), 1965, 1968); Hackett (1969); Lee (1969); Foong (1977). In Section 4.8, an examination of the centrifugates and tissue samples indicated a reduction in the cofactor level within the cutting which corresponds with the reduction in rooting ability after centrifugation. This again indicates a possible role of these cofactors in root formation.

Little is known of the mode of action of these cofactors. Hess (1965) and Fadl and Hartmann (1967 a & b) suggested that IAA may act by forming complexes with them.

Catechol, the phenolic auxin rooting cofactor, appeared to act partially by protecting and enhancing the IAA - aspartate conjugation (Hess, 1969). Ryugo and Breen (1974) agreed with Hess, that IAA - aspartate may be the first step tp promoting root initiation by IAA. They postulated that IAA forms the aspartate conjugate, which is then incorporated into specific proteins necessary for root initiation. These views are in accordance with ideas

on the mode of action of other auxin - induced growth promotion (Galston, 1967; Kobayashi and Yamaki, 1972; Morris et al, 1969; Masuda, 1965; Pilet and Braun, 1967; Tautvydas and Galston, 1970). Present experiments can not support these hypothesis any further, until an biochemical analysis of the nature of these cofactors is carried out.

Contrary to many reports (Gregory and Overbeek, 1945; Hess, 1966, 1968; Hackett, 1969; Foong, 1977). Cofactor 4 was found to be weaker than cofactor 2 and that the seasonal fluctuation of cofactor 4 did not correspond with that of the rooting percentage as did cofactor 2. This may suggest a variation in the dependence on specific biochemical factors for rootability between different plant species.

A close parallel can also be drawn between the seasonal cycle of percentage rooting (Figure 4.5.1) and that of ABA level (Figure 4.7.3). Abscisic acid can be visualised as promoting root initiation, an idea shared by many others (Chin and Beevers, 1969; Basu et al, 1970; Hyun and Sung, 1968; Bojarczuk and Jankiewicz, 1975). Centrifugation only removed a small portion of the endogenous ABA (Figure 4.8.3) which resulted in a significant drop in percentage rooting, which had been accounted for by IAA and cofactor drop. A similar parallel can be drawn for ABA. And this may suggest that the endogenous ABA in the cuttings may be initially marginal, thus a slight drop in ABA brings it right below the critical level resulting in a significant drop in percentage rooting. In contrast to the present finding, there is a long list of evidence refuting this promotive role of ABA as presented by Heide (1968), Eliason (1969), Pierik and Stegmans (1975), Basu, Roy and Bose (1970). The contemporary hypothesis of taking IAA:ABA ratio as a measure of rooting potential has been cited in several paper (Howard, 1966, 1971; Fadl and Hartmann, 1967a; Nesterov, 1968; Howard and Nahlawi, 1969; Dound and

Carlson, 1972). The present project can not substantiate this hypothesis to any great extent.

Reports on the effect of IAN in promotion of root formation are relatively few. Several reseachers postulated that IAN exerts its promotive effect through the interconversion of IAN to IAA (Sin and Sung, 1968; Sung, 1969; Gur and Samish, 1968; Odom and Carpenter, 1965) but from present data, no such conclusion can be drawn. The two peaks for the IAN level in August and January correspond to the lowest and highest rooting percentage of the cutting respectively. Tustin (1976) also found no correlation between IAN content with the seasonal changes in root formation of Malus sylvestris M.

In the June collection, IAA application was found to be effective in increasing the rooting potential of leafless cuttings (Treatment 1 and 7, Table 4.3.2). This suggested that the leaves may be the likely source of IAA as suggested by many researchers (Bouillenne and Went, 1933; Bouillenne and Bouillenne-Warland, 1955; Girouard, Hess, 1964; Okoro and Grace, 1976). But result from experiment 4.9 shows that IAA can only partially improve the rooting of leafless cuttings and that IAA/leaf effect is a synergistic one and not an additive effect. This further indicates that it is impossible to postulate that the role of leaves is solely an auxin producer, in the process of root formation.

Many workers view the role of leaves as a nutritive one (Zimmerman and Hitchcock, 1928; Altman, 1972; Bilan, 1974; Ohta and Furukawa, 1975; Porlingis and Therios, 1976). Haissig (1974c) further concluded that the role of auxin is to stimulate the initiation of root formation, whereas nutritive materials are essential as the building blocks for root formation. But this nutritive role of leaf can not explain the lack of response of leafless cutting to IBA and sucrose treatment in experiment 4.9.2.

The alternative rhizocaline hypothesis suggested by Cooper (1936, 1938) can be a more suitable explanation of present result. Went (1938) concluded that both auxin and rhizocaline are essential for root formation in cuttings. Auxin causes redistribution and accumulate of the rhizocaline at the base of the cutting. This may account for the synergistic effect of IAA and leaves.

From available literature and findings of the present project, it can be appreciated that adventitious root formation is a very complex process. Adventitious root formation is the result of a multiple of interactions of a well balanced hormones, rooting cofactors, inhibitors, nutritional factors and intrinsic enzymic factors, which it-self depends on anatomical and environmental influences. (Plant, 1940; Skoog and Miller, 1956; Heide, 1964, 1965 and 1967; Gautheret, 1969; Read and Hoysler, 1969; Mitsuhashi, Shibaska and Shimokoriyama, 1969; Saniewicz, Novak and Rudnicki, 1974).

6.2 ROOT CUTTINGS

The present experiment clearly demonstrated the feasibility of propagating Abbott from their own root pieces. This study has also revealed that the success of the technique is affected by a number of external environmental factors as well as internal conditions.

Root thickness itself is of considerable importance for shoot production. Way et al (1955) and Turovskaya (1969) suggested root thickness seems to be related to the reserves of assimulates available for regeneration. Robinson and Schwabe (1977) further suggested that the root thickness and food reserves interrelated with the ratio of bark to wood in the roots; a higher proportion of bark, probably indicating greater storage capacity, therefore increasing the survival and

regenerative potential.

Having a single piece of long (15 cm) root cuttings was found to be less productive than cutting it into several smaller pieces (5 cm). This was because by cutting a root piece into 3 segments, one generated two more proximal surfaces where shoot regeneration can occur. When tissue segments are cut, the physiological status is disturbed. This may cause a redistribution of some substance, probably auxin, thus accounting for the different response observed at previously adjacent surfaces. Hartmann and Kester (1975). This correlation of polarity of shoot regeneration with auxin acropetal movement is further affirmed by the fact that any proximal application of IAA suppressed the shoot regeneration capacity significantly, whereas distal application did not interfere with bud formation at the proximal end. It is therefore logical to visualise that further segmentation of a unit length of root piece would increase the productivity, as long as the segmented root pieces still have sufficient food reserve in them for shoot regeneration.

The shoot regeneration polarity is also expressed in several other ways: -

i) shoots only form at proximal cut end.

ii) newly formed shoot does not have any root, but when shoot is detached, the adventitious root will form shortly.

iii) Changing the position of the cuttings with respect to gravity does not alter this tendency. Cuttings planted upside down did not shoot at all.

In attached roots, auxin from the aerial part of the tree would normally prevent bud initiation, but this supply is cut off on detachment of the root, the depletion of auxin will allowing bud initiation. Farmer (1962) regerded this phenomenon as an extension of apical

dominance, and similar effects were shown by Maimi (1968) on poplar roots and by Sterrett and Chappell (1967) on black locust.

The present work supports the view of Wenger (1953) who stated that the effect of season is important and appears to be related to the prevailing growth phase of the plant. Similar seasonal fluctuation in respberry roots was observed by Hudson (1954, 1955) who found district 'on' and 'off' periods during Winter and Summer respectively. Hartmann and Kester (1975) concluded that best results are likely to be attained if the root pieces are taken from young stock plants in late Winter or early Spring when the roots are well supplied with stored foods but before new growth starts. By contrast, horse-radish and dandelion root cuttings have a constantly high (near 100%) shoot regenerating capacity throughout the year (Hudson, 1955; William and Hudson, 1956).

The seasonal fluctuation in shoot forming capacity may be attributed to carbohydrate reserves in the cuttings. A rapid and pronounced increase in polysaccharide from September to November followed by gradual hydrolysis and translocation during Winter was observed by Mochizuki and Hanada (1957), Priestley (1962), Hansen (1967), Kaphya (1968) and Quinlan (1969). During leaf fall, sugars evidently moved into the roots and were rapidly converted to polysaccharides. The importance of Autumn foliage to carbohydrate accumulation in the roots was also established by Priestley (1964). In January, 5% sucrose treatment had significantly ($P<0.01$) improved shoot regeneration, thus supporting the hypothesis of carbohydrate reserve significance.

Hormonal control is yet another possible mechanism which may interact with carbohydrate supply. IAA was found to be inhibitory and GA did not show any effect while cytokinin exhibited a strong promotive effect

on shoot regeneration (Table 6.5.2 - 6.5.4). Robinson and Schwabe (1977) also pointed out that a suitable balance between carbohydrate and IAA is probably required for the regeneration of apple shoots from root cuttings. The strong interaction of sucrose and BAP (Table 6.5.6) confirm the results of Robinson and Schwabe (1977).

To further eluidate the mechanism of shoot regeneration, it is advisible for future workers to determine the endogenous hormonal regulators as well as food substrate in the cuttings.

The present experiment, demonstrated the feasibility of growing Abbott from root cutting and a brief investigation into the mechanism of shoot regeneration. To really grow the plant from root cuttings one still has to assess the subsequent survival rate of the young shoot regenerated in this way from the cutting.

APPENDIX I

Buffer solutions

1) Phosphate - citrate Buffer for the Wheat Coleoptile Bioassay.

K_2HPO_4	4.48 gm.
Citric acid monohydrate	2.547 gm.

dissolve in 250 ml distilled water. This buffer is a 10X solution strength. For use, dilute 1 in 10 and add 2 gm. of sucrose per 100 ml buffer pH = 5.3

2) Burstrom Basal Solution

K_2HPO_4	10^{-1} mole litre^{-1}
$Ca(NO_3)_2$	10^{-4} mole litre^{-1}
$MgSO_4$	10^{-5} mole litre^{-1}
Glucose	16 gm litre^{-1}

0/1 M citric acid was added to the solution until a final pH of 5.6 was reached.

APPENDIX 2

SUMMARY OF STATISTICAL ANALYSIS

1) Propagation of Hayward cuttings under polythene tent/mist on 26.11.78. ANOVA for percentage rooting

	df	ss	ms	VR	F test
Block	7	17	2.4286	1.696	NS
IBA	1	220.625	220.625	154.434	XXX
mist	1	0.5	0.5	0.340	NS
IBA X mist	1	17.875	17.875	12.512	XX
error	21	30	1.4286		
Total	31	186			

2) Effect of wounding on percentage rooting of Hayward cuttings on 25.11.77

	df	ss	ms	VR	F test
Block	3	1.1875	0.3958	2.280	NS
IBA	1	52.5625	52.5625	302.779	XXX
Wound	1	27.5625	27.5625	158.77	XXX
IBA X wound	1	5.0625	5.0625	2.5945	NS
error	9	1.5625	0.1736		
Total	15	87.9375			

3) VARIETAL DIFFERENCE

(3.1)

5.5.77	df	ss	ms	VR	F test
Treat.	2	1	0.5	0.294	NS
block	5	14	2.8	1.647	NS
error	10	17	1.7		
	17	32			

(3.2)

26.1.78	df	ss	ms	VR	F test
Treat.	2	1	0.5	0.3489	NS
Block	5	6.6667	1.333	0.9302	NS
error	10	14.333	1.433		
	17	22			

4) Effect of bottom heat on percentage rooting of Hayward cuttings

(4.1) 26.1.78

26.1.78	df	ss	ms	VR	F test
Block	7	1.5	0.2143	0.333	NS
IBA	1	242	242	376.444	¥¥¥
B.Heat	1	18	18	28	¥¥¥
IBA X B.Heat	1	12.5	12.5	19.444	¥¥¥
error	21	13.5	0.6429		
Total	31	287.5			

(4.2)

5.5.77	df	ss	ms	VR	F test
Block	7	4.5	0.64	0.931	NS
IBA	1	98	98	141.931	¥¥¥
B.Heat	1	18	18	26.07	¥¥¥
IBA X B.Heat	1	12.5	12.5	18.1	¥¥¥
error	21	14.5	0.69		
Total	31				

(4.3) ANOVA table for the percentage rooting of Abbott cuttings when treated with different IBA concentration

5.5.77	df	ss	ms	VR	F Test
Treatment	7	57.235	8.1764	5.458	¥¥
Block	7	8.36	1.1942	0.797	NS
Error	49	73.405	1.498		
Total	63	139			

(4.4) ANOVA Table for the mean number of roots per cuttings when treated with different IBA concentration

5.5.77	df	ss	ms	VR	F Test
Treatment	7	724.464	103.495	11.789	¥¥¥
Block	7	120.092	17.156	1.954	NS
Error	49	430.151	8.779		
Total		1274.707			

The effect of varying concentration of IBA on Abbott stem cutting

(25.11)

	df	ss	ms	VR	F Test
Treatment	2	34.6667	17.3333	104.010	XXX
Block	2	0.6667	0.3334	2.001	NS
Error	4	0.666	0.1667		
Total	8	36			

(25.1)

	df	ss	ms	VR	F Test
Treatment	3	388.25	96.083	99.65	XXX
Block	7	7.5	1.0714	1.111	NS
Error	21	20.25	0.9642		
Total	31	316			

5) Seasonal fluctation in rooting ability

(5.1) Seasonal changes in percnetage rooting of Hayward cuttings treated with IBA

	df	ss	ms	VR	F Test
Treatment	5	458.583	91.717	177.516	XXX
Block	5	3.25	0.65	1.26	NS
Error	25	12.917	0.517		
Total	35	474.75			

(5.2) Seasonal changes in percentage rooting of Hayward cuttings not treated with IBA

	df	ss	ms	VR	F Test
Treatment	5	1.917	0.383	2.091	NS
Block	5	0.25	0.05	0.2727	NS
Error	25	4.583	0.183		
Total	35	6.75			

(5.3) Seasonal changes in percentage rooting of Abbott cuttings treated with IBA

	df	ss	ms	VR	F Test
Treatment	5	475.583	95.117	73.735	XXX
Block	5	2.917	0.583	0.452	NS
Error	25	32.250	1.29		
Total	35	510.75			

(5.4) Seasonal changes in percentage rooting of Abbott cuttings not treated with IBA

	df	ss	ms	VR	F Test
Treatment	5	3.6	0.72	3.8	X
Block	5	0.2666	0.053	0.279	NS
Error	25	4.733	0.189		
Total	35	8.6			

6) Effect of bud on percentage rooting on Abbott cuttings

(6.1) Harvesting Date - 6.5.77

	df	ss	ms	VR	F test
Block	8	0.3889	0.0486	1.1048	NS
IBA	1	0.1111	0.1111	2.525	NS
Bud	1	0.11111	0.1111	2.525	NS
IBA X Bud	1	0.2223	0.2223	5.0523	*
error	24	1.0556	0.0440		
Total	35	1.889			

(6.2) Harvesting Date - 7.6.77

	df	ss	ms	VR	F test
Block	8	1	0.125	1.2	NS
IBA	1	0.5	2	19.23	**
Bud	1	0.5	2	19.23	**
IBA X bud	1	1	1	9.615	*
Error	24	2.5	0.104		
Total	35	5.5			

7) The effect of IAA/IBA/Centrifugation on percentage rooting of Bruno cuttings on 5.5.77

	df	ss	ms	VR	F test
Block	2	2.2000	1.1	0.686	NS
Main effect					
IBA	5	27.333	5.467	3.407	*
IAA	1	98.000	98	61.086	***
Cent.	1	3.556	3.556	3.317	
2 factors interaction					
Cent/IBA	5	2.112	0.4224	0.263	NS
Cent/IAA	1	0.889	0.889	0.554	NS
IAA/IBA	5	8.333	1.667	1.039	NS
3 factors interaction					
IAA/IBA/Cent.	5	1.779	0.356	0.222	NS
Error	46	73.798	1.604		
Total	71	218.000			

8) The role of leaf/bud/IAA on percentage rooting of Abbott cutting 20.1.78

	df	ss	ms	VR	F test
Block	7	1.337	0.191		NS
Main effect					
IAA	1	168.750	168.750	156.136	***
Leaf	1	225.334	225.334	208.491	***
Bud	1	0.16	0.16	0.148	NS
2 factors interaction					
IAA/Leaf	1	126.750	126.750	117.276	***
IAA/Bud	1	0.59	0.59	0.546	NS
Leaf/Bud	1	0.173	0.173	0.160	NS
3 factors interaction					
leaf/bud/IAA	1	0.243	0.243	0.225	NS
Error	33	35.666	1.081		
Total	47	558.753			

APPENDIX 3

Statistical Analysis for rootings ANOVA for root thickness 1.4.77

	df	ss	ms	VR	F Test
Block	7	1.96	0.28	0.61109	NS
Treatment	2	11.583	5.7915	12.6397	**
Error	14	6.415	0.4582		
Total	23	19.958			

ANOVA for root length and polarity

a) 11.7.77.

	df	ss	ms	VR	F Test
Block	9	4.525	0.503	0.829	NS
Treatment	3	106.875	35.625	58.74	***
Error	27	16.375	0.606		
Total	39	127.775			

b) 31.9.77

	ddf	ss	ms	VR	F Test
Treat.	2	83.467	41.733	27.617	***
Block	9	17.2	1.9111	1.265	NS
Error	18	27.2	1.511		
Total	29	127.867			

ANOVA for percentage rooting of Abbott root cuttings at various season

	df	ss	ms	VR	F Test
Block	4	0.3	0.075	0.046	NS
Treat	3	108.55	36.183	22.041	***
Error	12	19.7	1.642		
Total	19	128.55			

ANOVA For IBA Treatment

	df	ss	ms	VR	F Test
Block	3	4.917	1.639	0.831	NS
Treat.	2	26.167	13.084	6.634	***
Error	6	11.833	1.972		
Total	11	42.917			

ANOVA For GA Treatment

	df	ss	ms	VR	F Test
Block	3	3.000	1	0.48	NS
Treat.	2	0.167	0.084	0.04	NS
Error	6	12.5	2.083		
Total	11	15.667			

ANOVA For BAP Effect

	df	ss	ms	VR	F Test
Block	3	6.000	2	0.6667	NS
Treat.	2	40.667	20.333	6.777	*
Error	6	18.000	3		
Total	11	64.667			

ANOVA For BAP and Sucrose Effect

	df	ss	ms	VR	F Test
Block	3	4.333	1.444	1.256	NS
BAP	2	52.75	26.375	22.935	***
Sucrose	1	28.167	28.167	24.493	***
BAP X Sucrose	2	82	41	35.652	***
Error	15	17.25	1.15		
Total	23	184.5			

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